"Methylation Revisited – Part 2 Identifying and Eliminating the Major Epimutagens to Optimize Methylation – Simply!" Online April 24, 2021 (9 AM – 2 PM EDT)

HEALTH kaizen OPTIMIZING HEALTH

kaizen is a long-term approach that systematically seeks continuous improvement by means of small, incremental changes; improving efficiency and quality.

Educating, Equipping, and Empowering Clinicians and their Patients to Mitigate Chronic Disease and Optimize Health!

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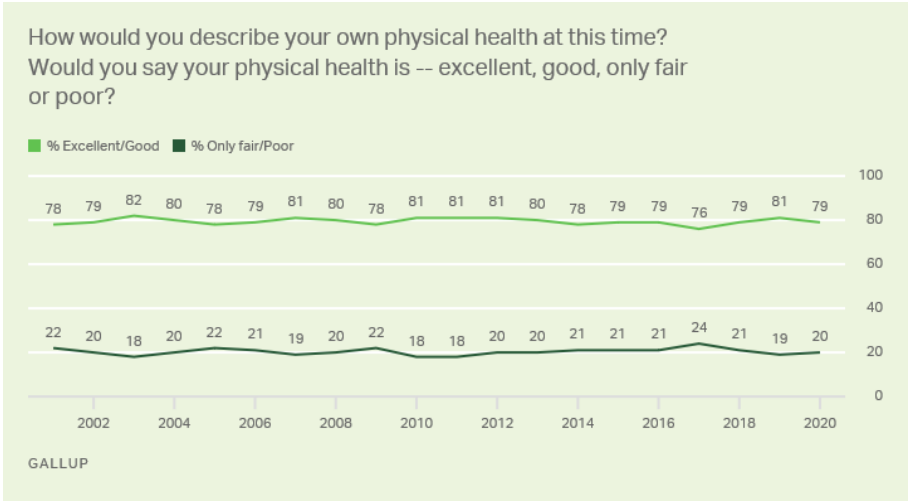

Downloaded March 29, 2021, from – https://news.gallup.com/poll/1648/personal-health-issues.aspx

2

Yet Over 70% of Americans have at least 1 chronic health condition. Chronic diseases cause 7 out of 10 premature deaths—many of which are completely preventable and account for 86% of our nation's healthcare costs.
And 40% have 2 or more.

3

6 key Questions:

- How do we know the patient has a deficiency?……
  Without A Doubt!
- Will a particular nutrient help the condition?……
  Without A Doubt!
- Is the Offending mechanism removed?……
  Without A Doubt!
- When to change the protocol?……
  Without A Doubt!
- When is the deficiency corrected?……
  Without A Doubt!
- When to introduce Life-time Aging Gracefully Protocols?
  Without A Doubt!

The aim of this Seminar is how to identify, assess and treat Chronic Degenerative Diseases, Correcting Biochemical, Functional and Metabolic Health Issues – Naturally, Safely and Effectively!….

~ Without A Doubt! ~

4

Seminar Objectives:

Have fun!

Take home this today's information and put it to use Monday morning!

Gain the understanding and confidence with Methylation Principles and effectively treat patients suffering from chronic degenerative diseases.

A clear and relevant understanding of undertaking a simple, concise, and systematic approach to methylation and managing chronic degenerative diseases.

Aging with Grace and Dying with Dignity!

~ Without A Doubt~

5

Disclaimer

The information presented is presented solely for educational purposes, and it is up to the clinician to determine appropriate intervention and/or appropriate referral to other health care practitioners.

Procedures and products recommended should not be construed as a claim or representation that such procedure or product will constitute a cure.

Treatment and Nutritional recommendations presented may be considered "off-label" per FDA definitions.

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Seminar Overview - Notices

Clinicians must use their own personal judgment, training, knowledge and experience to formulate and direct 'Individualized patient treatment' (Bioindividuality).

Some of the procedures or information presented may be beyond your scope of practice, depending upon your licensure, training, state, etc.. Please consult your state board for clarification performing new procedures.

Warning – portions of this presentation may contain imperceptibly dry humor, no humor at all or may not even be perceived to be funny at all.

Sometimes thoughts just pop into my head and exit my mouth! - Don't say you weren't warned

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Our goal is to recognize and identify patterns of internal chemistry, toxicity and nutritional deficiencies, as early as possible, which if corrected now will lead to Optimum Health and an improved quality of life.

If left uncorrected, they may become full-blown diseases later. Perhaps, requiring dangerous drugs and/or surgery in an attempt to prolong life, and most likely diminishing quality of life to a mere survival mode.

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2 Case study examples describing underlying core foundational issues set the stage for unraveling the chronic disease Conundrum

- **Infection causing thyroid malfunction**
- **Genetic "Parkinson-like" disorder**

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Deb (DOB – 10/13/62) – originally seen in our office 4/2014 – presenting complaints: insomnia, anxiety/depression, fatigue, tinnitus

10/2017 – she had some labs (no CBC)– all WNL, later in the the month she presented to her local clinic with laryngitis (CBC Mono 10%)

12/21/17 – labs TSH .06
1/8/18 US – Dx– Enlarged heterogeneous and hyperemic thyroid gland without discrete nodularity
2/5/18 – TSH 113.1, TPO antibodies >900
2/26/18 – TSH 0.1
3/14/18 – TSH 35.2
4/30/18 TSH 0.04
6/1/18 – TSH 0.3
8/1/18 – TSH – 0.5
9/12/18 – TSH – 3.9 (mono 9%)
11/6/18 – TSH – 4.4 (mono 9%)

10

Gary – DOB 9/10/52 – 64 yoa First presented to our office 2/16/17 co: unsteadiness, poor balance, poor visual focus, Extreme fatigue, constipation and dry mouth. Had been diagnosed 1 year before by UI as MSA. Was on carbidopa-levodopa tid.

Treated Microbiome originally – rapid, significant improvement.
7/18/17 – Monocytes 11% (<10%), lymph 12% (20-40%)
11/8/17 – Monocytes 13%, lymph 10%
1/31/18 – Monocytes 13%, lymph 16%
5/8/18 – Monocytes 15%, lymph 18%
10/11/18 – Monocytes 12%, (Abs mono – 1.0 lab < .9) lymph 16%
11/7/18 – Feeling GREAT! Eyes are focused most of the time, balance getting more steady.

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Common Presenting Complaints:

- Anxiety/Depression

Our Goal:

Achieve maximum or optimal human potential by:

- ❖Restoring BALANCE:
  - Removing the interferences
  - Correcting the deficiencies

Identifying and Correcting the biochemical, functional and metabolic disturbances robbing our patients of their full potential for optimal health.

- Pain
- Sleep disturbances
- Weight gain/loss

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We are on a mission to improve the health of individuals and the families we have the privilege of serving.

To help those with chronic illness regain, maintain and enhance the quality of life for this, as well as future generations –

Safely, Effectively and Naturally.

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4 Major Factors: Epimutagens
- Microbiome – Stealth Infections
- Acid/Alkaline Balance
- Blood Sugar Regulation
- Stress

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Applying a Systematic, Simple, and Effective Approach to Chronic Diseases: Unraveling the Gordian Knot* of Chronic Diseases

*The Gordian Knot is the legend of Phrygian Gordium associated with Alexander the Great. It is often used as a metaphor for disentangling an "impossible" knot solved easily by thinking outside the box ("cutting through the Gordian knot").

<u>Seminar Objective</u>: To present practitioners a systematic approach for developing effective methods to evaluate, assess, and treat chronic health conditions; utilizing innovative nutritional therapeutic options to support improved health outcomes.

By thinking outside of the box, the practitioner will be able to “unravel” the mystery of the Gordian Knot in the management of chronic diseases.

The Kaizen Way ~ Without A Doubt!!!

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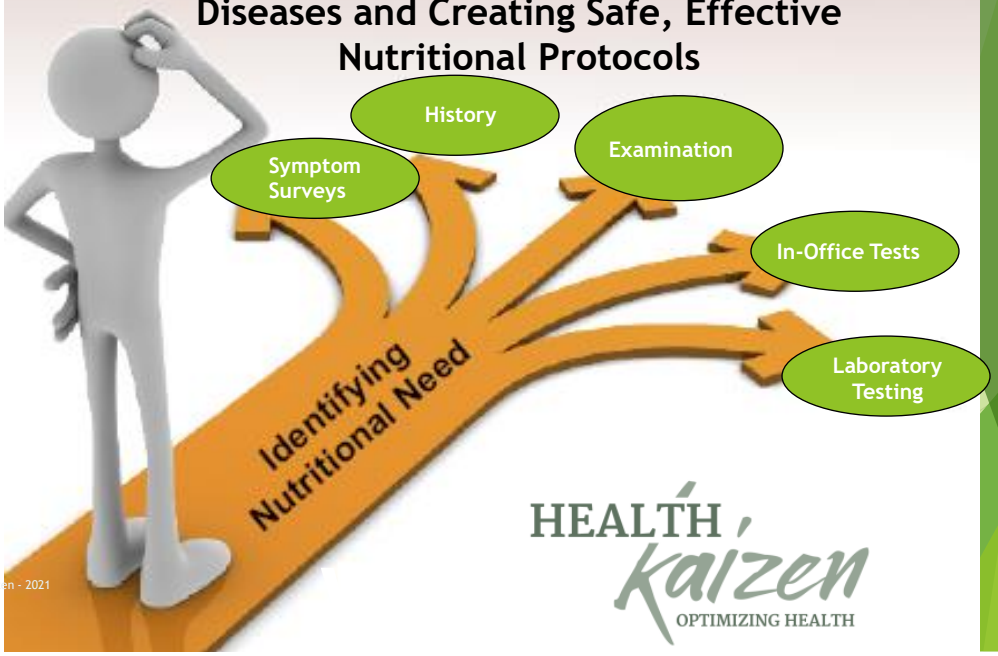
Supporting and Managing Chronic Degenerative Diseases and Creating Safe, Effective Nutritional Protocols



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"Everyone has a physician inside him or her; we just have to help in its work. The natural healing force within each one of us is the greatest force in getting well."

Hippocrates

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Research

Diseases linked to unhealthful diet and lifestyle choices, such as diabetes and cancer, are the leading causes of death in the United States, according to data published in *JAMA*. Researchers compared mortality for hundreds of causes and risk factors and found that heart disease, lung cancer, high BMI, and high blood pressure and blood sugar were all among the top risk factors for mortality. Dietary risk factors surpassed tobacco use as the leading cause of death. The authors note differences in risk factors at the state level and recommend targeted approaches to address these issues for disease prevention.

JAMA April 10, 2018

JAMA. 2018;319(14):1444-1472. doi:10.1001/jama.2018.0158

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The New York Times

Can Eating Organic Food Lower Your Cancer Risk?

In a study, those who ate more organic produce, dairy, meat and other products had 25 percent fewer cancer diagnoses over all, especially lymphoma and breast cancer.

By Roni Caryn Rabin

Oct. 23, 2018

People who buy organic food are usually convinced it's better for their health, and they're willing to pay dearly for it. But until now, evidence of the benefits of eating organic has been lacking.

Now a new French study that followed 70,000 adults, most of them women, for five years has reported that the most frequent consumers of organic food had 25 percent fewer cancers over all than those who never ate organic. Those who ate the most organic fruits, vegetables, dairy products, meat and other foods had a particularly steep drop in the incidence of lymphomas, and a significant reduction in postmenopausal breast cancers.

The magnitude of protection surprised the study authors. "We did expect to find a reduction, but the extent of the reduction is quite important," said Julia Baudry, the study's lead author and a researcher with the Center of Research in Epidemiology and Statistics Sorbonne Paris Cité of the French National Institute of Health and Medical Research. She noted the study does not prove an organic diet causes a reduction in cancers, but strongly suggests "that an organic-based diet could contribute to reducing cancer risk."

The study, published Monday in JAMA Internal Medicine, was paid for entirely by public and government funds.

Nutrition experts from Harvard who wrote a commentary accompanying the study expressed caution, however, criticizing the researchers' failure to test pesticide residue levels in participants in order to validate exposure levels. They called for more long-term government-funded studies to confirm the results.

"From a practical point of view, the results are still preliminary, and not sufficient to change dietary recommendations about cancer prevention," said Dr. Frank B. Hu, one of the authors of the commentary and the chairman of the department of nutrition at Harvard's T.H. Chan School of Public Health.

https://www.nytimes.com/2018/10/23/well/eat/can-eating-organic-food-lower-your-cancer-risk.html 1/3

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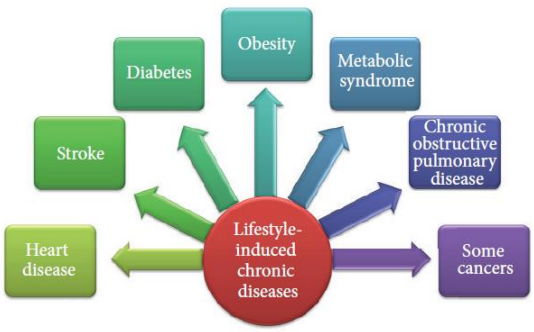

FIGURE 1: Lifestyle-induced chronic disease.

Hindawi Publishing Corporation, The Scientific World Journal; Volume 2013, Article ID 129841, 14 pages. http://dx.doi.org/10.1155/2013/129841

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Methylation

- VITAL Metabolic Process!
- Occurs in every cell of the body – Billions of times per second.
- Can affect EVERY organ.
- Turn genes on and off, important in the development of cancer.
- Process chemicals and toxins - Bio transformation.
- Process and metabolize hormones – estrogen.
- Make neurotransmitters – dopamine, serotonin, and epinephrine.
- Produce energy – ATP, CoQ10, and carnitine.
- Important for myelination.
- Synthesis of DNA and RNA.
- Necessary for healthy immune system – NK and T cells.

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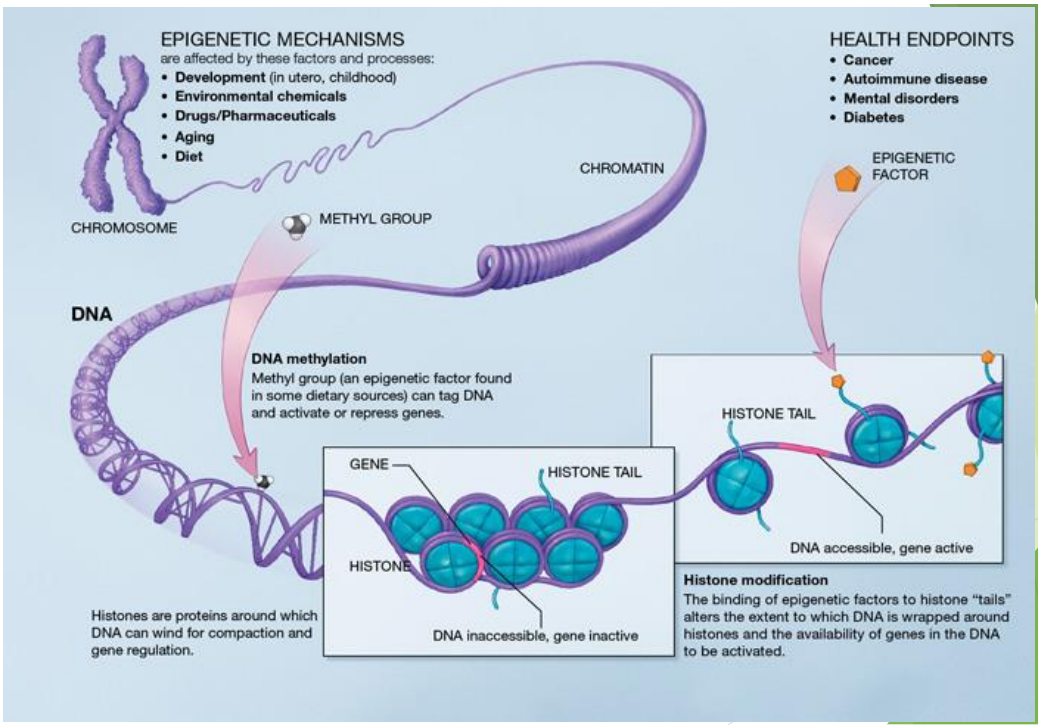

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EPIGENETICS

Epigenetics is the study of how these chemical reactions occur and the factors that influence them.

Coined by C. H. Waddington in 1942 as a portmanteau of the words epigenesis and *genetics*

Recent findings in epigenetics shed new light on the regulation of gene expression: The most frequently studied epigenetic mechanisms are:

- DNA methylation
- Histone modifications and
- MicroRNA

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EPIGENETICS

Epigenetics is considered an important mechanism in the unknown etiology of many diseases. Over the past decades, epigenetic studies mainly have been focused on embryonic development, aging, and cancer.

More recently, epigenetics has been highlighted in many other processes, such as inflammation, immune diseases, obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular diseases, and neurodegenerative diseases.

This is due to alterations in epigenetic modifications by external or internal environmental factors and their ability to change gene expression.

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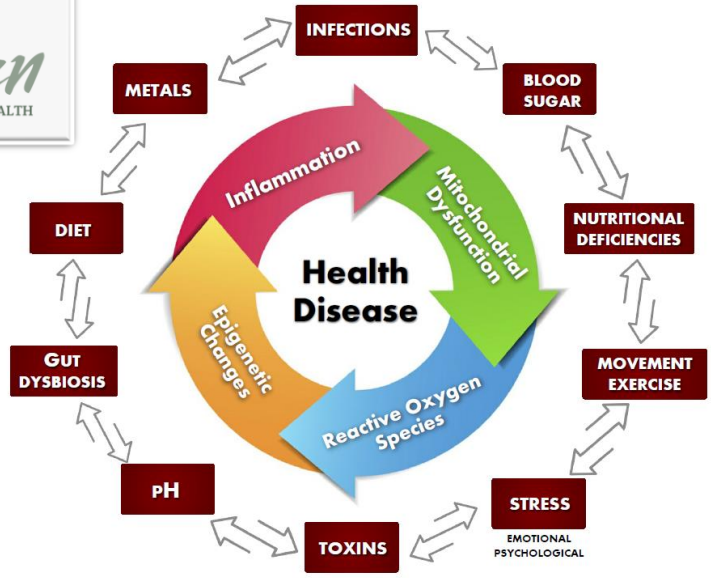

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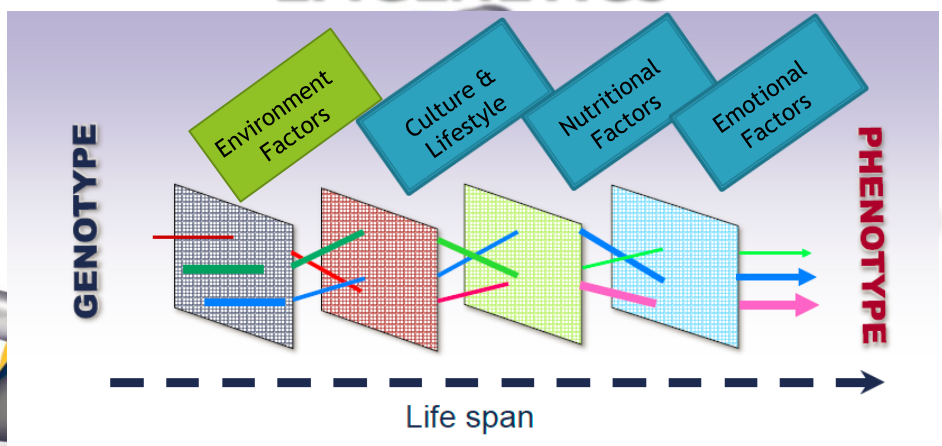
EPIGENETICS



Because of these filters, we are not slaves to our genes nor are we victims of genetic determinism.

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Epigenesis –

Theory that the features of an organism arise from an interaction between genetic and environmental influences.

Nature vs. Nurture

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EPIGENESIS:

Where Nature and Nurture Meet!!

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epimutagen

epi- + *mutagen*

Noun - epimutagen (*plural* **epimutagens**)

Any material/process that causes **epimutagenesis, in other words, anything that will alter "Genetic Expression!!"**

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CDC Centers for Disease Control and Prevention

The National Institute for Occupational Safety and Health (NIOSH)

Promoting productive workplaces through safety and health research NIOSH

Exposome and Exposomics

Overview

What is the exposome?

Success in mapping the human genome has fostered the complementary concept of the "exposome". The exposome can be defined as the measure of all the exposures of an individual in a lifetime and how those exposures relate to health. An individual's exposure begins before birth and includes insults from environmental and occupational sources. Understanding how exposures from our environment, diet, lifestyle, etc. interact with our own unique characteristics such as genetics, physiology, and epigenetics impact our health is how the exposome will be articulated.

Exposomics is the study of the exposome and relies on the application of internal and external exposure assessment methods. Internal exposure relies on fields of study such as genomics, metabonomics, lipidomics, transcriptomics and proteomics. Commonalities of these fields include 1) use of biomarkers to determine exposure, effect of exposure, disease progression, and susceptibility factors, 2) use of technologies that result in large amounts of data and 3) use of data mining techniques to find statistical associations between exposures, effect of exposures, and other factors such as genetics with disease. External exposure assessment relies on measuring environmental stressors. Common approaches include using direct reading instruments, laboratory-based analysis, and survey instruments. The extent to which internal and external exposure assessment will contribute to our understanding of the exposome is being debated as each approach has certain merits.

A key factor in describing the exposome is the ability to accurately measure exposures and effect of exposures. Many of the "omics" technologies have the potential to further our understanding of disease causation and progression. Metabonomics and adductomics (DNA and protein adduct measurement) have been used in the past to establish exposure-disease relationships. Research is needed to determine the utility of the "omics" technologies in defining the exposome.

Recently, some thought leaders in the exposome have pushed to narrow the focus to include only the study of metabonomics. Many of these small molecular weight compounds act as signals to regulate biological systems. They show promise in deciphering disease mechanisms. While metabonomics has great potential to contribute to the study of the exposome, it has not been established that it is the only approach needed to clearly articulate the important aspects of the exposome.

Why should we study the exposome?

One of the promises of the human genome project was that it could revolutionize our understanding of the underlying causes of disease and aid in the development of preventions and cures for more diseases. Unfortunately, genetics has been found to account for only about 10% of diseases, and the remaining causes appear to be from environmental causes. So to understand the causes and eventually the prevention of disease, environmental causes need to be studied.

What are the challenges of advancing exposomics?

"The exposome can be defined as the measure of all the exposures of an individual in a lifetime and how those exposures relate to health. An individual's exposure begins before birth and includes insults from environmental and occupational sources."

"Unfortunately, genetics has been found to account for only about 10% of diseases, and the remaining causes appear to be from environmental causes. So to understand the causes and eventually the prevention of disease, environmental causes need to be studied."

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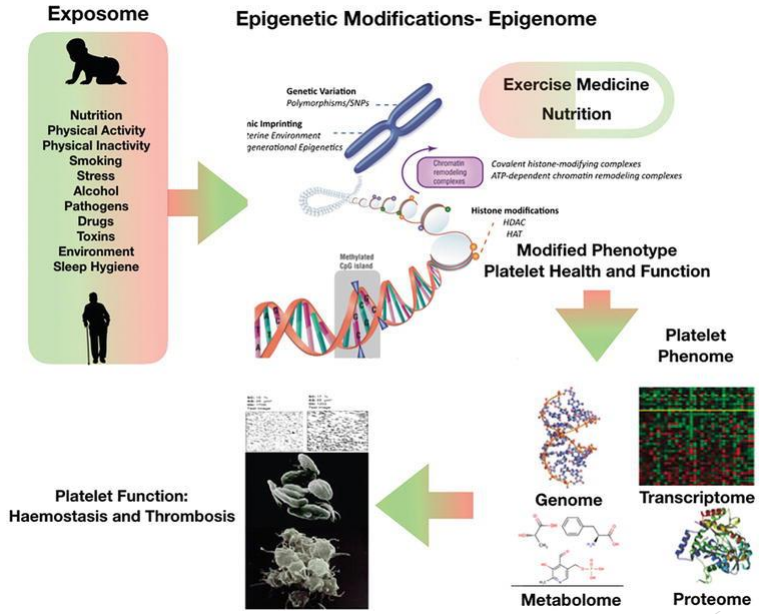

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The 'Balance' between Health/Disease:

INFLAMMATION ⟺ ***REACTIVE OXYGEN SPECIES***

MITOCHONDRIAL (DYS)FUNCTION ➡ ***HEALTH/DISEASE***

EPIMUTAGENS

- Mitochondrial Health
- Digestion
- pH – Acid/Alkaline Balance
- Sugar Regulation
- Microbiome
  - Pathogens – Stealth Infections
    - ✓ Viral loads
    - ✓ Bacterial loads
    - ✓ Fungal infections
    - ✓ Parasites
- Gut – Brain Connection
- Stress
- Hydration
- Eliminating and Mitigating the negative factors:
  - Diet/ Lifestyle
  - Heavy metals
  - Toxins
- Electrical/Energetic
- Exercise
- Structural
- Hormonal Balance
- Neurotransmitters
- Vitamin/Mineral Balance & Phytochemicals
- Essential Fatty Acids
- Emotional/Psychological
- Sleep
- Spiritual Congruence

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Published in final edited form as:
Epigenomics. 2012 October ; 4(5): 503–509. doi:10.2217/epi.12.41.

… "evidence suggests that epigenetic changes (i.e. set of reversible, heritable changes in gene function or other cell phenotype that occurs without a change in DNA sequence), may affect the aging process and may be one of the central mechanisms by which aging predisposes to many age-related diseases. The total number of altered methylation sites increases with increasing age….

Epigenomics. 2012 October; 4(5): 503-509. doi:10.2217/epi.12.41.

with increasing age, such that they could serve as marker for chronological age. This article systematically highlights the advances made in the field of epigenomics and their contribution to the understanding of the complex physiology of aging, lifespan and age-associated diseases.

Keywords

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SCIENTIFIC REPORTS

OPEN Human leukocyte telomere length is associated with DNA methylation levels

SUBJECT AREAS:

"…… epigenetic 'signature' of chronological age is related to telomere length shortening. It is well-known that there is wide inter-individual variation for the risk of age-related disease in people of the same chronological age. Loci at methylation levels are associated with both chronological age and telomere length may thus be of particular relevance to the investigation of factors that influence successful aging…."

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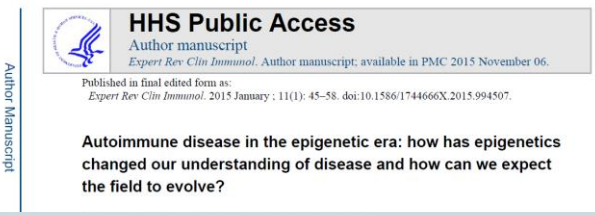
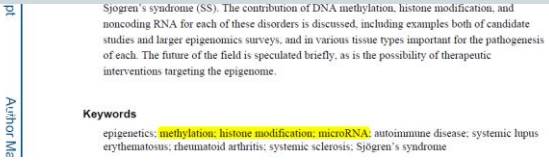


The contribution of DNA methylation, histone modification, and noncoding RNA for each of these disorders is discussed, including examples both of candidate studies and larger epigenomics surveys, and in various tissue types important for the pathogenesis of each. The future of the field is speculated briefly, as is the possibility of therapeutic interventions targeting the epigenome.

Expert Rev Clin Immunol. 2015 January ; 11(1): 45–58.
doi:10.1586/1744666X.2015.994507.



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NUTRIGENOMICS

Nutrigenomics is a branch of nutritional genomics and is the study of the effects of foods and food constituents (vitamins, minerals, enzymes, phytochemicals) have on gene expression.

This means that **Nutrigenomics** is research focusing on identifying and understanding molecular-level interaction between nutrients and other dietary bioactives with the genome.

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NUTRIGENOMICS

- **Diet can definitely alter the epigenetic state** of the genome leading to dramatic deprogramming or reprogramming of large numbers of genes in metabolic pathways and physiological systems.
- This may affect the incidence of long-latency, late-stage, diseases such as CVD, IDDM ,neurodegenerative diseases, cancer, etc.
- **Foods contain many inhibitors and stimulators of chromatin remodeling systems (**DNA methylases and histone acetylases and deacetylases), making Nutritional intervention a plausible way to **"Reprogram" the epigenome** to promote health and prevent disease processes.

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ASN 2013 ANNUAL MEETING SYMPOSIUM SUMMARY

ABSTRACT: Epigenetics can be defined as inheritable and reversible phenomena that affect gene expression without altering the underlying base pair sequence. Epigenomics is the study of genome-wide epigenetic modifications. Because gene expression changes are critical in both normal development and disease progression, epigenetics is widely applicable to many aspects of biological research. The influences of nutrients and bioactive food components on epigenetic phenomena such as DNA methylation and various types of histone modifications have been extensively investigated. Because an individual's epigenetic patterns are established during early gestation and are changed and personalized by environmental factors during our lifetime, epigenetic mechanisms are quite important in the development of transgenerational and adult obesity as well as in the development of diabetes mellitus. Aging and cancer demonstrate profound genome-wide DNA methylation changes, suggesting that nutrition may affect the aging process and cancer development through epigenetic mechanisms.

Adv. Nutr. 4: 530–532, 2013


submitted for publication in an upcoming issue of Advances of Nutrition.
[2] Author disclosures: J. A. Martinez is a partner of the FOOD4ME project (about nutrigenetics) funded by the European Union. S.-W. Choi, K. J. Claycombe, S. Fritz, and K. L. Schalinske, no conflicts of interest.
* To whom correspondence should be addressed. E-mail: sang.choi@tufts.edu.
[1] Abbreviations used: DMR, differentially methylated region; DNMT1, DNA methyltransferase; GNMT, glycine N-methyltransferase; HCC, hepatocellular carcinoma; HF, high fat; IgG, insulin-like growth factor 2; LP, low protein; ZDF, Zucker diabetic fatty.
is associated with greater body weight

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Epigenetics and Bacterial Infections

Hélène Bierne[1,2,3], Mélanie Hamon[1,2,3], and Pascale Cossart[1,2,3]

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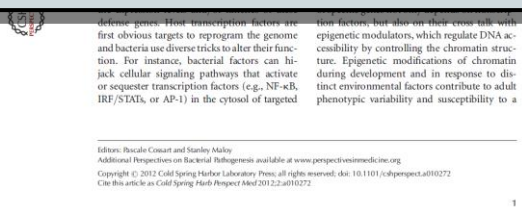
[3]INRA, USC2020, Paris F-75015, France
Correspondence: helene.bierne@pasteur.fr

Summary – "…Thus, pathogenic bacteria can be considered as potential epimutagens able to reshape the epigenome. Their effects might generate specific, long-lasting imprints on the host cells, leading to a memory of infection that influences immunity and might be at the origin of unexplained diseases."

Cold Spring Harb Perspect Med 2012:2:a010272


defense genes. Host transcription factors are first obvious targets to reprogram the genome and bacteria use diverse tricks to alter their function. For instance, bacterial factors can hijack cellular signaling pathways that activate or sequester transcription factors (e.g., NF-κB, IRF/STATs, or AP-1) in the cytosol of targeted

tion factors, but also on their cross-talk with epigenetic modulators, which regulate DNA accessibility by controlling the chromatin structure. Epigenetic modifications of chromatin during development and in response to distinct environmental factors contribute to adult phenotypic variability and susceptibility to a

Editors: Pascale Cossart and Stanley Maloy
Additional Perspectives on Bacterial Pathogenesis available at www.perspectivesinmedicine.org

Cite this article as *Cold Spring Harb Perspect Med* 2012;2:a010272

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Trends Microbiol. 2010 Oct; 18(10): 439–447. doi: 10.1016/j.tim.2010.07.003

Epigenetic reprogramming of host genes in viral and microbial pathogenesis

Konstantinos Paschos and Martin J. Allday

Summary – "…This article reviews examples of viruses and bacteria known or thought to induce epigenetic changes in host cells, and how this might contribute to disease."

Trends Microbiol. 2010 Oct; 18(10): 439–447.
doi: 10.1016/j.tim.2010.07.003

chronic diseases associated with microbial persistence; they might also explain so-called 'hit-and-run' phenomena in infectious disease pathogenesis.
PMCID: PMC3089700

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Fungal Genetics and Biology 132 (2019) 103253

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journal homepage: www.elsevier.com/locate/yfgbi

Review

Epigenetic mechanisms of drug resistance in fungi

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ARTICLE INFO

Keywords:
Epimutation
Long non-coding RNA
Histone modification
Antifungal drug
Candida albicans
Mucor

ABSTRACT

The emergence of drug-resistant fungi poses a continuously increasing threat to human health. Despite advances in preventive care and diagnostics, resistant fungi continue to cause significant mortality, especially in immunocompromised patients. Therapeutic resources are further limited by current usage of only four major classes of antifungal drugs. Resistance against these drugs has already been observed in pathogenic fungi requiring the development of much needed newer antifungal drugs. Epigenetic changes such as DNA or chromatin modifications alter gene expression levels in response to certain stimuli, including interaction with the host in the case of fungal pathogens. These changes can confer resistance to drugs by altering the expression of target genes or genes encoding drug efflux pumps. Multiple pathogens share many of these epigenetic pathways; thus, targeting epigenetic pathways might also identify drug target candidates for the development of broad-spectrum antifungal drugs. In this review, we discuss the importance of epigenetic pathways in mediating drug resistance in fungi as well as in the development of anti-fungal drugs.

1. Fungal diseases and antifungal resistance

Fungal diseases impact species across the plant and animal kingdoms, influencing food security, plant and wildlife extinctions, and human health worldwide (Brown et al., 2012; Fisher et al., 2012). Fungal infections are estimated to affect more than one billion people, with an estimated 150 million people suffering from severe or life-threatening forms of the disease (Bongomin et al., 2017). Furthermore, the overall incidence of fungal infections appears to be increasing, with newly emerging pathogens affecting all kingdoms of life including humans.

One of the factors contributing to the severity of fungal infections is the rapid emergence of antifungal drug resistance. There are several barriers to the effective treatment of fungal infections with antifungal drugs. First, the antifungal drugs available to treat human fungal pathogens are limited, with only four major classes of drugs clinically available: azoles, polyenes, echinocandins, and a nucleotide analog (McCarthy et al., 2017; Roemer and Krysan, 2014). Secondly, there is overlap between the antifungal agents employed to counter human and plant fungal infections. Similar mechanisms operate in both plant and human fungal pathogens to drive drug resistance (Fisher et al., 2018). Moreover, human fungal pathogens can also acquire azole-resistance by being exposed to agricultural azoles (Berger et al., 2017; Chowdhary et al., 2013). Finally, many species of fungi are intrinsically resistant to some antifungal classes, and the mechanisms underlying this resistance have yet to be fully elucidated (Cowen et al., 2014; Perlin et al., 2017).

Acquired antifungal resistance has been studied across many important pathogens, and mechanisms of resistance have been identified for all four classes of antifungals. These include several well-studied genetic mechanisms, including mutations, aneuploidy, upregulation of stress response pathways, and biofilm formation (Cowen et al., 2014; Perlin et al., 2017; Robbins et al., 2017). However, the study of epigenetic pathways is beginning to shed new light on the role of epigenetic factors in already existing or novel mechanisms through which fungi can develop antifungal resistance.

2. Epigenetic mechanisms in fungi

Epigenetics refers to cellular changes mediated by factors other than DNA sequence modifications. These changes do not alter DNA sequences or protein coding but instead transiently affect the expression of target genes. Broadly, epigenetic modifications can be categorized into two main mechanisms: RNA-based and chromatin-based.

RNA-based pathways include RNA interference (RNAi) and non-coding RNAs. RNAi is a mechanism mediated by small RNAs (sRNAs) produced through a core RNAi pathway involving RNA-dependent RNA

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"Epigenetic changes such as DNA or chromatin modifications alter gene expression levels in response to certain stimuli, including interaction with the host in the case of fungal pathogens."

Fungal Genetics and Biology 132 (2019) 103253

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Experimental Parasite Infection Causes Genome-Wide Changes in DNA Methylation

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Fastq raw reads of methylation sequencing are deposited at the NIH genetic sequence database (GenBank) with the accession ID PRJNA605637.

Abstract

Parasites are arguably among the strongest drivers of natural selection, constraining hosts to evolve resistance and tolerance mechanisms. Although, the genetic basis of adaptation to parasite infection has been widely studied, little is known about how epigenetic changes contribute to parasite resistance and eventually, adaptation. Here, we investigated the role of host DNA methylation modifications to respond to parasite infections. In a controlled infection experiment, we used the three-spined stickleback fish, a model species for host–parasite studies, and their nematode parasite *Camallanus lacustris*. We showed that the levels of DNA methylation are higher in infected fish. Results furthermore suggest correlations between DNA methylation and shifts in key fitness and immune traits between infected and control fish, including respiratory burst and functional trans-generational traits such as the concentration of motile sperm. We revealed that genes associated with metabolic, developmental, and regulatory processes (cell death and apoptosis) were differentially methylated between infected and control fish. Interestingly, genes such as the *neuropeptide FF receptor 2* and the *integrin alpha 1* as well as molecular pathways including the Th1 and Th2 cell differentiation were hyper-methylated in infected fish, suggesting parasite-mediated repression mechanisms of immune responses. Altogether, we demonstrate that parasite infection contributes to genome-wide DNA methylation modifications. Our study brings novel insights into the evolution of vertebrate immunity and suggests that epigenetic mechanisms are complementary to genetic responses against parasite-mediated selection.

Key words: **DNA methylation, epigenetics, host–parasite interactions, reduced representation bisulfite sequencing, three-spined stickleback.**

Introduction

Evolutionary theory predicts that the adaptive potential of a population primarily relies on its genomic variation (Frankham et al. 2002). In the case of rapid environmental changes, individuals are unlikely to be preadapted to survive under the new conditions and, as such, phenotypic plasticity may play a central role in population rescue (Merilä and Hendry 2014). Phenotypic plasticity refers to the capacity of a genotype to produce different phenotypes under different environmental conditions and is mostly modulated by the regulation of gene expression (West-Eberhard 2003). Resolving the molecular basis of phenotypic plasticity could hence be the missing piece of the puzzle for a better understanding of the adaptive potential of populations or species (Eizaguirre and Baltazar-Soares 2014; Rey et al. 2020).

Epigenetic mechanisms are important environment-modulated mechanisms possibly accelerating adaptive responses to selection (Gugger et al. 2016; Artemov et al. 2017; Metzger and Schulte 2017). Although several epigenetic pathways can facilitate phenotypic plasticity (e.g., histone modifications, chromatin remodeling, and small interfering RNAs), the addition of a methyl group to cytosine nucleotides is probably the best characterized to date (Skvortsova et al. 2018). Although there exists DNA methylation resetting mechanisms in the early embryo (Potok et al. 2013; Seisenberger et al. 2013), recent evidence suggests that reprograming may be incomplete and acquired DNA methylation states may be transmitted from parents to offspring (Metzger and Schulte

"We showed that the levels of DNA methylation are higher in infected fish."

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Epigenetics and environmental chemicals

Summary – "For several exposures, it has been proved that chemicals can alter epigenetic marks and that the same or similar epigenetic alterations can be found in patients with the disease of concern or in diseased tissues...."

Curr Opin Pediatr: 2009 pril; 21(2): 243-251

Introduction

Identifying the effects of environmental exposures on human health is a major objective of life sciences and biomedical research. In environmental health, the recognition that exposures could produce DNA mutations represented a major landmark for risk assessment and prevention.[1] Consequently, chemical agents have been categorized according to their capability to alter the DNA sequence. Such information has been fundamental to determine environmental risks and shape current regulatory efforts for exposure reduction.[2] Recent evidence suggests that the molecular influence of the environment may extend well beyond the interaction with the DNA sequence.[3,4] Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence.[5] Epigenetic mechanisms

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CLINICAL EPIGENETICS

Open Access

ciation exposures in early life and epigenetic variation: a potential link to disease susceptibility?

Alexander Vaiserman

Abstract

A growing body of evidence suggests that the risk of development and progression of a variety of human chronic diseases depends on epigenetic modifications triggered by environmental cues during early life sensitive stages. Exposures to environmental factors such as adverse nutritional, psychological, and social conditions, as well as pollutants and substance abuse in early life, have been shown to be important determinants of epigenetic programming of chronic pathological conditions in human populations. Over the past years, it has become increasingly clear due to the epigenome-wide association studies (EWASs) that early life adverse environmental events may trigger widespread and persistent alterations in transcriptional profiling. Several candidate genes have been identified underlying these associations. In this context, DNA methylation is the most intensively studied epigenetic phenomenon. In this review, the clinical and epidemiological evidence for the role of epigenetic factors in mediating the link between early life experiences and long-term health outcomes are summarized.

Keywords: Developmental programming, Chronic disease, Epigenetic modification, DNA methylation, Environmental xenobiotic, Nutrition, Stress

Introduction

During the past decades, the burden of chronic diseases is rapidly increasing worldwide. Adult lifestyle factors are the main risk contributors. It is, however, increasingly clear that unfavorable events during early development may also play a crucial role in the pathogenesis of chronic pathological conditions. The Developmental Origins of Health and Disease concept suggests that adverse exposures early in life may reprogram an individual for immediate adaptation to prenatal and/or neonatal environmental perturbations but enhance the risk of subsequent pathologies including cancer, type 2 diabetes (T2D), cardiovascular (CVD), and neurodegenerative disease [1].

The mechanisms implicated in developmental programming of chronic disorders are poorly understood, but epigenetic mechanisms are likely involved. In mammals, the epigenome undergoes major epigenetic modifications throughout the gametogenesis and early embryogenesis [2, 3]. In the early embryo, a dramatic reduction in methylation takes place; the methylation levels reach their minimum at the early blastocyst stage (32–64 cells). This process of epigenetic reprogramming throughout early embryogenesis erases gamete-specific methylation patterns inherited from the parents and is crucial to establishing pluripotency. After implantation, a wave of de novo methylation occurs. Another demethylation–remethylation cycle of epigenetic reprogramming takes place in the primordial germ cells which are the embryonic progenitors of oocytes and sperm. However, these processes differ in primordial germ cells and in embryos. In primordial germ cells, demethylation is close to absolute (with the exception of a few resistant retroelements), whereas in early embryos, methylation of imprinted gene regions is preserved, enabling parent-of-origin-specific gene expression in later tissues [2]. Early embryonic maintenance is especially critical in the context of developmental programming because this process is sensitive to

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BioMed Central

"A growing body of evidence suggests that the risk of development and progression of a variety of human chronic diseases depends on epigenetic modifications triggered by environmental cues during early life sensitive stages. Exposures to environmental factors such as adverse nutritional, psychological, and social conditions, as well as pollutants and substance abuse in early life, have been shown to be important determinants of epigenetic programming of chronic pathological conditions in human populations."

Vaiserman Clinical Epigenetics (2015) 7:96
DOI 10.1186/s13148-015-0130-0

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OPEN

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www.nature.com/tp

ORIGINAL ARTICLE

Epigenomic profiling of men exposed to early-life stress reveals DNA methylation differences in association with current mental state

… "Our results support the concept that DNA methylation differences may be important in the pathogenesis of psychiatric disease."

Transl Psychiatry (2014) 4, e448; doi:10.1038/tp2014.94

Translational Psychiatry (2014) **4**, e448; doi:10.1038/tp.2014.94; published online 23 September 2014

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Format: Abstract

Full text links

ELSEVIER FULL-TEXT ARTICLE

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Epigenetic Changes in Chronic Inflammatory Diseases.

Fogel O[1], Richard-Miceli C[2], Tost J[3].

Author information

Abstract

The number of people diagnosed with chronic inflammatory diseases has increased noteworthy in the last 40 years. Spondyloarthritis (SpA), inflammatory bowel diseases (IBD), and psoriasis are the most frequent chronic inflammatory diseases, resulting from a combination of genetic predisposition and environmental factors. Epigenetic modifications include DNA methylation, histone modifications, and small and long noncoding RNAs. They are influenced by environmental exposure, life-style, and aging and have recently been shown to be altered in many complex diseases including inflammatory diseases. While epigenetic modifications have been well characterized in other diseases such as cancer and autoimmune diseases, knowledge on changes in inflammatory diseases is lagging behind with some disease-specific differences. While the DNA methylation profile of different cell types in patients with IBD has been relatively well described, less is known on changes implicated in psoriasis, and no systematic genome-wide studies have so far been performed in SpA. In this chapter, we review in detail the reported changes in patterns of DNA methylation and posttranslational histone modifications in chronic inflammatory diseases highlighting potential connections between disease-associated pathophysiological changes such as the dysbiosis of the microbiome or genetic variations associated with disease susceptibility and the epigenome. We also discuss important parameters of meaningful epigenetic studies such as the use of well defined, disease-relevant cell populations, and elude on the potential future of engineering of the epigenome in inflammatory diseases.

KEYWORDS: Behcet's disease; Crohn's disease; DNA methylation; EWAS; Epigenetics; Histone modifications; Inflammatory bowel disease; Psoriasis; Spondyloarthritis; Ulcerative colitis

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[Indexed for MEDLINE]

"…in chronic inflammatory diseases highlighting potential connections between disease-associated pathophysiological changes such as the dysbiosis of the microbiome or genetic variations associated with disease susceptibility and the epigenome."

Adv Protein Chem Struct Biol. 2017;106:139-189.
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CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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J Dent Res 90(1):9-17, 2011

Epigenetic Mechanisms in Inflammation

ABSTRACT

Epigenetic modifications occur in response to environmental changes and play a fundamental role in gene expression following environmental stimuli. Major epigenetic events include methylation and acetylation of histones and regulatory factors, DNA methylation, and small non-coding RNAs. Diet, pollution, infections, and other environmental factors have profound effects on epigenetic modifications and trigger susceptibility to diseases. Despite a growing body of literature addressing the role of the environment on gene expression, very little is known about the epigenetic pathways involved in the modulation of inflammatory and anti-inflammatory genes. This review summarizes the current knowledge about epigenetic control mechanisms during the inflammatory response.

KEY WORDS: epigenetics, histone modifications, DNA methylation, inflammation.

INTRODUCTION

Epigenetics is defined as the study of mitotically and meiotically heritable changes in gene function that are not dependent on DNA sequence (Feinberg, 2007). The molecular basis of epigenetic processes is complex and involves modifications of histones, methylation of DNA, positioning of histone variants, and gene regulation by non-coding RNAs. Epigenetic modifications are potentially reversible, and, therefore, a thorough understanding of [illegible] may identify new therapeutic targets for disease.

Moreover, the overall epigenetic state of an organism, is just as important as the genome to normal development. Importantly, environmental factors (nutrients, toxins, infections, hypoxia) can have profound effects on the epigenetic signature (Fig. 1) and trigger susceptibility to disease (Barros and Offenbacher, 2009; Safronova and Morita, 2010). For example, recent studies have shown that the fetal environment can cause changes in the epigenome, with long-term consequences for gene regulation and age-related diseases (Thompson and Einstein, 2010). The studies by Bobetsis *et al.* (2006) showed that periodontal infection can lead to placental-fetal exposure and, when coupled with a fetal inflammatory response, leads to preterm delivery.

INFLAMMATION

Inflammation is a complex physiological response of an organism to harmful stimuli, such as pathogens, damaged cells, or irritants. In acute inflammation, the initial response of the body to a stimulus is achieved by increasing the migration of leukocytes and plasma from the blood to the injured areas. When inflammation has a slow onset and persists for a long period of time, it becomes chronic. The symptoms in chronic inflammation are not as severe as in acute inflammation, but the condition is persistent. Chronic inflammation underlies many diseases, including periodontal disease and diabetes mellitus (Dunning, 2009).

The complexity of the inflammatory response requires the development of a sophisticated regulatory network to carry out functions at signal-specific and gene-specific levels (Medzhitov and Horng, 2009). This network involves the activation of specific genes for antimicrobial defense, immune response, and tissue repair and remodeling (Medzhitov, 2008). Macrophages play critical roles in diverse chronic diseases, including cancer and allergic responses, and analysis of recent data indicates that chromatin modifications are mechanistically important in the acquisition of the macrophage phenotype (Khansari *et al.*, 2009). Transcription factors of the NF-κB, FOXP3, IRF, and STAT families along with epigenetic phenomena, including DNA methylation and covalent histone modifications, have been shown to be critical in the regulation of inflammatory genes (Medzhitov and Horng, 2009). In addition, several of these regulatory factors are controlled by epigenetic mechanisms in T-cells and monocytes (Lal *et al.*, 2009; Wells, 2009; Wierda *et al.*, 2010).

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"Major epigenetic events include methylation and acetylation of histones and regulatory factors, DNA methylation, and small non-coding RNAs. Diet, pollution, infections, and other environmental factors have profound effects on epigenetic modifications and trigger susceptibility to diseases. Despite a growing body of literature addressing the role of the environment on gene expression, very little is known about the epigenetic pathways involved in the modulation of inflammatory and anti-inflammatory genes."

J Dent Res 90(1):9-17, 2011

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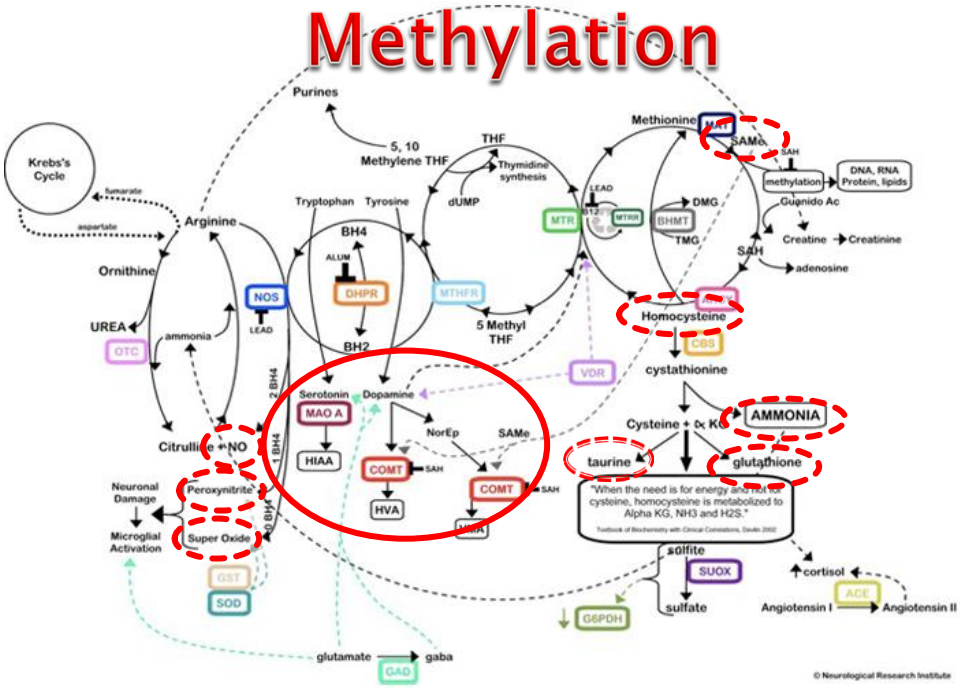
Methylation
Purines
Krebs's Cycle
fumarate
aspartate
Arginine
Ornithine
UREA
OTC
ammonia
NOS
LEAD
Citrulline + NO
Tryptophan
Tyrosine
BH4
ALUM
DHPR
BH2
2 BH4
1 BH4
5, 10 Methylene THF
THF
Thymidine synthesis
dUMP
MTHFR
5 Methyl THF
MTR
MTRR
B12
Methionine
SAMe
SAH
DMG
TMG
BHMT
methylation
DNA, RNA Protein, lipids
Guanido Ac
Creatine
Creatinine
adenosine
Homocysteine
CBS
cystathionine
VDR
Serotonin
Dopamine
MAO A
HIAA
NorEp
SAMe
COMT
HVA
Cysteine + 0x KG
AMMONIA
taurine
glutathione
"When the need is for energy and not for cysteine, homocysteine is metabolized to Alpha KG, NH3 and H2S."
Neuronal Damage
Peroxynitrite
Microglial Activation
Super Oxide
GST
SOD
sulfite
SUOX
sulfate
G6PDH
cortisol
ACE
Angiotensin I
Angiotensin II
glutamate
GAD
gaba
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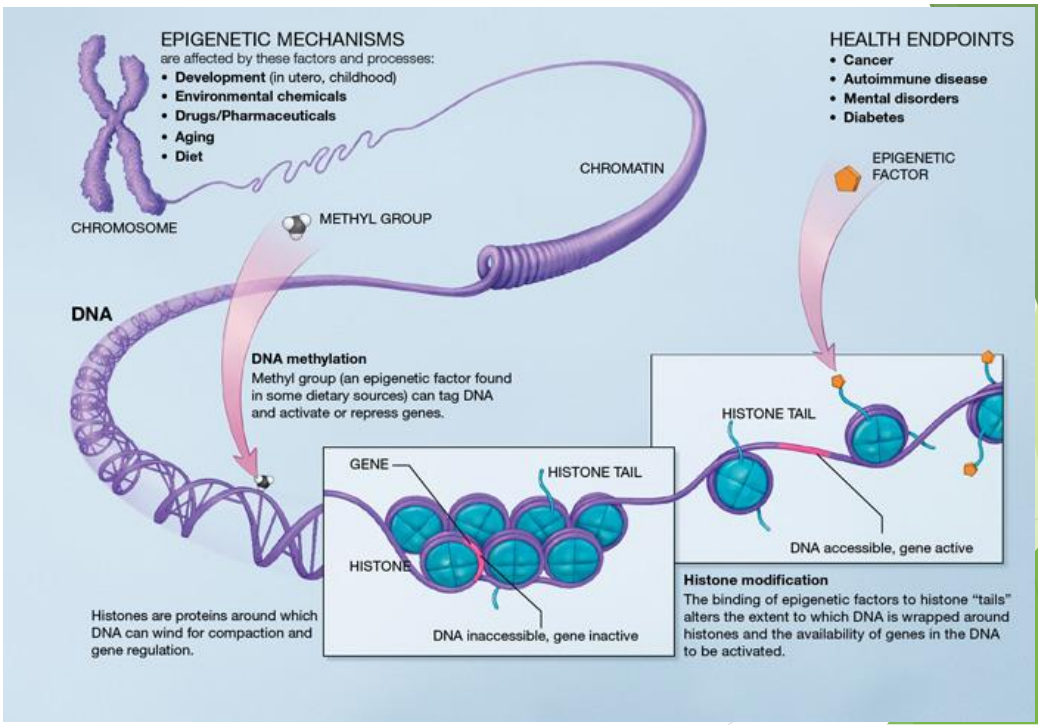
EPIGENETIC MECHANISMS
are affected by these factors and processes:
• Development (in utero, childhood)
• Environmental chemicals
• Drugs/Pharmaceuticals
• Aging
• Diet
HEALTH ENDPOINTS
• Cancer
• Autoimmune disease
• Mental disorders
• Diabetes
CHROMOSOME
CHROMATIN
METHYL GROUP
EPIGENETIC FACTOR
DNA
DNA methylation
Methyl group (an epigenetic factor found in some dietary sources) can tag DNA and activate or repress genes.
GENE
HISTONE TAIL
HISTONE
DNA inaccessible, gene inactive
HISTONE TAIL
DNA accessible, gene active
Histones are proteins around which DNA can wind for compaction and gene regulation.
Histone modification
The binding of epigenetic factors to histone "tails" alters the extent to which DNA is wrapped around histones and the availability of genes in the DNA to be activated.

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Methylation - The Good

- Detoxification: without, it would lead to an accumulation of environmental toxins, such as heavy metals and environmental chemicals, causing inflammation
- Glutathione and taurine production: a decreased level of this molecule leads to increased free radical damage; inflammation
- Hormonal metabolism: may lead to an increase in certain hormonal metabolites
- Neurotransmitter – synthesis and metabolism (serotonin & dopamine)
- DNA and Histone synthesis (Thymine)
- Energy production – CoQ10, carnitine, creatine, ATP
- Myelin production
- Build and maintain cell membranes(Phosphatidylcholine)

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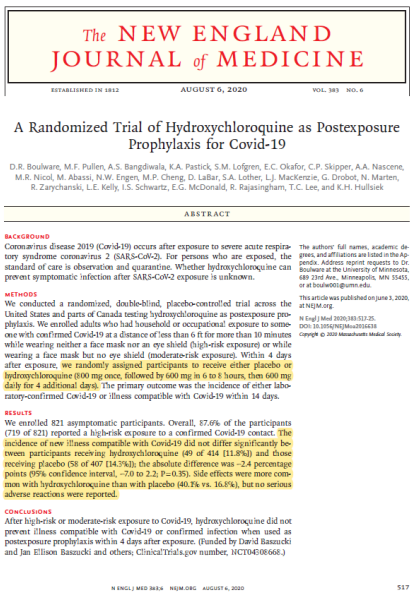
Methylation - The Good



The NEW ENGLAND JOURNAL of MEDICINE

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A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19

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ABSTRACT

BACKGROUND

Coronavirus disease 2019 (Covid-19) occurs after exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). For persons who are exposed, the standard of care is observation and quarantine. Whether hydroxychloroquine can prevent symptomatic infection after SARS-CoV-2 exposure is unknown.

METHODS

We conducted a randomized, double-blind, placebo-controlled trial across the United States and parts of Canada testing hydroxychloroquine as postexposure prophylaxis. We enrolled adults who had household or occupational exposure to someone with confirmed Covid-19 at a distance of less than 6 ft for more than 10 minutes while wearing neither a face mask nor an eye shield (high-risk exposure) or while wearing a face mask but no eye shield (moderate-risk exposure). Within 4 days after exposure, we randomly assigned participants to receive either placebo or hydroxychloroquine (800 mg once, followed by 600 mg in 6 to 8 hours, then 600 mg daily for 4 additional days). The primary outcome was the incidence of either laboratory-confirmed Covid-19 or illness compatible with Covid-19 within 14 days.

RESULTS

We enrolled 821 asymptomatic participants. Overall, 87.6% of the participants (719 of 821) reported a high-risk exposure to a confirmed Covid-19 contact. The incidence of new illness compatible with Covid-19 did not differ significantly between participants receiving hydroxychloroquine (49 of 414 [11.8%]) and those receiving placebo (58 of 407 [14.3%]); the absolute difference was −2.4 percentage points (95% confidence interval, −7.0 to 2.2; P=0.35). Side effects were more common with hydroxychloroquine than with placebo (40.1% vs. 16.8%), but no serious adverse reactions were reported.

CONCLUSIONS

After high-risk or moderate-risk exposure to Covid-19, hydroxychloroquine did not prevent illness compatible with Covid-19 or confirmed infection when used as postexposure prophylaxis within 4 days after exposure. (Funded by David Baszucki and Jan Ellison Baszucki and others; ClinicalTrials.gov number, NCT04308668.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Boulware at the University of Minnesota, 689 23rd Ave., Minneapolis, MN 55455, or at boulw001@umn.edu.

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"…we randomly assigned participants to receive either placebo or hydroxychloroquine (800 mg once, followed by 600 mg in 6 to 8 hours, then 600 mg daily for 4 additional days)."

"The incidence of new illness compatible with Covid-19 did not differ significantly between participants receiving hydroxychloroquine (49 of 414 [11.8%]) and those receiving placebo (58 of 407 [14.3%]); the absolute difference was −2.4 percentage points (95% confidence interval, −7.0 to 2.2; P = 0.35). Side effects were more common with hydroxychloroquine than with placebo (40.1% vs. 16.8%), but no serious adverse reactions were reported."

"We randomly assigned participants in a 1:1 ratio to receive either hydroxychloroquine or placebo."

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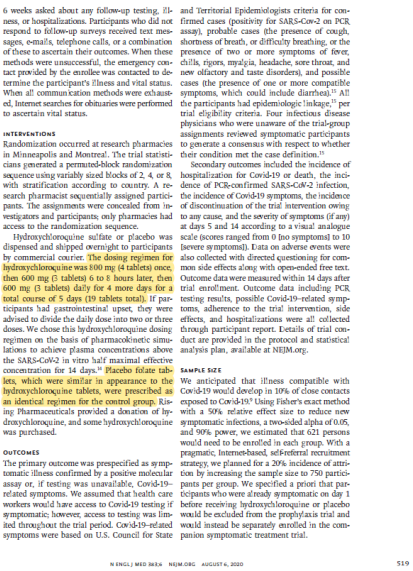
Methylation - The Good



HYDROXYCHLOROQUINE AS PROPHYLAXIS FOR COVID-19

6 weeks asked about any follow-up testing, illness, or hospitalizations. Participants who did not respond to follow-up surveys received text messages, e-mails, telephone calls, or a combination of these to ascertain their outcomes. When these methods were unsuccessful, the emergency contact provided by the enrollee was contacted to determine the participant's illness and vital status. When all communication methods were exhausted, Internet searches for obituaries were performed to ascertain vital status.

INTERVENTIONS

Randomization occurred at research pharmacies in Minneapolis and Montreal. The trial statisticians generated a permuted-block randomization sequence using variably sized blocks of 2, 4, or 8, with stratification according to country. A research pharmacist sequentially assigned participants. The assignments were concealed from investigators and participants; only pharmacies had access to the randomization sequence.

Hydroxychloroquine sulfate or placebo was dispensed and shipped overnight to participants by commercial courier. The dosing regimen for hydroxychloroquine was 800 mg (4 tablets) once, then 600 mg (3 tablets) 6 to 8 hours later, then 600 mg (3 tablets) daily for 4 more days for a total course of 5 days (19 tablets total). If participants had gastrointestinal upset, they were advised to divide the daily dose into two or three doses. We chose this hydroxychloroquine dosing regimen on the basis of pharmacokinetic simulations to achieve plasma concentrations above the SARS-CoV-2 in vitro half maximal effective concentration for 14 days.[9] Placebo folate tablets, which were similar in appearance to the hydroxychloroquine tablets, were prescribed as an identical regimen for the control group. Rising Pharmaceuticals provided a donation of hydroxychloroquine, and some hydroxychloroquine was purchased.

OUTCOMES

The primary outcome was prespecified as symptomatic illness confirmed by a positive molecular assay or, if testing was unavailable, Covid-19–related symptoms. We assumed that health care workers would have access to Covid-19 testing if symptomatic; however, access to testing was limited throughout the trial period. Covid-19–related symptoms were based on U.S. Council for State and Territorial Epidemiologists criteria for confirmed cases (positivity for SARS-CoV-2 on PCR assay), probable cases (the presence of cough, shortness of breath, or difficulty breathing, or the presence of two or more symptoms of fever, chills, rigors, myalgia, headache, sore throat, and new olfactory and taste disorders), and possible cases (the presence of one or more compatible symptoms, which could include diarrhea).[11] All the participants had epidemiologic linkage,[11] per trial eligibility criteria. Four infectious disease physicians who were unaware of the trial-group assignments reviewed symptomatic participants to generate a consensus with respect to whether their condition met the case definition.[11]

Secondary outcomes included the incidence of hospitalization for Covid-19 or death, the incidence of PCR-confirmed SARS-CoV-2 infection, the incidence of Covid-19 symptoms, the incidence of discontinuation of the trial intervention owing to any cause, and the severity of symptoms (if any) at days 5 and 14 according to a visual analogue scale (scores ranged from 0 [no symptoms] to 10 [severe symptoms]). Data on adverse events were also collected with directed questioning for common side effects along with open-ended free text. Outcome data were measured within 14 days after trial enrollment. Outcome data including PCR testing results, possible Covid-19–related symptoms, adherence to the trial intervention, side effects, and hospitalizations were all collected through participant report. Details of trial conduct are provided in the protocol and statistical analysis plan, available at NEJM.org.

SAMPLE SIZE

We anticipated that illness compatible with Covid-19 would develop in 10% of close contacts exposed to Covid-19.[9] Using Fisher's exact method with a 50% relative effect size to reduce new symptomatic infections, a two-sided alpha of 0.05, and 90% power, we estimated that 621 persons would need to be enrolled in each group. With a pragmatic, Internet-based, self-referral recruitment strategy, we planned for a 20% incidence of attrition by increasing the sample size to 750 participants per group. We specified a priori that participants who were already symptomatic on day 1 before receiving hydroxychloroquine or placebo would be excluded from the prophylaxis trial and would instead be separately enrolled in the companion symptomatic treatment trial.

"The dosing regimen for hydroxychloroquine was 800 mg (4 tablets) once, then 600 mg (3 tablets) 6 to 8 hours later, then 600 mg (3 tablets) daily for 4 more days for a total course of 5 days (19 tablets total)."

"Placebo folate tablets, which were similar in appearance to the hydroxychloroquine tablets, were prescribed as an identical regimen for the control group."

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DNA Methylation: The Original Anti-Virus Program

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Security and anti-virus software is a must-have accessory for the internet age, but it turns out that DNA methylation has been protecting us all from retroviral infections for quite a bit longer than any computer program. A talented research team lead by Richard Meehan from the University of Edinburgh (Scotland) applied HELP-seq analysis and DNA methylation mutants as a model to investigate how retrotransposon activation is selective and context dependent.
The team scoured the methylome data, and made a number of precise novel observations with respect to the specificity of activation; which classes of repeats are activated in mutants and which are not, and the effect of repeat activation in relation to neighboring genes.

Quote from - Richard Meehan from the University of Edinburgh

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Methylation plays a role in:

- ADD/ADHD
- Addictions
- Agnosia
- Allergies
- ALS
- Alzheimer's
- Asthma
- Autism
- Autoimmune disorders
- Bipolar
- Brain fog
- Cancer (several types)
- Cervical dysplasia
- Chronic fatigue
- Decreased telomere length
- Dementia
- Depression
- Endometriosis
- Epilepsy
- Fibromyalgia
- *Hyperhomocysteinuria*
- Inability to tolerate some medications
- Inflammation
- Intolerance for environmental toxins
- Irritable bowel syndrome
- Learning disabilities
- Low HDL
- Mitochondrial disease
- Mood instability
- Multiple sclerosis
- Myocardial infarction
- Parkinson's disease
- Placenta abrupta
- Pre-eclampsia
- Psychosis
- Rashes
- Spina bifida
- Stroke
- Thyroid dysfunction
- Tics
- Type I diabetes
- Vertigo
- Zollinger – Ellison Syndrome
- ???????

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January 4, 2016 – 4 Weeks later…

"Our daughter has severe and extensive eczema. She had it as a baby, and it has gotten progressively worse to the point where she couldn't even open her eyes, had many open sores all over, and woke up with bloody sheets every morning, at the age of 7. School was so difficult, as was sleeping, and finding friends. With the help of Dr. Peterson and his vast knowledge in allopathic medicine, our daughter's eczema has cleared up tremendously, from roughly 90% covered on her body to very little on her face, and maybe 20% covering her body. She gladly goes to school, feels good about her friendships, and is sleeping better during the night. This treatment really worked well for us, and it is so wonderful to see our daughter's beautiful blue eyes and fantastic smile once again."

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Methylation - The Ugly

"Hypo" or "Hyper" Methylation??

There are Two Types of aberrant Methylators:

- Hypomethylation
- Hypermethylation

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OPEN ACCESS Freely available online PLOS ONE

Alu and *LINE-1* Hypomethylation Is Associated with HER2 Enriched Subtype of Breast Cancer

So Yeon Park[1,2¤], An Na Seo[2¤], Hae Yoen Jung[2], Jae Moon Gwak[1], Namhee Jung[3], Nam-Yun Cho[3], Gyeong Hoon Kang[1,3]*

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Abstract

The changes in DNA methylation status in cancer cells are characterized by hypermethylation of promoter CpG islands and diffuse genomic hypomethylation. *Alu* and long interspersed nucleotide element-1 (*LINE-1*) are non-coding genomic repetitive sequences and methylation of these elements can be used as a surrogate marker for genome-wide methylation status. This study was designed to evaluate the changes of *Alu* and *LINE-1* hypomethylation during breast cancer progression from normal to pre-invasive lesions and invasive breast cancer (IBC), and their relationship with characteristics of IBC. We analyzed the methylation status of *Alu* and *LINE-1* in 145 cases of breast samples including normal breast tissue, atypical ductal hyperplasia/flat epithelial atypia (ADH/FEA), ductal carcinoma in situ (DCIS) and IBC, and another set of 129 cases of IBC by pyrosequencing. *Alu* methylation showed no significant changes during multistep progression of breast cancer, although it tended to decrease during the transition from DCIS to IBC. In contrast, *LINE-1* methylation significantly decreased from normal to ADH/FEA, while it was similar in ADH/FEA, DCIS and IBC. In IBC, *Alu* hypomethylation correlated with negative estrogen receptor (ER) status, and *LINE-1* hypomethylation was associated with negative ER status, *ERBB2 (HER2)* amplification and p53 overexpression. *Alu* and *LINE-1* methylation status was significantly different between breast cancer subtypes, and the HER2 enriched subtype had lowest methylation levels. In survival analyses, low *Alu* methylation status tended to be associated with poor disease-free survival of the patients. Our findings suggest that *LINE-1* hypomethylation is an early event and *Alu* hypomethylation is probably a late event during breast cancer progression, and prominent hypomethylation of *Alu* and *LINE-1* in HER2 enriched subtype may be related to chromosomal instability of this specific subtype.

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Introduction

Common epigenetic changes in cancer include CpG island hypermethylation of gene promoters and genome-wide hypomethylation of non-coding genomic regions. While promoter CpG island hypermethylation is an alternative mechanism for inactivating tumor suppressor genes, resulting in their transcriptional silencing [1,2], genome-wide hypomethylation is associated with genomic instability and hence facilitates tumor progression [3,4]. In breast cancer, promoter CpG island hypermethylation has been described for genes involved in all aspects of cellular function and was found to be associated with various histopathologic characteristics, including tumor grade [5,6], hormone receptor [7,8], HER2/neu status [9] and molecular subtype [10–13]. However, few studies have focused on genome-wide hypomethylation in breast cancer and its association with the clinicopathologic characteristics of breast cancer.

Genome-wide global hypomethylation affects repetitive transposable DNA elements and they reside mainly in the intergenic and intronic regions of the genome [14,15]. *Alu* and long interspersed nucleotide element-1 (*LINE-1*) are major components of repetitive transposable DNA elements, constituting approximately 17% and 11% of the human genome [14]. Because of their high frequencies in the genome, *Alu* and *LINE-1* methylation status serve as a useful surrogate marker for genome-wide methylation status. In normal cells, CpG sites within *Alu* and *LINE-1* are usually methylated, thus maintaining transcriptional inactivation and inhibiting retrotransposition [16]. However, hypomethylation of *Alu* and *LINE-1* is consistently found in many types of human cancers [17–23]. Hypomethylation of transposable elements such as *Alu* and *LINE-1* causes transcriptional activation, resulting in retrotransposition of the transposable element, chromosome alteration and thus genomic instability [18,24]. *Alu* and *LINE-1* hypomethylation have been reported as early events in the multistep carcinogenesis of colorectal cancer [17,20,22]. However, some controversies exist in other types of cancer [18,19,21]. Moreover, in breast cancer, studies on changes of *Alu* and *LINE-1*

"The changes in DNA methylation status in cancer cells are characterized by hypermethylation of promoter CpG islands and diffuse genomic hypomethylation."

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Letter to the Editor

BRCA2 Promoter Hypermethylation in Sporadic Breast Cancer

Rémy Bosviel, Julie Durif, Jiaoli Guo, Mourad Mebrek, Fabrice Kwiatkowski, Yves-Jean Bignon, and Dominique J. Bernard-Gallon

Dear Editor:

Breast cancer is a multifactorial disease. It is the first cause of mortality per cancer for the woman in the world (Jemal *et al.*, 2011). The implication of specific genes, such as *BRCA1* and *BRCA2* tumor suppressor genes, has been shown in mammary carcinogenesis. In sporadic breast cancers, specific modifications of *BRCA1* and *BRCA2* mRNA expression have been reported too (Bernard-Gallon *et al.*, 1999; Bieche *et al.*, 1999). Epigenetic modifications such as promoter hypermethylation of the oncosuppressor genes *BRCA1* and *BRCA2* can play a role in the oncogenesis of cancer (Esteller *et al.*, 2000). Indeed, hypermethylation of the CpG islands in the promoters of these genes involve their inactivation and therefore a higher risk of developing a tumor (Baylin and Ohm, 2006; Moelans *et al.*, 2011; Rahmatpanah *et al.*, 2009).

We have compared the DNA methylation rates of the *BRCA1* and *BRCA2* gene promoters using DNA isolated from blood samples from COSA (Cancer d'Ovaire et du Sein en Auvergne) patients suffering from breast or ovarian cancer and from a population of healthy women. To this aim, the QAMA method (Quantitative Analysis of Methylated Alleles) was used (Zeschnigk *et al.*, 2004) and adapted to *BRCA1* and *BRCA2* genes (Bosviel *et al.*, 2011). This method is based on bisulfite conversion of the nonmethylated cytosines and an analysis by qPCR using Taqman minor groove binder probes specific for methylated or nonmethylated target sites after conversion. Percentage of methylation is then obtained by calculating the difference between C_T values of the methylated DNA targeting probe and the nonmethylated DNA targeting probe (ΔC_T) and reporting the obtained values on a standard curve.

In a previous work, we reported that *BRCA1* methylation is significantly decreased in ovarian cancer by comparison with the control group. The comparison between the two different populations did not show any significant difference regarding *BRCA2* methylation but exhibited a trend in the decrease of *BRCA2* promoter methylation in peripheral blood DNA of sporadic ovarian cancer (Bosviel *et al.*, 2011).

Then we demonstrated a trend toward *BRCA1* promoter hypermethylation in PBCs of sporadic breast cancer patients by comparison with controls (Bosviel *et al.*, 2012). *BRCA1* promoter methylation in PBCs corresponded to 47.1% with CI 95% [46.1; 48.1] in breast cancer patients and to 45.9% with CI 95% [45.0; 46.8] in controls. Association between methylation level and clinicopathological features were evaluated using statistical tests. *BRCA1* promoter methylation in PBCs increased significantly in breast cancer patients by comparison with controls, with the age over 70 years old ($p=0.022$), in post menopausal status ($p=0.013$), with a BMI <20 ($p=0.0095$), or with a WHR ≤ 76.8 ($p=0.0027$). We also found an association of increased *BRCA1* promoter methylation in PBCs with AC/ACA genotype for the SNP $Thr^{594}Thr$ in *ESR* (estrogen receptor), known to be associated with breast cancer risk ($p=0.092$), due to the reduced presence of this genotype in this breast cancer case-control study.

Within this study, the objective was to compare the methylation of the CpG islands present in the *BRCA2* promoter in the same population of women suffering from breast cancer compared to the control population. In total, 873 breast samples belonging to COSA and 980 control samples were converted and the methylation rates measured. The complete database of this study is available as supplementary data (supplementary data are available online at www.liebertonline.com/omi).

BRCA2 promoter methylation mean in PBCs is 16.9% (CI95% [16.3; 17.4]) in breast cancer patients and 16.2% (CI95% [15.7; 16.8]) in controls. The statistical analysis of the mean methylation rates obtained for the *BRCA2* promoter did not reveal a significant difference ($p=0.1$) between the two populations (Fig. 1).

Significant differences in methylation rates between patients and healthy women were, however, obtained for different subclasses (Table 1). It reached 17.3% in breast cancer patients older than 70 years by comparison with control patients (14.7%) with $p=0.016$. In the subclass with an early menopause (before 48 years), the level was respectively 16.6% in breast cancer patients versus 15.4% in control patients ($p=0.028$).

Then, breast cancer patients showing a normal BMI (Body Mass Index) [20–25] exhibited a *BRCA2* promoter methylation in PBCs of 17.6% versus 16.3% for control patients ($p=0.019$).

Concerning the increase in the WHR (Waist-to-Hip Ratio) [81.7%–87.5%], the *BRCA2* promoter methylation in PBCs was 17.1% in COSA patients by comparison to control patients (15.4%) with $p=0.0046$. So, an android distribution of fat tissue revealed a higher methylation rate of the *BRCA2* promoter region.

Centre Jean Perrin, Département d'Oncogénétique, CBRV, CHNH, and ERTICA EA 4677, Clermont-Ferrand, France.

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BRCA1 methylation

BRCA1 methylation

The local hypermethylation of BRCA1 (Breast Cancer Susceptibility Protein1), a tumour suppressor gene, coupled with global DNA hypomethylaton at CpG is lands, which are characteristic of BRCA1related cancers of the breast and presumably also of the ovaries.

Mechanism

BRCA1 prevents global DNA hypomethylation by upregulating expression of DNMT1, which encodes a methylation maintenance enzyme that is a transcriptional target of BRCA1. Reduced expression of BRCA1 correlates with reduced levels of DNMT1 and reduced methylation of CpG islands.

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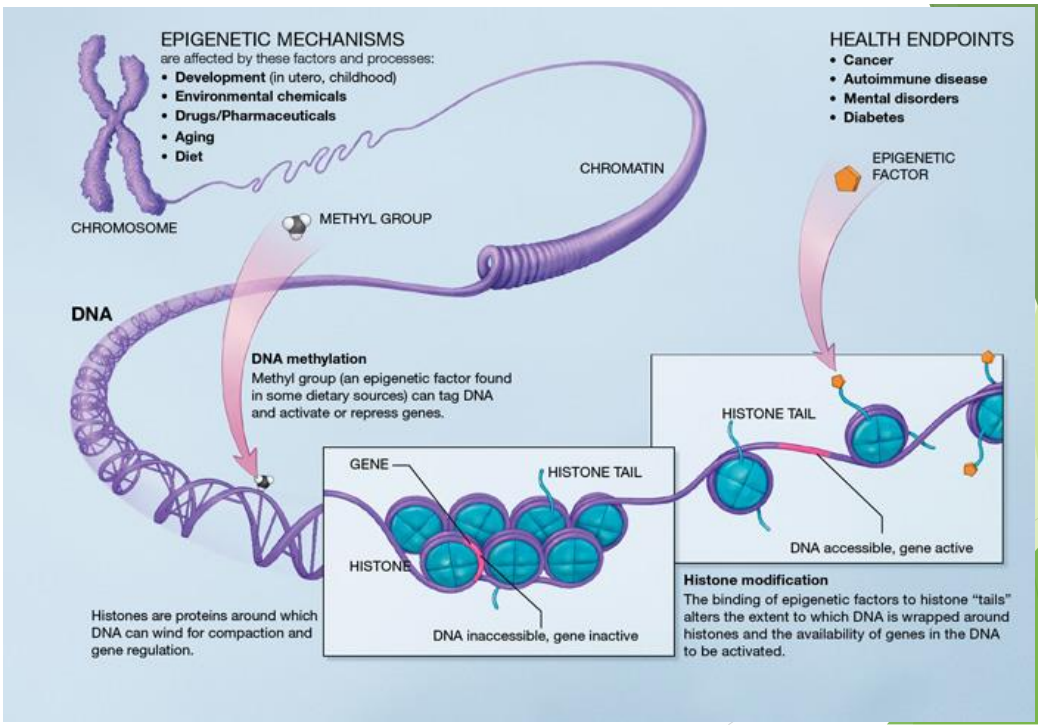

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Methylation

Considerations or factors which influence methylation: INFLAMMATION

- Bowel Health*
- Acid/Alkaline Balance*
- Blood sugar regulation*
- Stealth Infections*
- Lack of the necessary substrates and cofactors for methylation
  - B2, B3, B6, B12, Zn, Mg, cysteine, folate
- Medications
  - Antacids, metformin, methotrexate, nitrous oxide
- Environmental toxins – heavy metals and chemicals
  - Hg, Ar, Cu, Pb, Cd, acetylaldehyde
- Mental, Spiritual and psychological influences
  - Sleep, stress, lack of congruence
- Excessive substrate and cofactors causing inhibition
  - Niacin, folic acid
- Single nucleotide polymorphisms (SNP)
  - MTHFR, COMT, CBS, DHPR

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DNA Methylation, Cancer Susceptibility

DNA methylation is an important epigenetic mechanism of transcriptional control…Global hypomethylation can result in chromosome instability, and hypermethylation has been associated with the inaction of tumor suppressor genes. Dietary factors that are involved in one-carbon metabolism provide the most compelling data for the interaction of nutrients and DNA methylation because they influence the supply of methyl groups, and therefore the biochemical pathways of methylation processes.

Exp Biol Med 229:988–995, 2004

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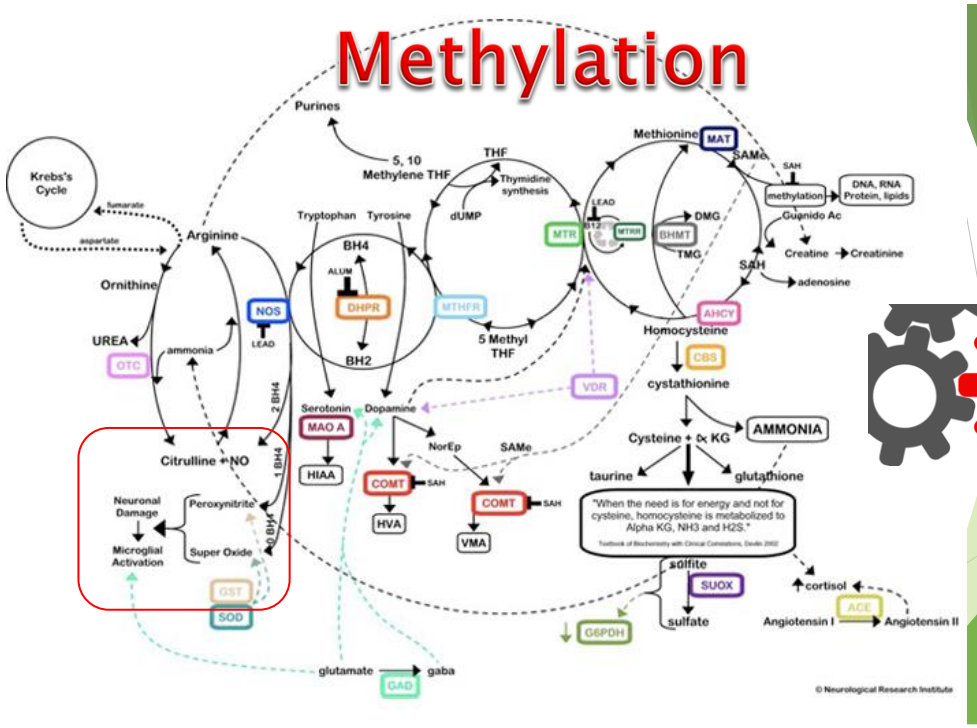

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Peroxynitrite - Free Radical

- Formed when L-arginine is not adequately converted to nitric oxide, or when NO reacts with Super Oxide.
- Has a profoundly devastating effect on mitochondria.
- Very short-lived free radical that is an initiator of cell death.
- The damaging affects of this free radical has profound effects on DNA, lipids and proteins either directly or indirectly.
- May be a benefactor in bacterial invasion due to its oxidant effect.

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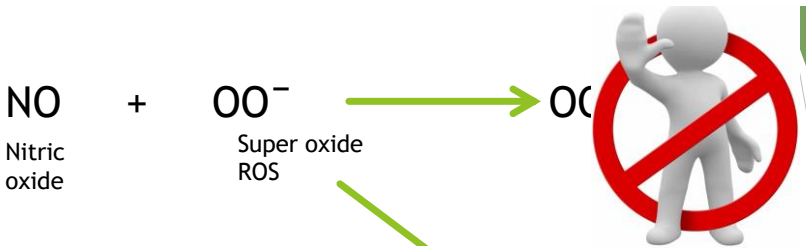

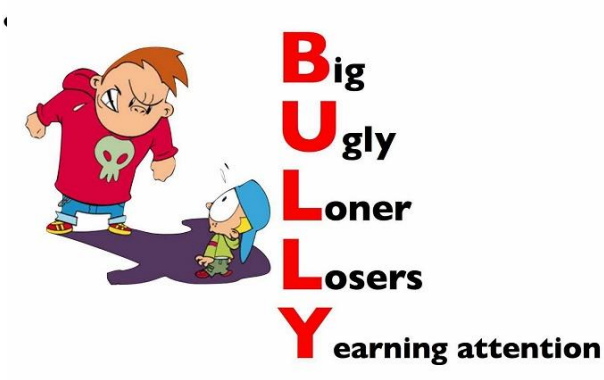

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Published in final edited form as:

"Recent evidence indicates that most of the cytoxicity attributed to NO is rather due to peroxynitrite prod betw ...he remo ther

"...peroxynitrite generation represents a crucial pathogenic mechanism in conditions such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders."

Physiol Rev. 2007 January: 87(1): 315–424

Physiol Rev. 2007 January; 87(1): 315-424

generation represents a crucial pathogenic mechanism in conditions such as stroke, myocardial infarction, chronic heart failure, diabetes, circulatory shock, chronic inflammatory diseases, cancer, and neurodegenerative disorders. Hence, novel pharmacological strategies aimed at removing peroxynitrite might represent powerful therapeutic tools in the future. Evidence supporting these novel roles of NO and peroxynitrite is presented in detail in this review.

r Manuscript

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Begin with the end in mind!!

Don't get lost in the forest!!

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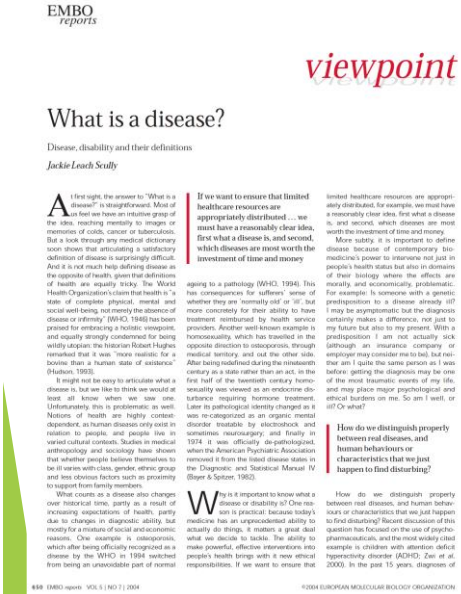
DISEASE DEFINED:

"A condition of the living animal or plant body or of one of its parts that impairs normal functioning and is typically manifested by distinguishing signs and symptoms."

Merriam Webster website



EMBO reports

viewpoint

What is a disease?

Disease, disability and their definitions

Jackie Leach Scully

At first sight, the answer to "What is a disease?" is straightforward. Most of us feel we have an intuitive grasp of the idea, reaching mentally to images or memories of colds, cancer or tuberculosis. But a look through any medical dictionary soon shows that articulating a satisfactory definition of disease is surprisingly difficult. And it is not much help defining disease as the opposite of health, given that definitions of health are equally tricky. The World Health Organization's claim that health is "a state of complete physical, mental and social well-being, not merely the absence of disease or infirmity" (WHO, 1948) has been praised for embracing a holistic viewpoint, and equally strongly condemned for being wildly utopian: the historian Robert Hughes remarked that it was "more realistic for a bovine than a human state of existence" (Hudson, 1993).

It might not be easy to articulate what a disease is, but we like to think we would at least, all know when we saw one. Unfortunately, this is problematic as well. Notions of health are highly context-dependent, as human diseases only exist in relation to people, and people live in varied cultural contexts. Studies in medical anthropology and sociology have shown that whether people believe themselves to be ill varies with class, gender, ethnic group and less obvious factors such as proximity to support from family members.

What counts as a disease also changes over historical time, partly as a result of increasing expectations of health, partly due to changes in diagnostic ability, but mostly for a mixture of social and economic reasons. One example is osteoporosis, which after being officially recognized as a disease by the WHO in 1994 switched from being an unavoidable part of normal

If we want to ensure that limited healthcare resources are appropriately distributed … we must have a reasonably clear idea, first what a disease is, and second, which diseases are most worth the investment of time and money

ageing to a pathology (WHO, 1994). This has consequences for sufferers' sense of whether they are 'normally old' or 'ill', but more concretely for their ability to have treatment reimbursed by health service providers. Another well-known example is homosexuality, which has travelled in the opposite direction to osteoporosis, through medical territory, and out the other side. After being redefined during the nineteenth century as a state rather than an act, in the first half of the twentieth century homosexuality was viewed as an endocrine disturbance requiring hormone treatment. Later its pathological identity changed as it was re-categorized as an organic mental disorder treatable by electroshock and sometimes neurosurgery, and finally in 1974 it was officially de-pathologized, when the American Psychiatric Association removed it from the listed disease states in the Diagnostic and Statistical Manual IV (Bayer & Spitzer, 1982).

Why is it important to know what a disease or disability is? One reason is practical: because today's medicine has an unprecedented ability to actually do things, it matters a great deal what we decide to tackle. The ability to make powerful, effective interventions into people's health brings with it new ethical responsibilities. If we want to ensure that limited healthcare resources are appropriately distributed, for example, we must have a reasonably clear idea, first what a disease is, and second, which diseases are most worth the investment of time and money.

More subtly, it is important to define disease because of contemporary biomedicine's power to intervene not just in people's health status but also in domains of their biology where the effects are morality, and economically, problematic. For example: Is someone with a genetic predisposition to a disease already ill? I may be asymptomatic but the diagnosis certainly makes a difference, not just to my future but also to my present. With a predisposition I am not actually sick (although an insurance company or employer may consider me to be), but neither am I quite the same person as I was before: getting the diagnosis may be one of the most traumatic events of my life, and may place major psychological and ethical burdens on me. So am I well, or ill? Or what?

How do we distinguish properly between real diseases, and human behaviours or characteristics that we just happen to find disturbing?

How do we distinguish properly between real diseases, and human behaviours or characteristics that we just happen to find disturbing? Recent discussion of this question has focused on the use of psychopharmaceuticals, and the most widely-cited example is children with attention deficit hyperactivity disorder (ADHD; Zwi et al, 2000). In the past 15 years, diagnoses of

"As the business literature shows, new clinical diagnoses are often welcomed primarily as opportunities for market growth"

2004 EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

viewpoint

science & society

As the business literature shows, new clinical diagnoses are often welcomed primarily as opportunities for market growth

Beighton *et al*, 1988; Grahame, 1992), some of the HCTDs are relatively benign whereas others have more severe consequences, and considerable investigation (genetic and other) is needed to make a differential diagnosis. Therefore having this characteristic can now be your entry card into a world of testing. Since childhood, then, I have moved from 'enviably flexible' to 'at risk of several unpleasant disorders'; a fairly major transformation, while my everyday experience of hypermobility has not changed at all.

If defining disease is difficult, disability is worse. There are problems even with deciding where to look. Does disability lie in the person? Or somewhere else? Where does the cut-off point between physical variation and disability lie? Is there in fact a cut-off point? Until recently the only coherent model for thinking about disability was a medical one, in which disability is seen as a normative pathology: a disease, degeneration, defect or deficit located in an individual. Exactly what constitutes disease, degeneration, defect or deficit here is decided by reference to a biomedical norm. It is therefore helpful to have a biomedical norm available, which might explain why the idea of 'disability' as a category arose in parallel with medical standardization.

… the personal experience of disability is not always predictable, and it can be very different from the experience of disease

Increasing dissatisfaction with the limitations of a purely medical perspective for comprehending the whole experience of disability has generated several alternatives based on the social model (Oliver, 1996; Shakespeare & Watson, 2002). The social model's fundamental criticism of the medical model is that it wrongly locates 'the problem' of disability in biological constraints, considering it only from the point of view of the individual and neglecting the social and systemic frameworks that contribute to it. The social model distinguishes between impairment (the biological substrate, such as impaired hearing) and the disabled experience. In this view the presence of impaired hearing is one thing, while the absence of subtitling on TV is quite another, and it is the refusal of society to make the necessary accommodations that is the real site of disability. A social model does not ignore biology, but contends that societal, economic and environmental factors are at least as important in producing disability.

… biomedicine's contemporary power means that it can no longer adopt ambient ideas about disease and disability without running into tricky areas of ambiguity and, potentially, ethical difficulties

On top of this, the personal experience of disability is not always predictable, and it can be very different from the experience of disease. Most sociological models of acute and chronic disease see it as a disruption to an ongoing personal identity (Bury, 1982). In part this was confirmed by a study that I carried out together with Christoph Rehmann-Sutter and Christine Rippberger in Switzerland between 1998 and 2001, in which we compared the attitudes of potential providers and potential consumers of future somatic gene therapy (Scully *et al*, 2004). People with multiple sclerosis clearly identified their illness as a disruption, "something that has happened to me." Many forms of disability are also experienced as disruptions, especially those that occur in the course of a person's life as a result of ageing, trauma or illness.

But our own and other research has shown that an impairment, especially one that is congenital or genetic, and is stable rather than progressive, can also form an important part of a person's identity. In our study, some people with impairments such as genetic deafness or achondroplasia made statements like: "If you take these [disabling] elements away from me, I wouldn't be X. I would no longer be that person." Strikingly, although most participants gave their ethnic group as Swiss, more than one Deaf participant chose "Deaf culture". To locate their primary identification with other people with their disability, even above their nationality, demonstrates its importance to their sense of identity.

The example of deafness is a particularly interesting one. Many culturally Deaf (the convention is to use lowercase 'deaf' to indicate the condition of hearing impairment, and uppercase 'Deaf' to indicate the cultural

Laboratory Findings
Center For Natural Medicine, P.A.

PATIENT NAME: Mary 65yoa

Test description	Date 10.04.18	Date 10.05.18	Homeostatic	Clinical	Units
Glucose	91	96	85.00 – 100.00	65.00 – 99.00	mg/dL
Hemoglobin A1C	-	5.4	4.90 – 5.50	4.80 – 5.60	%
Uric Acid			4.00 – 6.00	2.40 – 8.20	mg/dL
BUN			12.00 – 19.50	5.00 – 26.00	mg/dL
Creatinine	0.8	0.7	0.70 – 1.00	0.57 – 1.00	mg/dL
BUN/Creatinine Ratio	14	12	13.00 – 17.00	8.0 – 27.00	Ratio
Sodium			141.00 – 144.00	135.00 – 145.00	Mmol/L
Potassium	3.6	▶3.4	4.10 – 4.60	3.50 – 5.20	Mmol/L
Chloride	106	106	100.00 – 105.00	97.00 – 108.00	Mmol/L
Bicarbonate CO2	▶21	24	26.00 – 28.00	20.00 – 32.00	Mmol/L
Calcium	9.6	9.5	9.70 – 10.10	8.70 – 10.20	mg/dL
Phosphorous	-	3.6	3.60 – 4.10	2.50 – 4.50	mg/dL
Magnesium	-	1.8	2.20 – 2.80	1.60 – 2.60	mg/dL
Protein, Total	7.1	-	7.10 – 7.80	6.00 – 8.50	g/dL
Albumin	3.9	-	4.20 – 4.80	3.50 – 5.50	g/dL
Globulin			2.80 – 3.50	1.50 – 4.50	g/dL
A/G Ratio			1.20 – 1.60	1.10 – 2.50	Ratio
Total Bilirubin	0.4	-	0.50 – 0.70	0.10 – 1.20	mg/dL
Alkaline Phosphatase	60	-	60.00 – 80.00	25.00 – 150.00	Iu/L
LDH			120.00 – 160.00	100.00 – 250.00	Iu/L
AST (SGOT)	30	-	18.00 – 26.00	0.0 – 40.00	Iu/L
ALT (SGPT)	27	-	18.00 – 26.00	0.0 – 40.00	Iu/L
GGT			1.00 – 36.00	0.0 – 60.00	Iu/L
Iron			85.00 – 120.00	35.00 – 155.00	mcg/dL
Ferritin			25.00 – 225.00	13.00 – 150.00	ng/mL
Cholesterol, Total		199	185.00 – 200.00	100.00 – 199.00	mg/dL
Triglycerides		49	70.00 – 100.00	0.00 – 149.00	mg/dL
HDL Cholesterol		82	55.00 – 120.00	>39	mg/dL
LDL Cholesterol		87	60.00 – 125.00	0.00 – 99.00	mg/dL
TSH	2.84		1.00 – 2.50	0.45 – 4.5	mcIU/mL
Thyroxine (T4)			7.00 – 9.00	4.50 – 12.00	mcg/dL
T3 Uptake			27.00 – 35.00	24.00 – 39.00	%
T7 (Free Thyroxine Index)			2.60 – 3.60	1.20 – 4.90	
C-Reactive Protein			< 2.00	0.00 – 4.90	mg/L
White Blood Cell	6.00	5.31	5.00 – 8.00	4.00 – 10.50	x 10-3/uL
Red Blood Cell			4.50 – 5.50	3.80 – 5.10	X 10-6/uL
Hemoglobin	▶11.8	▶12.0	14.00 – 17.00	11.50 – 15.0	g/dL
Hematocrit			40.00 – 47.00	34.00 – 44.00	%
MCV	85	85.4	85.00 – 97.00	80.00 – 98.00	fL
MCH			27.00 – 32.50	27.00 – 34.00	pg
MCHC			32.00 – 34.00	32.00 – 36.00	g/dL
RDW			11.50 – 14.50	11.70 – 15.00	%
Platelets	207	196	170.00 – 300.00	140.00 – 415.00	x 10-3/uL
Polys			55.00 – 65.00	40.00 – 74.00	%
Lymphs			25.00 – 40.00	14.00 – 46.00	%
Monocytes			3.00 – 7.00	4.00 – 13.00	%
Eosinophils			0.00 – 3.00	0.00 – 7.00	%
Basophils			< 1.00	0.00 – 3.00	%
Sedimentation Rate (ESR)			0.00 – 8.00	0.00 – 30.00	mm/hr
Anion Gap	11	9			

CENTER FOR NATURAL MEDICINE

Discharge Recommendations:
- Statin
- ASA
- Heparin –generic
- Follow-up for Echocardiogram

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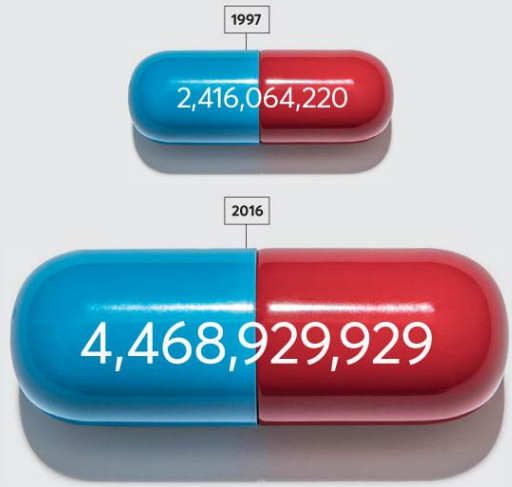
Pill Nation: The Rise of Rx Drug Use

The total number of prescriptions filled by all Americans, including adults and children, has increased by 85 percent over two decades, while the total U.S. population has increased by only 21 percent.



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IS A CHANGE IN THE HEALTH PARADIGM NEEDED??

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Health is:

"A condition in which all functions of the body and mind are normally active."

The World Health Organization defines health as a state of complete physical, mental, or social well-being and not merely the absence of infirmity.

(Taber Medical Dictionary & World Health Organization - 1948)

74

Health is:

Achieving maximum or optimal health potential by:

- ❖Restoring BALANCE
  - ➢ Removing interferences
  - ➢ Correcting deficiencies

Correcting the biochemical, functional and metabolic disturbances robbing our patients of Optimal Health and Aging Gracefully.

75

Tolle Causum - "Find the Cause"

To assist a patient towards wellness is to identify not just the constellation of symptoms, but to **truly** find the underlying cause of the symptoms.

Is it?:

- A deficiency of life-giving substances
  - Vitamin, mineral, phytochemical, amino acid, etc.
- An excess of bad exposures?
  - Toxins from pathogens, air, water, food, health and beauty products, etc.
- An unknown

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SUBMIT FORM

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Confidential Health History

Please provide the requested information to the best of your ability so that we will have a more complete understanding of your present health status as well as your future health needs. We know you could have chosen another doctor, we are honored you chose us and will work to earn that trust. **Thank you!**

What do you hope to achieve in your visit with us?

"If your patients knew what you know, they would do as you do."

3.

Have you made the decision to change? To do what it takes to get well? ☐ Yes ☐ No

The definition of insanity is: "to keep doing the same thing and expecting different results." If you keep following the same course of treatment you have been following will your results really change? Have you ever wondered if you are on the right path to achieving optimal health? Sometimes it requires taking a new and improved road to reach your destination. Most people I ask tell me they've made the decision to change. But how many people have truly decided to change? Very few! Why? Because there is a big difference between deciding something and having "reasons".

What bothers you the most?

77

The Progressive Effects of Malnutrition

Our goal is to recognize patterns of internal chemistry, toxicity and nutritional deficiencies which if corrected now will lead to optimum health and an improved quality of life.

If they are left uncorrected they may become full-blown diseases later, perhaps requiring dangerous drugs or surgery to prolong life and diminishing quality of life designed to a mere survival mode.

Taken from Malnutrition in the Elderly: A National Crisis
US Administration on Aging, US Department of Health and Human Services

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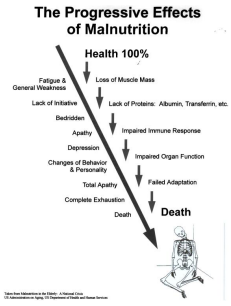
Progression of a Nutrient Deficiency

Deficiency Stage	Symptoms
1. Biochemical	➢ None YET!!! ➢ Inadequate supply of vitamins, minerals, enzymes, phytochemicals, cofactors, etc. ➢ pH ➢ Blood sugar ➢ Toxins ➢ Stealth Infections ➢ Stress ➢ ????????



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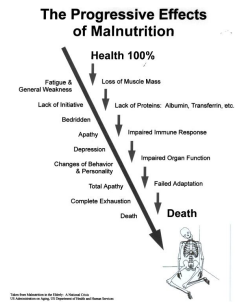
Progression of a Nutrient Deficiency

Deficiency Stage	Symptoms
2. Functional	➢ Still not discernable ➢ Limitation of physiological processes in the cells and organ systems



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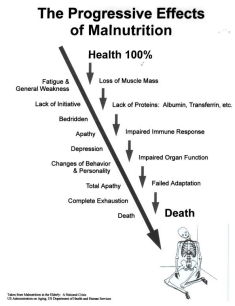
Progression of a Nutrient Deficiency

Deficiency Stage | **Symptoms**

3. Metabolic



- **Early signs of not feeling good: "Just not feeling my old self!"**
- **Indigestion, insomnia, constipation/diarrhea, malaise**

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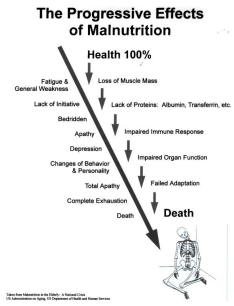
Progression of a Nutrient Deficiency

Deficiency Stage | **Symptoms**

4. Clinical

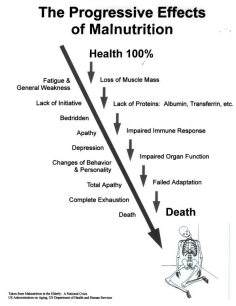


- **"Something is not right!"**
- **Fatigue, loss or gain of weight, hair loss, decrease in libido, dry skin**

82

Progression of a Nutrient Deficiency

Deficiency Stage	Symptoms
5. Pathological	➢ "What's wrong with me?" ➢ Specific disease processes such as an autoimmune, diabetes, cardiovascular or other organ disease and possibly cancer and eventually death.



Section 3 – The Impact of Chronic Conditions on Individuals and Their Caregivers

People With Chronic Conditions Report Not Receiving Adequate Information

The Solution:

HEALTH kaizen
OPTIMIZING HEALTH

Source: *Chronic Illness and Caregiving*, a survey conducted by Harris Interactive, Inc., 2000.

Journal of Inflammation Research

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REVIEW

Epigenetics of chronic inflammatory diseases

Eleni Stylianou

Consultant Biomedical Scientist and Bioinformaticist, North Royalton, OH, USA

Abstract: Chronic, noncommunicable, and inflammation-associated diseases remain the largest cause of morbidity and mortality globally and within the United States. This is mainly due to our limited understanding of the molecular mechanisms that underlie these complex pathologies. The available evidence indicates that studies of epigenetics (traditionally defined as the heritable changes to gene expression that are independent of changes to DNA) are significantly advancing our knowledge of these inflammatory conditions. This review will focus on epigenetic studies of three diseases, that are among the most burdensome globally: cardiovascular disease, the number one cause of deaths worldwide, type 2 diabetes and, Alzheimer's disease. The current status of epigenetic research, including the ability to predict disease risk, and key pathophysiological defects are discussed. The significance of defining the contribution of epigenetic defects to nonresolving inflammation and aging, each associated with these diseases, is highlighted, as these are likely to provide new insights into inflammatory disease pathogenesis.

Keywords: epigenetics, nonresolving inflammation, inflammatory diseases, atherosclerosis, type 2 diabetes, Alzheimer's disease

Introduction

Our fascination with inflammation is centuries old, yet the most recent figures indicate that inflammation-associated diseases remain the most common health problem worldwide and within the United States (http://www.who.int/chp/about/integrated_cd/en/ and https://www.cdc.gov/chronicdisease/index.htm). These chronic, noncommunicable, and complex pathologies include atherosclerosis, metabolic diseases such as type 2 diabetes (T2DM), and neurodegenerative disorders. Our knowledge of the molecular mechanisms that dysregulate a physiological, beneficial, inflammatory response, and render it pathological, remains limited.

Definition of the epigenetic changes that regulate genes associated with chronic inflammatory diseases is advancing both our ability to predict disease risk and our understanding of the underlying pathophysiological defects. Traditionally, epigenetics is defined as heritable changes to gene expression that are independent of changes to the DNA sequence.[1] Detailed discussion of the exceptions to this, eg, the dependence of DNA methylation on allele-specific single nucleotide polymorphisms (SNPs), is beyond the scope of this review but will be addressed briefly.[2]

This review will focus on explaining the current status of epigenetic research in three chronic disorders that are among the most burdensome worldwide. First:

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Abstract: Chronic, noncommunicable, and inflammation-associated diseases remain the largest cause of morbidity and mortality globally and within the United States. This is mainly due to our limited understanding of the molecular mechanisms that underlie these complex pathologies. The available evidence indicates that studies of epigenetics (traditionally defined as the heritable changes to gene expression that are independent of changes to DNA) are significantly advancing our knowledge of these inflammatory conditions. This review will focus on epigenetic studies of three diseases, that are among the most burdensome globally: cardiovascular disease, the number one cause of deaths worldwide, type 2 diabetes and, Alzheimer's disease. The current status of epigenetic research, including the ability to predict disease risk, and key pathophysiological defects are discussed. The significance of defining the contribution of epigenetic defects to nonresolving inflammation and aging, each associated with these diseases, is highlighted, as these are likely to provide new insights into inflammatory disease pathogenesis.

Keywords: epigenetics, nonresolving inflammation, inflammatory diseases, atherosclerosis, type 2 diabetes, Alzheimer's disease

Journal of Inflammation Research 2019:12 1–14

Pharmaceutical Research, Vol. 25, No. 9, September 2008 (© 2008)
DOI: 10.1007/s11095-008-9661-9

Expert Review

Cancer is a Preventable Disease that Requires Major Lifestyle Changes

Preetha Anand,[1] Ajaikumar B. Kunnumakara,[1] Chitra Sundaram,[1] Kuzhuvelil B. Harikumar,[1] Sheeja T. Tharakan,[1] Oiki S. Lai,[1] Bokyung Sung,[1] and Bharat B. Aggarwal[1,2]

"…, a disease commonly believed to be preventable. Only 5–10% of all cancer cases can be attributed to genetic defects, whereas the remaining 90–95% have their roots in the environment and lifestyle. The lifestyle factors include cigarette smoking, diet (fried foods, red meat), alcohol, sun exposure, environmental pollutants, infections, stress, obesity, and physical activity."

Pharmaceutical Research, Vol. 25, No. 9, September 2008

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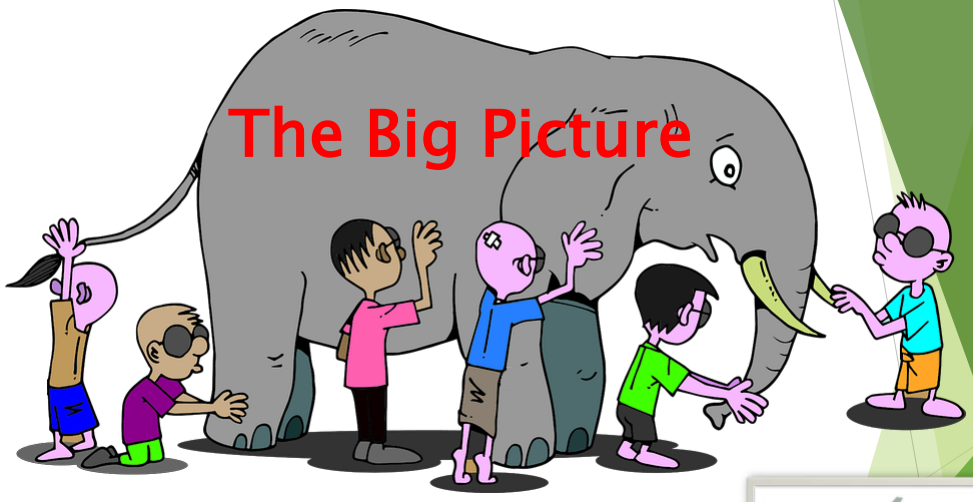
[2] To whom correspondence should be addressed. (e-mail: aggarwal@mdanderson.org)

of the US national cancer program as a "qualified failure," a judgment made 14 years after President Nixon's official declaration of the "War on Cancer." Even after an additional quarter century of extensive research, researchers are still trying to determine whether cancer is preventable and are asking "If it is preventable, why are we losing the war on cancer?" In this review, we attempt to answer this question by

The Big Picture



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Mitigating Chronic Disease/Inflammation: AGING GRACEFULLY!!!

- Evaluate and treat dysbiosis – treat "Leaky Gut"
  - ✓ Microbiome
  - ✓ Consider "Stealth Infections"
  - ✓ Consider Heavy Metal burden
  - ✓ Consider "Detoxification"
- Evaluate and balance pH
- Work on blood sugar regulation
- Evaluate biochemical, metabolic and physiological imbalances
- Discuss Diet/Lifestyle choices
- Ongoing Patient Education

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Chronic Inflammation

Chronic inflammation may be triggered by cellular stress, damage eventually leading to cellular dysfunction. It can be caused by excessive caloric intake, elevated blood sugars, and oxidative stress. It is now clear that the destructive capacity of chronic inflammation is unprecedented among physiologic processes. (Karin et al. 2006).

That danger of chronic, low-level inflammation is that this once beneficial process now becomes a silent, destructive process within the body! In fact, once stress-induced inflammation as triggered it may persist undetected for years, and even decades.

This process propagates cell dysfunction and eventually death throughout the body and is most likely the underlying pathophysiological process leading to most if not all of the major chronic diseases affecting our patients today.

In fact the term "INFLAMM-AGING" has been used to describe this destructive process associated with aging in the body.

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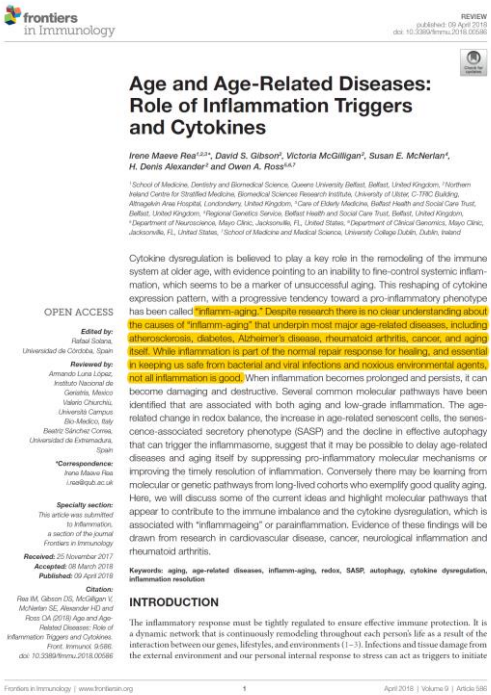

frontiers in Immunology

REVIEW

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Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines

Irene Maeve Rea[1,2,3], David S. Gibson[2], Victoria McGilligan[2], Susan E. McNerlan[4], H. Denis Alexander[2] and Owen A. Ross[5,6,7]*

[1] School of Medicine, Dentistry and Biomedical Science, Queens University Belfast, Belfast, United Kingdom, [2] Northern Ireland Centre for Stratified Medicine, Biomedical Sciences Research Institute, University of Ulster, C-TRIC Building, Altnagelvin Area Hospital, Londonderry, United Kingdom, [3] Care of Elderly Medicine, Belfast Health and Social Care Trust, Belfast, United Kingdom, [4] Regional Genetics Service, Belfast Health and Social Care Trust, Belfast, United Kingdom, [5] Department of Neuroscience, Mayo Clinic, Jacksonville, FL, United States, [6] Department of Clinical Genomics, Mayo Clinic, Jacksonville, FL, United States, [7] School of Medicine and Medical Science, University College Dublin, Dublin, Ireland

Cytokine dysregulation is believed to play a key role in the remodeling of the immune system at older age, with evidence pointing to an inability to fine-control systemic inflammation, which seems to be a marker of unsuccessful aging. This reshaping of cytokine expression pattern, with a progressive tendency toward a pro-inflammatory phenotype has been called "inflamm-aging." Despite research there is no clear understanding about the causes of "inflamm-aging" that underpin most major age-related diseases, including atherosclerosis, diabetes, Alzheimer's disease, rheumatoid arthritis, cancer, and aging itself. While inflammation is part of the normal repair response for healing, and essential in keeping us safe from bacterial and viral infections and noxious environmental agents, not all inflammation is good. When inflammation becomes prolonged and persists, it can become damaging and destructive. Several common molecular pathways have been identified that are associated with both aging and low-grade inflammation. The age-related change in redox balance, the increase in age-related senescent cells, the senescence-associated secretory phenotype (SASP) and the decline in effective autophagy that can trigger the inflammasome, suggest that it may be possible to delay age-related diseases and aging itself by suppressing pro-inflammatory molecular mechanisms or improving the timely resolution of inflammation. Conversely there may be learning from molecular or genetic pathways from long-lived cohorts who exemplify good quality aging. Here, we will discuss some of the current ideas and highlight molecular pathways that appear to contribute to the immune imbalance and the cytokine dysregulation, which is associated with "inflammageing" or parainflammation. Evidence of these findings will be drawn from research in cardiovascular disease, cancer, neurological inflammation and rheumatoid arthritis.

Keywords: aging, age-related diseases, inflamm-aging, redox, SASP, autophagy, cytokine dysregulation, inflammation resolution

OPEN ACCESS

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INTRODUCTION

The inflammatory response must be tightly regulated to ensure effective immune protection. It is a dynamic network that is continuously remodeling throughout each person's life as a result of the interaction between our genes, lifestyles, and environments (1–3). Infections and tissue damage from the external environment and our personal internal response to stress can act as triggers to initiate

…"inflamm-aging." Despite research there is no clear understanding about the causes of "inflamm-aging" that underpin most major age-related diseases, including atherosclerosis, diabetes, Alzheimer's disease, rheumatoid arthritis, cancer, and aging itself. While inflammation is part of the normal repair response for healing, and essential in keeping us safe from bacterial and viral infections and noxious environmental agents, not all inflammation is good."

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REVIEW ARTICLE OPEN

Macrophages in age-related chronic inflammatory diseases

Yumiko Oishi[1] and Ichiro Manabe[2]

Chronic inflammation is the common pathological basis for such age-associated diseases as cardiovascular disease, diabetes, cancer and Alzheimer's disease. A multitude of bodily changes occur with aging that contribute to the initiation and development of inflammation. In particular, the immune system of elderly individuals often exhibits diminished efficiency and fidelity, termed immunosenescence. But, although immune responses to new pathogens and vaccines are impaired, immunosenescence is also characterized by a basal systemic inflammatory state. This alteration in immune system function likely promotes chronic inflammation. Changes in the tissue microenvironment, such as the accumulation of cell debris, and systemic changes in metabolic and hormonal signals, also likely contribute to the development of chronic inflammation. Monocyte/macrophage lineage cells are crucial to these age-associated changes, which culminate in the development of chronic inflammatory diseases. In this review, we will summarize the diverse physiological and pathological roles of macrophages in the chronic inflammation underlying age-associated diseases.

npj Aging and Mechanisms of Disease (2016) **2**, 16018; doi:10.1038/npjamd.2016.18; published online 28 July 2016

INTRODUCTION

With advancing age, the immune system undergoes a dynamic change characterized by the coexistence of a smaller immune response to newly encountered pathogens or vaccine antigens, and an elevated systemic inflammatory state made manifest, for example, by elevated levels of proinflammatory cytokines, clotting factors and acute phase reactants.[1] This chronic activation of inflammation associated with aging has been termed inflammaging, and recent studies indicate it is involved in the development of such non-communicable diseases (NCDs) as cardiovascular and metabolic disease and cancer in the elderly. Although the chronic inflammation associated with NCDs does not necessarily lead by age-associated changes in the body, the observation that the prevalence of many NCDs increases with advancing age suggests a pathogenic link between inflammaging and age-associated diseases.

Chronic inflammation is a prolonged condition in which tissue injury and attempts at repair coexist, leading to tissue remodeling and dysfunction.[2] Although chronic inflammation may follow acute inflammation, in the most common NCDs of today it likely begins insidiously as a low-grade, smoldering response with no manifestation of the cardinal signs of inflammation (Dolor (pain), Calor (heat), Rubor (redness) and Tumor (swelling)). However, even low-grade inflammation may impair tissue function (Functio laesa). For instance, inflammatory signals interfere with insulin signaling.[3] Moreover, the continuous progression of tissue injury and repair promotes tissue remodeling (e.g., extensive fibrosis) that may eventually cause irreversible tissue dysfunction.[4] In fact, the severity of tissue remodeling determines the prognosis of some NCDs, such as heart failure and chronic kidney disease.[5]

A complex interplay between parenchymal cells within a tissue and the various cells in the stroma, including immune cells, vascular cells and fibroblasts, lead the processes of chronic inflammation under the influence of inputs from both the local microenvironment and the wider system. Of particular interest are monocyte–macrophage lineage cells, which act as major effector cells in chronic inflammatory processes during the pathological development of NCDs.[4] In this review, we will summarize the pathological connection between chronic inflammation and age-associated diseases, with a particular focus on what is currently known about the roles played by macrophages.

IMMUNOSENESCENCE AND AGE-ASSOCIATED DISEASES

It is often noted that elderly individuals are more vulnerable to infectious diseases. For instance, occult infection with tuberculosis and varicella zoster virus becomes evident, sometimes leading to life-threatening disease. Moreover, vaccines are often ineffective in older adults owing to inability of the adaptive immune system to generate protective immunity. Overall, these changes in the immune system, characterized by declining fidelity and efficiency, are termed immunosenescence. A key feature of immunosenescence is an imbalance between inflammatory and anti-inflammatory networks, leading to a complex presentation of impaired adaptive immune responses with concomitant persistent low-grade inflammation and a greater susceptibility to autoimmune responses.[6] The changes in the adaptive immune system are characterized by a decreases in naive T- and B cells, an increase in memory cells and a progressive reduction in the T-cell receptor (TCR) and B-cell receptor (BCR) repertoire.[7–9] By contrast, generation of myeloid cells is favored in aging animals.

Immunosenescence involves not only age-related changes intrinsic to immune cells, but also microenvironmental and systemic alterations. Although it is likely that cellular senescence is involved in some of these age-related alterations, many are likely induced independently of cellular senescence pathways.

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npj nature partner journals

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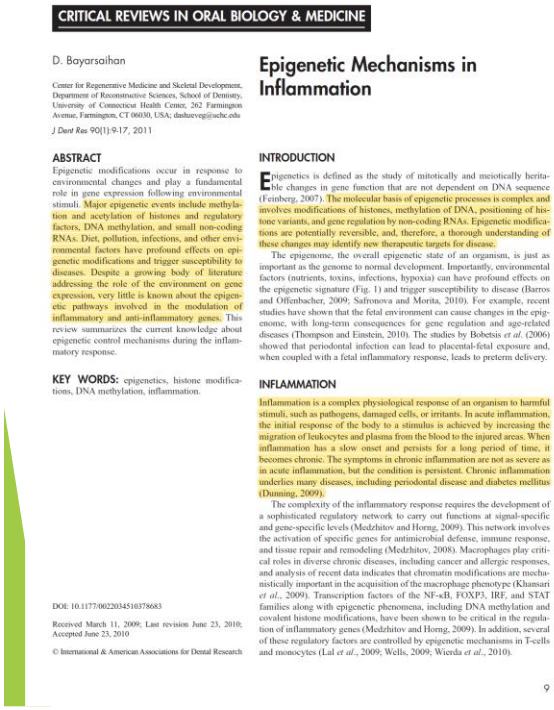

CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

D. Bayarsaihan

Center for Regenerative Medicine and Skeletal Development, Department of Reconstructive Sciences, School of Dentistry, University of Connecticut Health Center, 262 Farmington Avenue, Farmington, CT 06030, USA; dashuvsg@uchc.edu

J Dent Res 90(1):9-17, 2011

Epigenetic Mechanisms in Inflammation

ABSTRACT

Epigenetic modifications occur in response to environmental changes and play a fundamental role in gene expression following environmental stimuli. Major epigenetic events include methylation and acetylation of histones and regulatory factors, DNA methylation, and small non-coding RNAs. Diet, pollution, infections, and other environmental factors have profound effects on epigenetic modifications and trigger susceptibility to diseases. Despite a growing body of literature addressing the role of the environment on gene expression, very little is known about the epigenetic pathways involved in the modulation of inflammatory and anti-inflammatory genes. This review summarizes the current knowledge about epigenetic control mechanisms during the inflammatory response.

KEY WORDS: epigenetics, histone modifications, DNA methylation, inflammation.

INTRODUCTION

Epigenetics is defined as the study of mitotically and meiotically heritable changes in gene function that are not dependent on DNA sequence (Feinberg, 2007). The molecular basis of epigenetic processes is complex and involves modifications of histones, methylation of DNA, positioning of histone variants, and gene regulation by non-coding RNAs. Epigenetic modifications are potentially reversible, and, therefore, a thorough understanding of these changes may identify new therapeutic targets for disease.

The epigenome, the overall epigenetic state of an organism, is just as important as the genome to normal development. Importantly, environmental factors (nutrients, toxins, infections, hypoxia) can have profound effects on the epigenetic signature (Fig. 1) and trigger susceptibility to disease (Barros and Offenbacher, 2009; Safronova and Morita, 2010). For example, recent studies have shown that the fetal environment can cause changes in the epigenome, with long-term consequences for gene regulation and age-related diseases (Thompson and Einstein, 2010). The studies by Bobetsis *et al.* (2006) showed that periodontal infection can lead to placental-fetal exposure and, when coupled with a fetal inflammatory response, leads to preterm delivery.

INFLAMMATION

Inflammation is a complex physiological response of an organism to harmful stimuli, such as pathogens, damaged cells, or irritants. In acute inflammation, the initial response of the body to a stimulus is achieved by increasing the migration of leukocytes and plasma from the blood to the injured areas. When inflammation has a slow onset and persists for a long period of time, it becomes chronic. The symptoms in chronic inflammation are not as severe as in acute inflammation, but the condition is persistent. Chronic inflammation underlies many diseases, including periodontal disease and diabetes mellitus (Dunning, 2009).

The complexity of the inflammatory response requires the development of a sophisticated regulatory network to carry out functions at signal-specific and gene-specific levels (Medzhitov and Horng, 2009). This network involves the activation of specific genes for antimicrobial defense, immune response, and tissue repair and remodeling (Medzhitov, 2008). Macrophages play critical roles in diverse chronic diseases, including cancer and allergic responses, and analysis of recent data indicates that chromatin modifications are mechanistically important in the acquisition of the macrophage phenotype (Khansari *et al.*, 2009). Transcription factors of the NF-κB, FOXP3, IRF, and STAT families along with epigenetic phenomena, including DNA methylation and covalent histone modifications, have been shown to be critical in the regulation of inflammatory genes (Medzhitov and Horng, 2009). In addition, several of these regulatory factors are controlled by epigenetic mechanisms in T-cells and monocytes (Lal *et al.*, 2009; Wells, 2009; Wierda *et al.*, 2010).

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"Inflammation is a complex physiological response of an organism to harmful stimuli, such as pathogens, damaged cells, or irritants. In acute inflammation, the initial response of the body to a stimulus is achieved by increasing the migration of leukocytes and plasma from the blood to the injured areas. When inflammation has a slow onset and persists for a long period of time, it becomes chronic. The symptoms in chronic inflammation are not as severe as in acute inflammation, but the condition is persistent. Chronic inflammation underlies many diseases, including periodontal disease and diabetes mellitus (Dunning, 2009)."

J Dent Res 90(1):9-17, 2011

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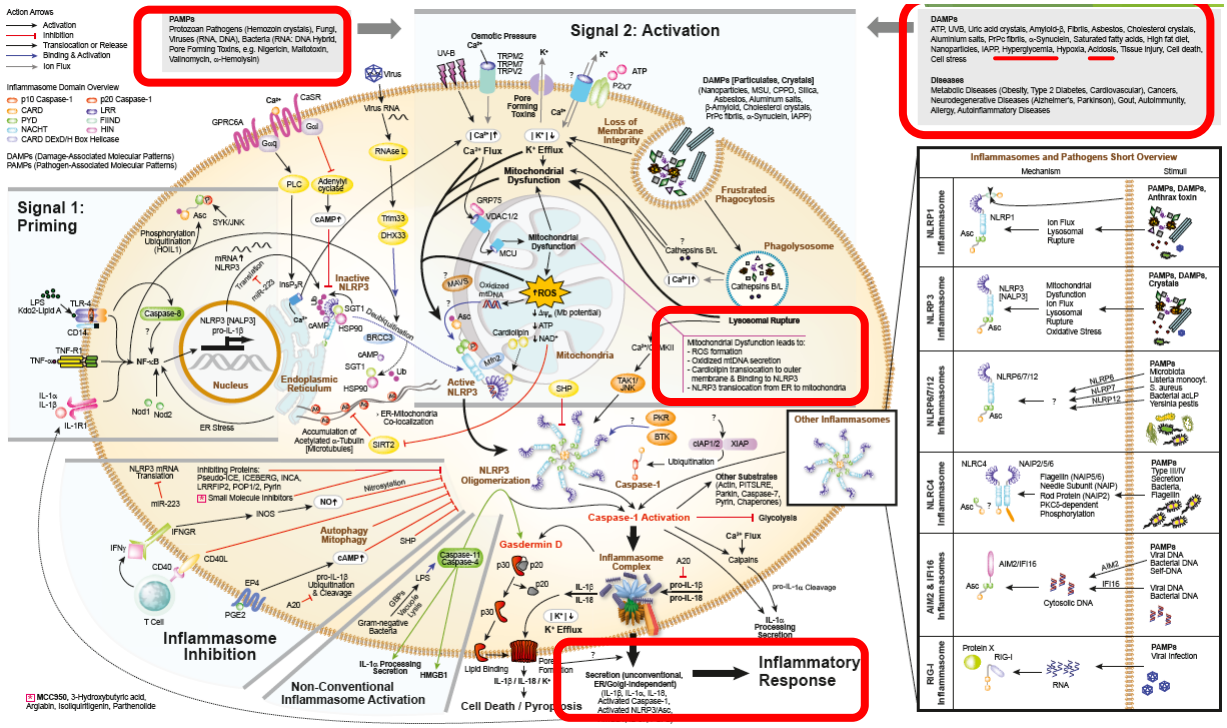
Signal 1: Priming
Signal 2: Activation
PAMPs
DAMPs
Inflammasomes and Pathogens Short Overview
Inflammasome Inhibition
Non-Conventional Inflammasome Activation
Inflammatory Response
Cell Death / Pyroptosis

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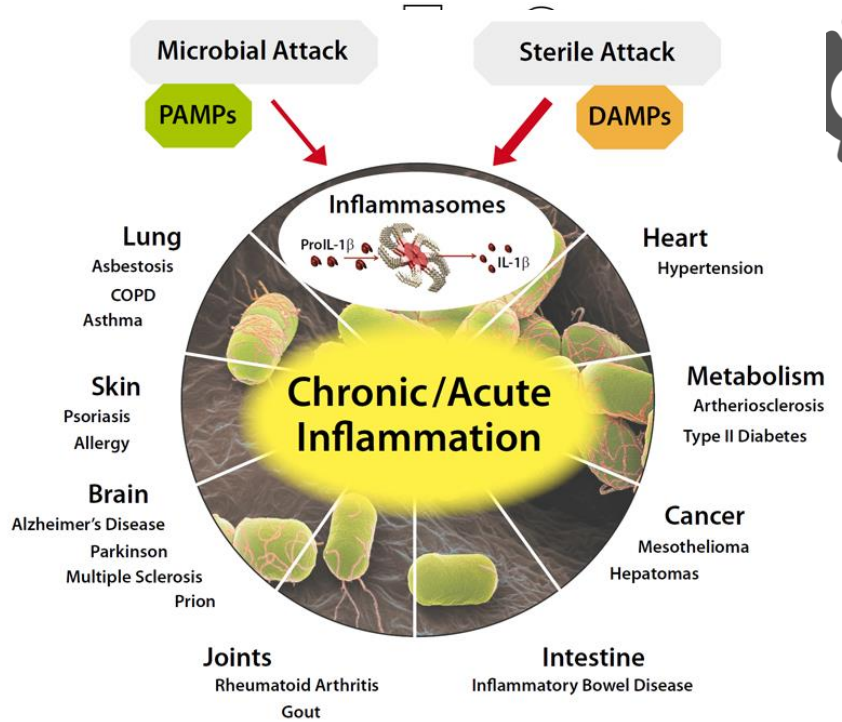
Microbial Attack
Sterile Attack
PAMPs
DAMPs
Inflammasomes
ProIL-1β
IL-1β
Chronic/Acute Inflammation
Lung
Asbestosis
COPD
Asthma
Heart
Hypertension
Skin
Psoriasis
Allergy
Metabolism
Artheriosclerosis
Type II Diabetes
Brain
Alzheimer's Disease
Parkinson
Multiple Sclerosis
Prion
Cancer
Mesothelioma
Hepatomas
Joints
Rheumatoid Arthritis
Gout
Intestine
Inflammatory Bowel Disease


SAND

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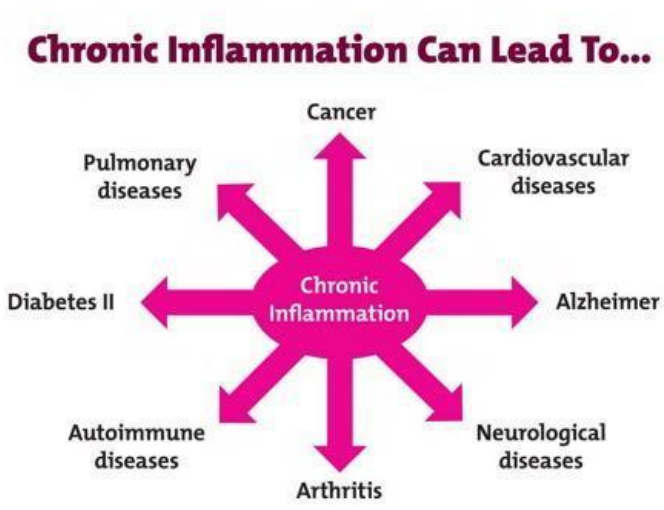

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Inflammation

My top candidates for chronic inflammation:

Aka Reactive Oxygen Species (ROS)

- Bowel dysbiosis – Microbiota/Microbiome
- Stealth Infections
- Glycation
- Acid/alkaline balance
- Diet/Lifestyle – Fast food, processed food
- Stress
- Toxicity
- Deficiencies
- Hormonal imbalances
- A combination of all of the above

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"Medical science has made such tremendous progress that there is hardly a healthy human left."

~ Aldous Huxley (1894 – 1963)

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4 Major Factors: Epimutagens

- **Microbiome - Stealth Infections**
- Acid/Alkaline Balance
- Blood Sugar Regulation
- Stress

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"Dr. Peterson never forget;
ALWAYS start with the bowel…"

Dr. Bernard Jensen - circa 1993

99

The 'Balance' between Health/Disease

100

The secret to longevity is in the microbiome and the gut

31 May 2018

Credit: McGill University

You are what you eat. Or so the saying goes. Science now tells us that we are what the bacteria living in our intestinal tract eat and this could have an influence on how well we age. Building on this, McGill University scientists fed fruit flies with a combination of probiotics and an herbal supplement called Triphala that was able to prolong the flies' longevity by 60 % and protect them against chronic diseases associated with aging.

The study, published in *Scientific Reports*, adds to a growing body of evidence of the influence that gut bacteria can have on health. The researchers incorporated a symbiotic—made of probiotics with a polyphenol-rich supplement—into the diet of fruit flies.

The flies fed with the synbiotic lived up to 66 days old—26 days more than the ones without the supplement. They also showed reduced traits of aging, such as mounting insulin resistance, inflammation and oxidative stress.

"Probiotics dramatically change the architecture of the gut microbiota, not only in its composition but also in respect to how the foods that we eat are metabolized," says Satya Prakash, professor of biomedical engineering in McGill's Faculty of Medicine and senior author of the study. "This allows a single probiotic formulation to simultaneously act on several biochemical signaling pathways to elicit broad beneficial physiological effects, and explains why the single formulation we present in this paper has such a dramatic effect on so many different markers".

The fruit fly is remarkably similar to mammals with about 70 % similarity in terms of their biochemical pathways, making it a good indicator of what would happen in humans, adds Prakash.

"The effects in humans would likely not be as dramatic, but our results definitely suggest that a diet specifically incorporating Triphala along with these probiotics will promote a long and healthy life."

The authors also say that the findings can be explained by the "gut-brain axis," a bidirectional communication system between microorganisms residing in the gastrointestinal tract—the microbiota—and the brain. In the past few years, studies have shown the gut-brain axis to be involved in neuropathological changes and a variety of conditions such as irritable bowel syndrome, neurodegeneration and even depression. Few studies, however, have successfully designed gut microbiota-modulating therapeutics having effects as potent or broad as the formulation presented in the new study.

Learning from traditional medicine

The herbal supplement used in the study, Triphala, is a formulation made from amalaki, bibhitaki and haritaki, fruits used as medicinal plants in Ayurveda, a form of traditional Indian medicine.

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The 'Balance' between Health/Disease:

INFLAMMATION

- Microbiome
  - What it is
  - Pathogens – Stealth Infections
    - ✓ Viral loads
    - ✓ Bacterial loads
    - ✓ Fungal infections
    - ✓ Parasites
  - Gut/brain connection
  - Oncobiome

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Hindawi

Review Article

The Central Role of the Gut Microbiota in Chronic Inflammatory Diseases

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The commensal microbiota is in constant interaction with the immune system, teaching immune cells to respond to antigens. Studies in mice have demonstrated that manipulation of the intestinal microbiota alters host immune cell homeostasis. Additionally, metagenomic sequencing analysis has revealed alterations in intestinal microbiota in patients suffering from inflammatory bowel disease, asthma, and obesity. Perturbations in the microbiota composition result in a deficient immune response and impaired tolerance to commensal microorganisms. Due to altered microbiota composition which is associated to some inflammatory diseases, several strategies, such as the administration of probiotics, diet, and antibiotic usage, have been utilized to prevent or ameliorate chronic inflammatory diseases. The purpose of this review is to present and discuss recent evidence showing that the gut microbiota controls immune system function and onset, development, and resolution of some common inflammatory diseases.

1. Introduction

Commensal microbiota consists of many microorganisms that cover all host mucosal surfaces, but most reside in the gastrointestinal tract, which is the subject of this review. Amazingly, although the human body is composed of approximately 100 trillion cells, only 10 trillion are human cells while 90 trillion are microbes. The genes of these microorganisms form our metagenome, known as our second genome [1]. Thus, it is not surprising that this large arsenal of gene products has a relevant role in body homeostasis [2, 3]. The relationship between the gut microbiota and its host plays a key role in immune system maturation, food digestion, drug metabolism, detoxification, vitamin production, and prevention of pathogenic bacteria adhesion [4]. One of the most important roles of the microbiota is the maturation of the immune system in the postnatal period. The first appearance of adaptive immunity in humans coincides with acquisition of a complex diet and microbiota, which suggests that mucosal immunity in the intestines has evolved to tolerate diverse microbes and food antigens.

Colonization of the gastrointestinal tract begins after birth, despite the fact that some researchers have discovered a small community of bacteria living in the placenta [5]. However, there is no convincing evidence demonstrating that such bacteria normally reach the fetus through the placenta. It

Due to altered microbiota composition which is associated to some inflammatory diseases, several strategies, such as administration of probiotics, diet, and antibiotic usage, have been utilized to prevent or ameliorate chronic inflammatory diseases.

Ferreira et al., Journal of Immunology Research, Volume 2014, September 2014

Engineering 3 (2017) 71–82

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Research
Microecology—Review

The Human Microbiota in Health and Disease

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Probiotics

ABSTRACT

Trillions of microbes have evolved with and continue to live on and within human beings. A variety of environmental factors can affect intestinal microbial imbalance, which has a close relationship with human health and disease. Here, we focus on the interactions between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases. We also review important advances in techniques associated with microbial research, such as DNA sequencing, metabonomics, and proteomics combined with computation-based bioinformatics. Current research on the human microbiota has become much more sophisticated and more comprehensive. Therefore, we propose that research should focus on the host-microbe interaction and on cause-effect mechanisms, which could pave the way to an understanding of the role of gut microbiota in health and disease, and provide new therapeutic targets and treatment approaches in clinical practice.

1. Introduction

More than 100 trillion symbiotic microorganisms live on and within human beings and play an important role in human health and disease. The human microbiota, especially the gut microbiota, has even been considered to be an "essential organ" [1], carrying approximately 150 times more genes than are found in the entire human genome [2]. Important advances have shown that the gut microbiota is involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity [3–6]. Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota [7,8] (Fig.1).

In recent decades, a tremendous amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease [7,9–23] via several mechanisms. First, the microbiota has the potential to increase energy extraction from food [24], increase nutrient harvest [9,10], and alter appetite signaling [25,26]. The microbiota contains far more versatile metabolic genes than are found in the human genome, and provides humans with unique and specific enzymes and biochemical pathways [9]. In addition, a large proportion of the metabolic microbiotic processes that are beneficial to the host are involved in either nutrient acquisition or xenobiotic processing, including the metabolism of undigested carbohydrates and the biosynthesis of vitamins [10]. Second, the human microbiota also provides a physical barrier, protecting its host against foreign pathogens through competitive exclusion and the production of antimicrobial substances [11–13]. Finally, the microbiota is essential in the development of the intestinal mucosa and immune system of the host [14,16]. For example, germ-free (GF) animals have abnormal numbers of several immune cell types, deficits in local and systemic lymphoid structures, poorly formed spleens and lymph

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"Here, we focus on the interactions between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases."

"…Therefore, we propose that research should focus on the host-microbe interaction and on cause-effect mechanisms, which could pave the way to an understanding of the role of gut microbiota in health and disease, and provide new therapeutic targets and treatment approaches in clinical practice."

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http://dx.doi.org/10.1016/J.ENG.2017.01.008

Fig. 1. Human microbial symbiosis has a close relationship with diseases of different systems.

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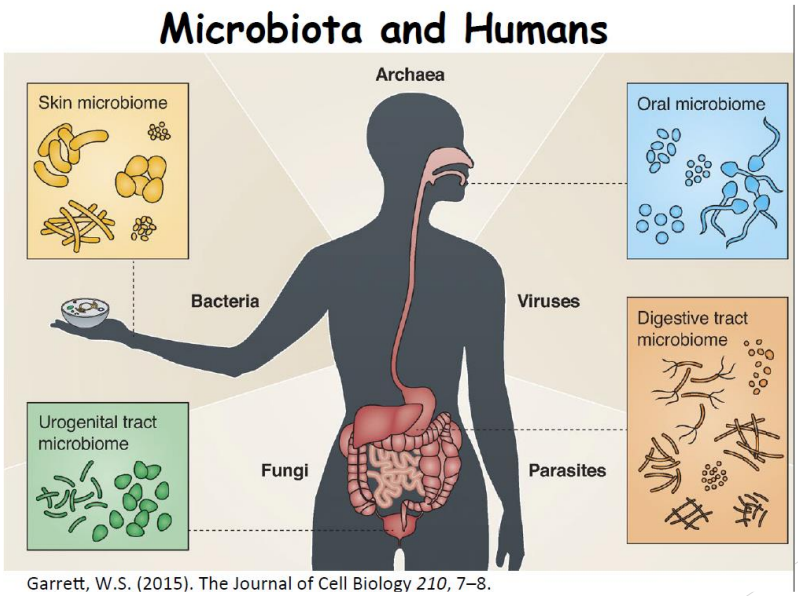

Garrett, W.S. (2015). The Journal of Cell Biology *210*, 7–8.

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Definition of MICROBIOME

1: a community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment and especially the collection of microorganisms living in or on the human body. Your body is home to about 100 trillion bacteria and other microbes, collectively known as your *microbiome*.

- … what's arguably become the hottest area of medicine: *microbiome* research, an emerging field that's investigating how the bacteria that live in and on our bodies affect our health.

2: the collective genomes of microorganisms inhabiting a particular environment and especially the human body. They form one community among the many that make up the human *microbiome*: the full genetic complement of bacteria and other organisms at home on your skin, gums, and teeth, in your genital tract, and especially in your gut.

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Definition of Microbiota

: the microscopic organisms of a particular environment

: MICROBIOME: It's very possible that the master key to unlocking chronic disease will turn out to be the health and composition of the *microbiota* in your gut.

Microbiotic

adjective

the *microbiotic* environment of the gut

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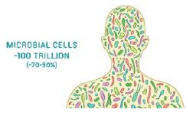
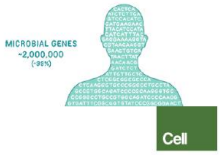
Humans are a composite of microorganisms



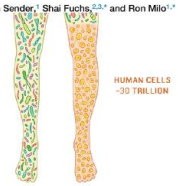


Leading Edge
Commentary

Cell

Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans

Ron Sender,[1] Shai Fuchs,[2,3,*] and Ron Milo[1,*]



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MEDPAGE TODAY®

Gastroenterology > General Gastroenterology

18 Common Drugs Tied to Altered Gut Microbiome

— PPIs, oral antidiabetics, antibiotics, and laxatives had most impact

by Diana Swift, Contributing Writer
October 23, 2019

Almost of half of 41 common drug classes were associated with alterations of the microbiota of the human gut, Dutch researchers reported.

Extensive changes in taxonomic structure, metabolic activity, and resistome (antibiotic-resistant genes) were seen in human fecal samples following use of 18 of 41 common drug categories, with the four most frequent culprits being proton pump inhibitors (PPIs), metformin, antibiotics, and laxatives, reported Arnau Vich Vila, MSc, of the University Medical Center Groningen at United European Gastroenterology Week in Barcelona.

Vich Vila and colleagues performed metagenomics sequencing on 1,883 fresh frozen fecal samples from three independent cohorts: a population-based group, patients with inflammatory bowel disease, and patients with irritable bowel syndrome, intermixed with healthy controls.

Differences between drug users and non-users were assessed by looking at the effect of single medication use and also factoring in the use of multiple drugs by each participant. Cohort-specific results were combined in a meta-analysis using inverse variance.

"Our work highlights the importance of considering the role of the gut microbiota when designing treatments and also points to new hypotheses that could explain certain side-effects associated with medication use," Vich Vila said. These associations need to be functionally investigated in light of the importance of the gut microbiota in health and the widespread use of many drugs.

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APJAI Asian Pacific Journal of Allergy and Immunology

REVIEW ARTICLE

The potential impact of gut microbiota on your health: Current status and future challenges

Sittaya Sirisinha

Abstract

Our health and probably also our behaviors and mood depend not only on what we eat or what we do (lifestyle behaviors), but also on what we host. It is well established for decades that all vertebrates including humans are colonized by a wide array of bacteria, fungi, eukaryotic parasites and viruses, and that, at steady state (homeostasis), this community of microbes establishes a friendly mutual relationship with the host. The term microbiota was originally meant to represent an ecological community of commensals and potentially pathogenic microbes that live within our bodies, but it is now used interchangeably with the term microbiome which was initially meant to represent a collective genome of the microbiota. Although the number of microbes that live in or on our body was previously estimated to outnumber that of their hosts by 10 to 1, the latest estimate put the ratio to be closer to 1:1. On the other hand, their collective genomes (microbiome) outnumber those of the host by 100-200 times. It is not surprising therefore that these microbes not only provide the host with a variety of metabolic impact, but can also modulate tissue integrity and immune defense, all of which lead to a healthy ecosystem (symbiosis) that is unfavorable for colonization and invasion of pathogens. Microbiota is well known for its role in development and education of immune system. However, its link with diseases is less known and it is only recently that there is a surge of interest in the potential impact of microbiota on human health and disease. The diversity and composition of microbiota (healthy microbiota profile) are dynamics, depending not only on the host physical status, genotype and immune phenotype, but also on the environmental factors like diet, antibiotic usage and lifestyle behaviors. These environmental factors may adversely alter gut ecosystem (dysbiosis) that is frequently associated with increased susceptibility to infectious as well as to non-communicable diseases like obesity, metabolic syndromes (e.g., diabetes and cardiovascular diseases), allergy and other inflammatory diseases. Emerging evidence from more recent studies also demonstrate the existence of a bidirectional communication route linking gut and microbiota with brain, thus suggesting that these microbes may play a role in neurological disorders as well as in host perception, behavior and emotional response. However, whether the observed alteration of the microbiota profile in these diverse conditions is the cause or the consequence of the disease remains to be established. These observations imply that it may be possible to design new strategies for the management of diseases by manipulating gut microbiota. The common practice now available is the use of probiotics to rehabilitate gut ecosystem. The microbiota-based therapeutics like fecal transplantation for the treatment of recurrent antibiotic-resistant *Clostridium difficile* infection is now under clinical trial and reported to be highly successful. In the next decade, we will probably see even more exciting approaches, for example, using advanced microbiota engineering technologies to create "intelligent" or "smart" bacteria for use in diagnosis, prevention, prediction and treatment of inflammatory diseases and possibly of some gastrointestinal cancers. The microbiota-based therapeutics together with personalized medicine may be the most accurate and optimal strategy for the future treatment of some difficult-to-manage diseases. However, many challenges remain to be solved before the translational potential of this new knowledge can be implemented clinically. In this review, I highlight some important recent developments and advances that contribute to our understanding in the role of microbiota in human health and disease and on how to best manipulate the microbiome to promote greater human health.

Keywords: microbiota, microbiome, gut homeostasis, dysbiosis, microbiota-based therapeutics.

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"…all vertebrates including humans are colonized by a wide array of bacteria, fungi, eukaryotic parasites and viruses, and that, at steady state (homeostasis), this community of microbes establishes a friendly mutual relationship with the host. The term microbiota was originally meant to represent an ecological community of commensals and potentially pathogenic microbes that live within our bodies, but it is now used interchangeably with the term microbiome which was initially meant to represent a collective genome of the microbiota."

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"The microbiota-based therapeutics like fecal transplantation for the treatment of recurrent antibiotic-resistant *Clostridium difficile* infection is now under clinical trial and reported to be highly successful."

Asian Pac J Allergy Immunol 2016;34:249-264
DOI 10.12932/AP0803

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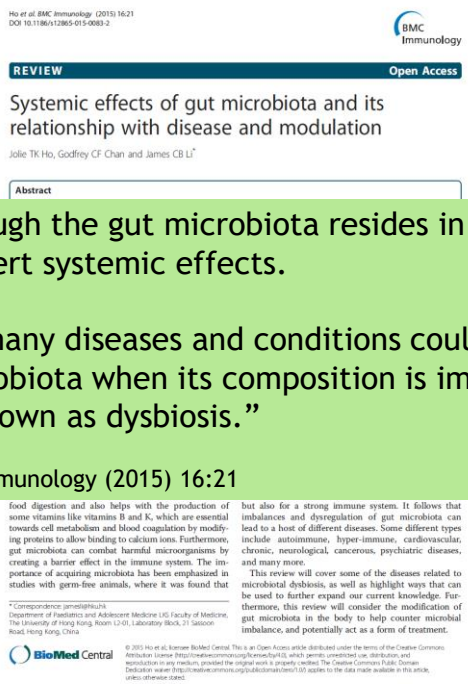

Ho et al. BMC Immunology (2015) 16:21
DOI 10.1186/s12865-015-0083-2

BMC Immunology

REVIEW Open Access

Systemic effects of gut microbiota and its relationship with disease and modulation

Jolie TK Ho, Godfrey CF Chan and James CB Li*

Abstract

food digestion and also helps with the production of some vitamins like vitamins B and K, which are essential towards cell metabolism and blood coagulation by modifying proteins to allow binding to calcium ions. Furthermore, gut microbiota can combat harmful microorganisms by creating a barrier effect in the immune system. The importance of acquiring microbiota has been emphasized in studies with germ-free animals, where it was found that

but also for a strong immune system. It follows that imbalances and dysregulation of gut microbiota can lead to a host of different diseases. Some different types include autoimmune, hyper-immune, cardiovascular, chronic, neurological, cancerous, psychiatric diseases, and many more.

This review will cover some of the diseases related to microbiotal dysbiosis, as well as highlight ways that can be used to further expand our current knowledge. Furthermore, this review will consider the modification of gut microbiota in the body to help counter microbial imbalance, and potentially act as a form of treatment.

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"…and although the gut microbiota resides in the intestines, it is able to exert systemic effects.

Therefore, many diseases and conditions could be impacted by the gut microbiota when its composition is imbalanced, otherwise known as dysbiosis."

Ho et al, BMC Immunology (2015) 16:21

Gut"

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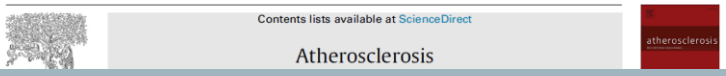

Contents lists available at ScienceDirect

Atherosclerosis

atherosclerosis

Intervention at the level of the microbiota appears to attenuate symptoms in these inflammatory syndromes with probiotic treatment, such as Lactobacilli, playing a uniquely beneficial role in restoring intestinal health, decreasing inflammation, and reducing cardiovascular disease. This review will discuss obesity, T1DM, RA, and SLE in the context of how each unique microbiome profile contributes to elevated cardiovascular risk

https://doi.org/10.1016/j.atherosclerosis.2018.02.036

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Progress in Retinal and Eye Research 64 (2018) 84–95

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Progress in Retinal and Eye Research

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Epigenetics, microbiota, and intraocular inflammation: New paradigms of immune regulation in the eye

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Age-related macular degeneration
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Gut microbiota
Epigenetic therapy

ABSTRACT

Sight threatening immune responses that damage the eye characterize intraocular inflammatory diseases. These diseases including uveitis and age-related macular degeneration are worryingly common and quality of life shattering. Genetic studies in past decades significantly advanced our understanding of the etiology of these devastating diseases. Unfortunately, patient genetics alone failed to adequately explain disease origin, susceptibility, and progression. Non-genetic factors such as the epigenetic regulation of ocular diseases and the environmental factors triggering intraocular inflammation offer new insight into intraocular inflammatory disorders. Importantly, mounting evidence is signaling that dysbiosis of human microbiota leads to rapid epigenomic reprograming of host cells and results in the onset of many diseases. In this review, we discuss how epigenetic mechanisms and microbiota may cooperate to initiate and perpetuate ocular inflammation. Lastly, we propose that the discovery of intraocular microbiota presents a significant shift in thought affecting current approaches to the diagnosis, treatment, and prevention of intraocular inflammatory diseases such as uveitis and age-related macular degeneration. The geographical and genetic background difference in both disease presentation and genetic association of intraocular inflammatory diseases may be due to the variation of intraocular microbiota.

1. Epigenetics

1.1. What is epigenetics?

In the course of biological study, many researchers found numerous biological phenomena could not be explained by genetic principles alone. Conrad Waddington (1905–1975) proposed the word "epigenetics" phenomena in 1942 (Waddington, 1942). The prefix "*epi*" of Greek origin means "over, outside of, or around". Therefore, the epigenetics refers to the study of phenomena "in addition to" genetics (Waddington, 1968). Examples of epigenetics include DNA methylation, post-translational protein modification of histones around which DNA is wrapped, and large scale differences in genome structure. Epigenetic functions are of fundamental importance during literally all biological processes (Goldberg et al., 2007), and an explosion of research efforts in past decades explored these important non-genetic functions.

The concept of epigenetics has evolved gradually from a general definition to a category of molecular mechanisms controlling the "in addition to genetic" phenomena. It was first defined broadly as "the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being" (Waddington, 1942). Later, Holliday defined epigenetics as "the study of the mechanisms of temporal and spatial control of gene activity during the development of complex organisms" (Holliday, 1990), Russo et al. defined epigenetics as "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" (Russo and Riggs, 1996). Bird defined epigenetics as "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" (Bird, 2007). Broadly, epigenetics bridges gaps between genotype and phenotype and provides a conceptual explanation for why the same genotype can result in various stable and heritable phenotypes (Wu and Morris, 2001). In particular, epigenetics constitutes the molecular events controlling gene expression and activity without changes of DNA sequence. These molecular events include covalent and noncovalent modifications of DNA and histones that shape/reshape the chromatin structure according to environmental cues (Allis and Reinberg, 2006). Therefore, the study of chemical reactions shaping chromatin accessibility, regulating output of genetic information in terms of expression, and the signals from the environment that coordinate these chemical reactions represents the fundamental aspects of epigenetic research.

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REVIEW ARTICLE OPEN

Macrophages in age-related chronic inflammatory diseases

Yumiko Oishi[1] and Ichiro Manabe[2]

Chronic inflammation is the common pathological basis for such age-associated diseases as cardiovascular disease, diabetes, cancer and Alzheimer's disease. A multitude of bodily changes occur with aging that contribute to the initiation and development of inflammation. In particular, the immune system of elderly individuals often exhibits diminished efficiency and fidelity, termed immunosenescence. But, although immune responses to new pathogens and vaccines are impaired, immunosenescence is also characterized by a basal systemic inflammatory state. This alteration in immune system function likely promotes chronic inflammation. Changes in the tissue microenvironment, such as the accumulation of cell debris, and systemic changes in metabolic and hormonal signals, also likely contribute to the development of chronic inflammation. Monocyte/macrophage lineage cells are crucial to these age-associated changes, which culminate in the development of chronic inflammatory diseases. In this review, we will summarize the diverse physiological and pathological roles of macrophages in the chronic inflammation underlying age-associated diseases.

npj Aging and Mechanisms of Disease (2016) **2,** 16018; doi:10.1038/npjamd.2016.18; published online 28 July 2016

INTRODUCTION

With advancing age, the immune system undergoes a dynamic change characterized by the coexistence of a smaller immune response to newly encountered pathogens or vaccine antigens, and an elevated systemic inflammatory state made manifest, for example, by elevated levels of proinflammatory cytokines, clotting factors and acute phase reactants.[1] This chronic activation of inflammation associated with aging has been termed inflammaging, and recent studies indicate it is involved in the development of such non-communicable diseases (NCDs) as cardiovascular and metabolic disease and cancer in the elderly. Although the chronic inflammation associated with NCDs does not necessarily lead by age-associated changes in the body, the observation that the prevalence of many NCDs increases with advancing age suggests a pathogenic link between inflammaging and age-associated diseases.

Chronic inflammation is a prolonged condition in which tissue injury and attempts at repair coexist, leading to tissue remodeling and dysfunction.[2] Although chronic inflammation may follow acute inflammation, in the most common NCDs of today it likely begins insidiously as a low-grade, smoldering response with no manifestation of the cardinal signs of inflammation (Dolor (pain), Calor (heat), Rubor (redness) and Tumor (swelling)). However, even low-grade inflammation may impair tissue function (Functio laesa). For instance, inflammatory signals interfere with insulin signaling.[3] Moreover, the continuous progression of tissue injury and repair promotes tissue remodeling (e.g., extensive fibrosis) that may eventually cause irreversible tissue dysfunction.[4] In fact, the severity of tissue remodeling determines the prognosis of some NCDs, such as heart failure and chronic kidney disease.[5]

A complex interplay between parenchymal cells within a tissue and the various cells in the stroma, including immune cells, vascular cells and fibroblasts, lead the processes of chronic inflammation under the influence of inputs from both the local microenvironment and the wider system. Of particular interest are monocyte–macrophage lineage cells, which act as major effector cells in chronic inflammatory processes during the pathological development of NCDs.[4] In this review, we will summarize the pathological connection between chronic inflammation and age-associated diseases, with a particular focus on what is currently known about the roles played by macrophages.

IMMUNOSENESCENCE AND AGE-ASSOCIATED DISEASES

It is often noted that elderly individuals are more vulnerable to infectious diseases. For instance, occult infection with tuberculosis and varicella zoster virus becomes evident, sometimes leading to life-threatening disease. Moreover, vaccines are often ineffective in older adults owing to inability of the adaptive immune system to generate protective immunity. Overall, these changes in the immune system, characterized by declining fidelity and efficiency, are termed immunosenescence. A key feature of immunosenescence is an imbalance between inflammatory and anti-inflammatory networks, leading to a complex presentation of impaired adaptive immune responses with concomitant persistent low-grade inflammation and a greater susceptibility to autoimmune responses.[6] The changes in the adaptive immune system are characterized by a decreases in naive T- and B cells, an increase in memory cells and a progressive reduction in the T-cell receptor (TCR) and B-cell receptor (BCR) repertoire.[7–9] By contrast, generation of myeloid cells is favored in aging animals.

Immunosenescence involves not only age-related changes intrinsic to immune cells, but also microenvironmental and systemic alterations. Although it is likely that cellular senescence is involved in some of these age-related alterations, many are likely induced independently of cellular senescence pathways.

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"Chronic inflammation is the common pathological basis for such age-associated diseases as cardiovascular disease, diabetes, cancer and Alzheimer's disease."

"Monocyte/macrophage lineage cells are crucial to these age-associated changes, which culminate in the development of chronic inflammatory diseases. In this review, **we will summarize the diverse physiological and pathological roles of macrophages in the chronic inflammation underlying age associated diseases."**

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Cells of myeloid lineage including monocytes, macrophages and dendritic cells play an important role in the initial infection and therefore contribute to its pathogenesis throughout the course of infection. This is mainly because these cells are critical immune cells responsible for a wide range of both innate and adaptative immune functions.

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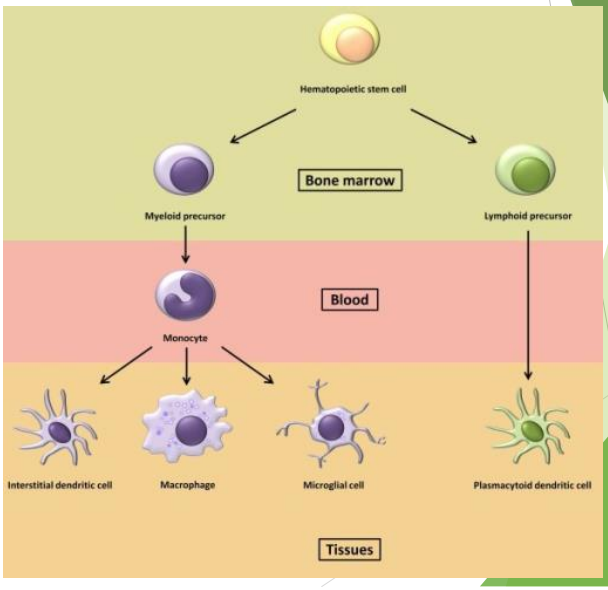

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Cell Host & Microbe
Short Article

Gut Dysbiosis Promotes M2 Macrophage Polarization and Allergic Airway Inflammation via Fungi-Induced PGE_2

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SUMMARY

Although imbalances in gut microbiota composition, or "dysbiosis," are associated with many diseases, the effects of gut dysbiosis on host systemic physiology are less well characterized. We report that gut dysbiosis induced by antibiotic (Abx) treatment promotes allergic airway inflammation by shifting macrophage polarization in the lung toward the alternatively activated M2 phenotype. Adoptive transfer of alveolar macrophages derived from Abx-treated mice was sufficient to increase allergic airway inflammation. Abx treatment resulted in the overgrowth of a commensal fungal *Candida* species in the gut and increased plasma concentrations of prostaglandin E_2 (PGE_2), which induced M2 macrophage polarization in the lung. Suppression of PGE2 synthesis by the cyclooxygenase inhibitors aspirin and celecoxib suppressed M2 macrophage polarization and decreased allergic airway inflammatory cell infiltration in Abx-treated mice. Thus, Abx treatment can cause overgrowth of particular fungal species in the gut and promote M2 macrophage activation at distant sites to influence systemic responses including allergic inflammation.

INTRODUCTION

Imbalances in gut microbiota composition, described as "dysbiosis," are caused by many factors, including host genetics, lifestyle, and exposure to microorganisms or various medical procedures (Round and Mazmanian, 2009). Dysbiosis has been associated not only with intestinal inflammation (Elinav et al., 2011; Mazmanian et al., 2008) but also with many diseases outside the gut, such as atopic dermatitis, allergy, obesity, and diabetes (Arumugam et al., 2011; Henao-Mejia et al., 2012; Penders et al., 2007; Vijay-Kumar et al., 2010). However, how the gut dysbiosis influences host immunity outside the gastrointestinal tract is largely unknown.

Several examples of the systemic influence of the commensal bacteria on peripheral immune responses have recently been provided. Peptidoglycan from orally inoculated *E. coli*-enhanced killing of *Streptococcus pneumoniae* and *Staphylococcus aureus* by bone-marrow-derived neutrophils in a Nod1-dependent manner (Clarke et al., 2010). Short-chain fatty acids (SCFAs), which were produced by fermentable dietary fiber induced by commensal bacteria, protect against the development of inflammatory diseases including colitis, arthritis, and allergy (Maslowski et al., 2009). However, the vast majority of these studies on interplay between commensal microbiota and systemic immune responses have focused on gut bacteria but not other microbes such as fungi or viruses.

Although more than 99% of microbiota consists of bacteria, fungi, most of which are *Candida* species, are also detectable in gastrointestinal sections of about 70% of healthy human adults (Cohen et al., 1969). Dysbiosis can result from a loss of beneficial commensal bacteria and an overgrowth of fungi (Giuliano et al., 1987; Samonis et al., 1990). *Candida* infection can induce production of inflammatory mediators by host cells. *Candida* also produces ligands for pattern recognition receptors (PRRs), including β-glucans, chitin, mannans, β-(1,2)-linked oligomannosides, and fungal nucleic acids, which stimulate innate immune responses. In addition, *Candida* produces proinflammatory substances such as alcohol (Santelmann and Howard, 2005) and prostaglandin (PGE_2) (Noverr et al., 2001). Several studies have suggested that gut fungi can influence inflammatory disorders such as inflammatory bowel disease (Iliev et al., 2012; Ott et al., 2008) or allergic airway inflammation (Noverr et al., 2004). However, although the study of the fungal microbiota is a rapidly emerging field, the mechanisms by which gut dysbiosis-driven fungal overgrowth in the gut affects host immune responses remain poorly understood.

Here we provide the evidence that *Candida* overgrowth promotes M2 macrophage polarization via PGE_2, which plays a critical role in the increased allergic airway inflammatory cell infiltration.

"Although imbalances in gut microbiota composition, or "dysbiosis," are associated with many diseases, the effects of gut dysbiosis on host systemic physiology are less well characterized. We report that gut dysbiosis induced by antibiotic (Abx) treatment promotes allergic airway inflammation by shifting macrophage polarization in the lung toward the alternatively activated M2 phenotype."

"Thus, Abx treatment can cause overgrowth of particular fungal species in the gut and promote M2 macrophage activation at distant sites to influence systemic responses including allergic inflammation."

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Defining M1 and M2 Macrophages

This classification is based upon macrophage polarization rather than macrophage location.

M1 macrophages are classically activated, typically by IFN-γ or lipopolysaccharide (LPS), and produce proinflammatory cytokines, phagocytize microbes, and initiate an immune response. M1 macrophages produce nitric oxide (NO) or reactive oxygen intermediates (ROI) to protect against bacteria and viruses.

M2 macrophages are alternatively activated by exposure to certain cytokines such as IL-4, IL-10, or IL-13. M2 macrophages will produce either polyamines to induce proliferation or proline to induce collagen production. These macrophages are associated with wound healing and tissue repair.

There are three types of M2 macrophages: M2a, M2b, and M2c

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Mini-review

Metabolic changes in tumor cells and tumor-associated macrophages: A mutual relationship

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ABSTRACT

In order to adapt to the reduced availability of nutrients and oxygen in the tumor microenvironment and the increased requirements of energy and building blocks necessary for maintaining their high proliferation rate, malignant cells undergo metabolic changes that result in an increased production of lactate, nitric oxide, reactive oxygen species, prostaglandins and other byproducts of arachidonic acid metabolism that influence both the composition of the inflammatory microenvironment and the function of the tumor-associated macrophages (TAMs). In response to cues present in the TME, among which products of altered tumor cell metabolism, TAMs are also required to reprogram their metabolism, with activation of glycolysis, fatty acid synthesis and altered nitrogen cycle metabolism. These changes result in functional reprogramming of TAMs which includes changes in the production of cytokines and angiogenetic factors, and contribute to the tumor progression and metastasis. Understanding the metabolic changes governing the intricate relationship between the tumor cells and the TAMs represents an essential step towards developing novel therapeutic approaches targeting the metabolic reprogramming of the immune cells to potentiate their tumoricidal potential and to circumvent therapy resistance.

Introduction

Tumor microenvironment (TME) consists of a complex mixture of malignantly transformed cells, immune cells, and stromal cells which fulfill different functions. Some of these cells, including the dendritic cells (DC), $CD8^+$ and $CD4^+$ T-lymphocytes, natural killer (NK) cells, are activated in order to contain the tumor and prevent immune evasion and progression of the disease. Other immune cells such as tolerogenic DC's, regulatory T-cells (Treg), myeloid derived suppressor cells (MDSC), tumor-associated neutrophils and tumor associated macrophages (TAM), on the other hand, promote tumor growth, progression, invasion, angiogenesis and suppress the antitumoral immune responses [1].

During the last decades, it has become clear that in many tumors a proinflammatory TME promotes cancer development, progression and metastasis [2–5]. Moreover, the composition of the

Abbreviations: TME, tumor microenvironment; DC, dendritic cells; NK, natural killer; Treg, regulatory T-cells; MDSC, myeloid derived suppressor cells; TAM, tumor associated macrophages; RNS, reactive nitrogen species; OXPHOS, oxidative phosphorylation; ATP, adenosine triphosphate; NAD, nicotinamide adenine dinucleotide; NADH, reduced form of nicotinamide adenine dinucleotide; GLUT1, glucose transporter 1; MCT1, monocarboxylic acid transporter 1; MCT4, monocarboxylic acid transporter 4; CAIX, carbonic anhydrase IX; VEGF, vascular endothelial growth factor; PPP, pentose phosphate pathway; FA, fatty acid; TCA, tricarboxylic acid; acetyl CoA, acetyl coenzyme A; $FADH_2$, flavin adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; NO, nitric oxide; HETE, hydroxieicosyticotetraenoic acid; COX, cyclooxygenase; LO, lipoxygenase; PPARs, peroxisome proliferator-activated receptors; SREBP1, sterol regulatory element binding protein 1; TLR4, toll like receptor 4; PGE2, prostaglandin E2; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin; FAO, fatty acid oxidation; PDHA, pyruvate dehydrogenase kinase 4; IFN, interferon; HK2, hexokinase-2; PI3K, phosphatidyl-inositol-3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; PDCA, pancreatic ductal adenocarcinoma; ETM, epithelial-to-mesenchymal; 2DG, 2-deoxiglucose; REDD1, regulated in development and in DNA damage response 1; E-FABP, epithelial fatty acid binding proteins; iNOS, inducible nitric oxide synthase; ARG1, arginase; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; F1,6P, fructose-1,6-diphosphate; G3P, Glucose-3-phosphate; TG, Triglycerides; αKG, α-ketoglutarate; Gln, Glutamine.

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"In order to adapt to the reduced availability of nutrients and oxygen in the tumor microenvironment and the increased requirements of energy and building blocks necessary for maintaining their high proliferation rate, malignant cells undergo metabolic changes that result in an increased production of lactate, nitric oxide, reactive oxygen species, prostaglandins and other byproducts of arachidonic acid metabolism that influence both the composition of the inflammatory microenvironment and the function of the tumor-associated macrophages (TAMs)."

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Cancer Cell
Review

Macrophage Regulation of Tumor Responses to Anticancer Therapies

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Tumor-associated macrophages (TAMs) promote key processes in tumor progression, like angiogenesis, immunosuppression, invasion, and metastasis. Increasing studies have also shown that TAMs can either enhance or antagonize the antitumor efficacy of cytotoxic chemotherapy, cancer-cell targeting antibodies, and immunotherapeutic agents—depending on the type of treatment and tumor model. TAMs also drive reparative mechanisms in tumors after vascular-targeting agents. Here, we discuss the biological significance and clinical implications of these findings, with an emphasis on novel approaches that effectively target TAMs to increase the efficacy of such therapies.

Introduction

Macrophages phagocytose microbes and present antigens to T cells, therefore constituting a first line of defense against invading pathogens. They also regulate tissue growth, homeostasis, repair, and remodeling via their expression of numerous cytokines, chemokines, growth factors, proteolytic enzymes, and scavenger receptors (Gordon and Martinez, 2010; Murray and Wynn, 2011). As such, macrophages play a central role in developmental processes, such as tissue morphogenesis and vascular and neuronal patterning, but also in pathophysiological responses, like inflammation and organ healing/regeneration (Mantovani et al., 2013; Nucera et al., 2011; Pollard, 2009).

In selected organs of the adult mouse, the origin of tissue macrophages can be traced back to fetal macrophages that appear before the onset of definitive hematopoiesis (Schulz et al., 2012). In inflamed and remodeling tissues, elevated macrophage turnover is sustained largely from hematopoietic progenitor cells (HPCs), which proliferate and differentiate into promonocytes in the bone marrow (BM) before they are shed into the circulation as monocytes. These then undergo final differentiation into macrophages as they extravasate in the target tissues (Shi and Pamer, 2011). During inflammation and tumor growth, BM-derived HPCs may also accumulate at extramedullary sites, such as the spleen, which can become an important site of monocyte production (Cortez-Retamozo et al., 2012).

Once resident in tissues, macrophages acquire a distinct, tissue-specific phenotype in response to signals present within individual microenvironments. The exact combination of such tissue-specific cues dictates both the differentiation and activation status of these cells. Two extreme forms of the latter are generally referred to as "classical" (or M1) and "alternative" (or M2) activation, which parallel Th1/Th2 programming of adaptive immune cells (Biswas and Mantovani, 2010; Mantovani et al., 2002). During acute inflammation, macrophages are M1-activated by toll-like receptor (TLR) agonists and Th1 cytokines (e.g., interferon [IFN]-γ). This enhances their ability to kill and phagocytose pathogens, upregulate proinflammatory cytokines (e.g., interleukin [IL]-1β, IL-12, and tumor necrosis factor-α [TNF-α]) and reactive molecular species, and present antigens via major histocompatibility complex (MHC) class II molecules (Biswas and Mantovani, 2010; Mantovani et al., 2002). Alternatively, Th2 cytokines, like IL-4 and 13, stimulate monocytes/macrophages to express an M2 activation state. This is characterized by higher production of the anti-inflammatory cytokine, IL-10; lower expression of proinflammatory cytokines; amplification of metabolic pathways that can suppress adaptive immune responses; and the upregulation of cell-surface scavenger receptors, such as mannose receptor (MRC1/CD206) and hemoglobin/aptoglobin scavenger receptor (CD163). As such, M2 macrophage activation may facilitate the resolution of inflammation and promote tissue repair (including angiogenesis) after the acute inflammatory phase (Biswas and Mantovani, 2010; Gordon and Martinez, 2010). In healthy tissues, macrophages often express a mixed M1/M2 phenotype; hence "M1" and "M2" polarization should be regarded as extreme ends of a continuum of activation states, with their exact point on the scale depending on the precise mix of local signals present in a given microenvironment (Biswas and Mantovani, 2010; Lawrence and Natoli, 2011; Sica and Mantovani, 2012).

Tumor-Associated Macrophages

Macrophages are a major cellular component of murine and human tumors, where they are commonly termed tumor-associated macrophages (TAMs). In this article, we specifically review the role of these cells and their monocyte precursors in tumor responses to anticancer therapies. Other tumor-infiltrating myeloid cells not discussed here include neutrophils, eosinophils, and activated dendritic cells (DCs) (de Visser et al., 2006). Tumors also recruit a variety of immature myeloid cells, often referred to as myeloid-derived suppressor cells (MDSCs), which comprise precursors of both the monocyte-DC (mononuclear) and neutrophil (granulocytic) lineages and are commonly identified by their expression of Gr1 (Ly6C/G) and immunosuppressive activity. Mononuclear MDSCs can further mature into TAMs (Coffelt et al., 2010; Gabrilovich et al., 2012). Finally,

"Tumor-associated macrophages (TAMs) promote key processes in tumor progression, like angiogenesis, immunosuppression, invasion, and metastasis. Increasing studies have also shown that TAMs can either enhance or antagonize the antitumor efficacy of cytotoxic chemotherapy, cancer-cell targeting antibodies, and immunotherapeutic agents—depending on the type of treatment and tumor model. TAMs also drive reparative mechanisms in tumors after radiotherapy or treatment with vascular-targeting agents. Here, we discuss the biological significance and clinical implications of these findings, with an emphasis on novel approaches that effectively target TAMs to increase the efficacy of such therapies."

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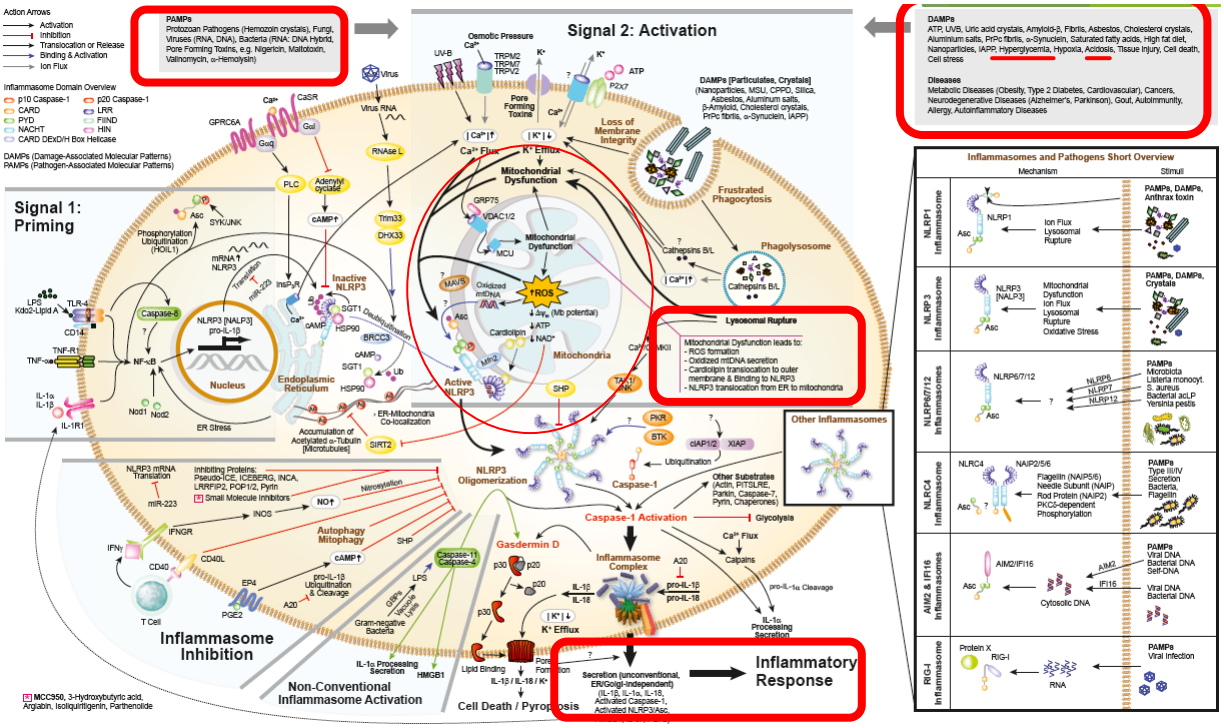

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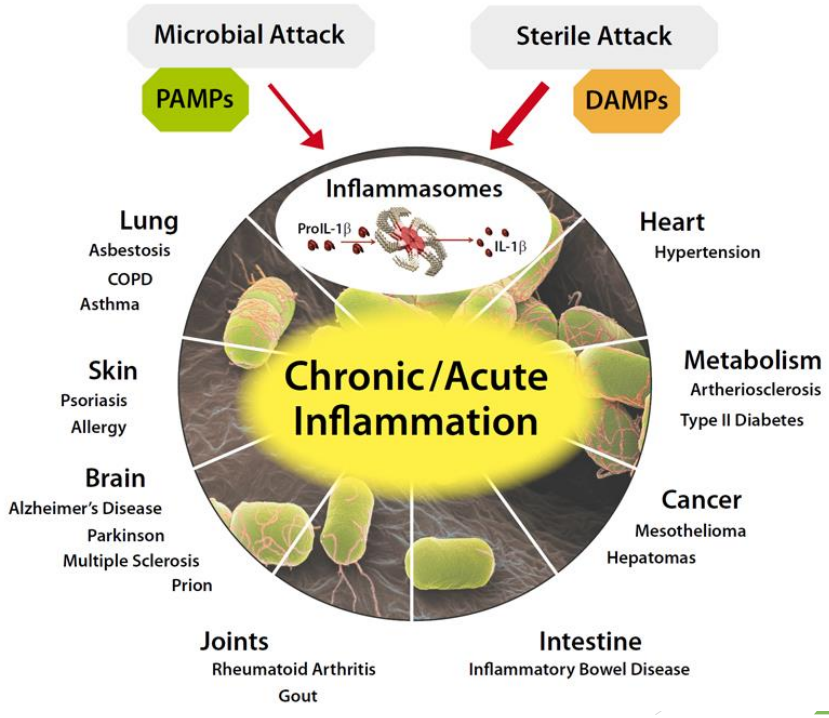

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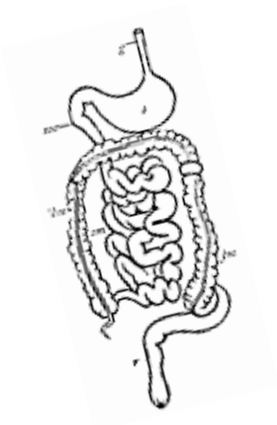
Do You Have The 'Guts' To Be Healthy???



"Death begins in the colon."

Hippocrates

http://www.youtube.com/watch?v=gnZEge78_78&feature=player_embedded#

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Cell Host & Microbe
Perspective

Defining a Healthy Human Gut Microbiome: Current Concepts, Future Directions, and Clinical Applications

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Indigenous microbiota are an essential component in the modern concept of human health, but the composition and functional characteristics of a healthy microbiome remain to be precisely defined. Patterns of microbial colonization associated with disease states have been documented, but the health-associated microbial patterns and their functional characteristics are less clear. A healthy microbiome, considered in the context of body habitat or body site, could be described in terms of ecologic stability (i.e., ability to resist community structure change under stress or to rapidly return to baseline following a stress-related change), by an idealized (presumably health-associated) composition or by a desirable functional profile (including metabolic and trophic provisions to the host). Elucidation of the properties of healthy microbiota would provide a target for dietary interventions and/or microbial modifications aimed at sustaining health in generally healthy populations and improving the health of individuals exhibiting disrupted microbiota and associated diseases.

Introduction

The nature of microbial colonization of humans is being increasingly understood through regional microbiome projects that are linked as a single global network, such as the International Human Microbiome Consortium, the European Commission's Metagenomics of the Human Intestinal Tract project, the US National Institutes of Health's Human Microbiome Project, and the Canadian Microbiome Initiative, among others. These projects are focused on the identity, genetic potential, and metabolic activities of microbes (bacteria, viruses, archaea, and eukaryotes) associated with numerous body sites. Although there is agreement that microbes are important to human health, with the exception of defined pathogens, the roles that these microbes play in health and disease remain to be fully elucidated. Different patterns of microbial colonization associated with disease states compared to healthy controls have been documented (Table 1), although a causal relationship has not been established. However, the patterns of microbial colonization associated with health are more difficult to define. Definition of healthy microbiota would provide a target for interventions aimed at sustaining health in the generally healthy populations and improving the health status of people exhibiting disrupted microbiota and diseases associated with these disruptions. However, the definition of a healthy human microbiome is not yet defined.

This Perspective focuses on key concepts related to defining a healthy gut microbiome. Briefly, it addresses how a healthy microbiome is defined and the current evidence that relates the microbiome to human health. Many factors must be addressed before considering dietary or therapeutic interventions that target the microbiome. The microbial composition, metabolic activities (Figure 1A), host responses to microbes (Figure 1B) including genetic susceptibility to disease, and environmental factors (such as diet) that may influence host response may all impact the success of any intervention. A better understanding of factors that shape microbiome structure and function can result in defining specific microbial community properties that can be targeted through interventions with diet, functional foods, chemicals (drugs), or live organisms. Pathways to achieve this goal are discussed, including how the "health" of a gut microbiome might be assessed. Although the technical aspects of sampling and analysis are important considerations in defining a healthy microbiome, those topics are beyond the scope of this paper. The reader is referred to several excellent references regarding human sampling (Grice et al., 2009; Jalanka-Tuovinen et al., 2011; Mai et al., 2011; Saulnier et al., 2011) and analysis (Kuczynski et al., 2012).

Definition of a Healthy Microbiome

How is a healthy microbiome defined? From the ecologic standpoint, the stability of a community (bacterial or otherwise) can be thought of as a functional property descriptive of the health of that community. Stability refers to the ability of a community to

"A healthy microbiome, considered in the context of body habitat or body site, could be described in terms of ecologic stability (i.e., ability to resist community structure change under stress or to rapidly return to baseline following a stress-related change), by an idealized (presumably health-associated) composition or by a desirable functional profile (including metabolic and trophic provisions to the host). Elucidation of the properties of healthy microbiota would provide a target for dietary interventions and/or microbial modifications aimed at sustaining health in generally healthy populations and improving the health of individuals exhibiting disrupted microbiota and associated diseases."

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A large-scale survey of the postmortem human microbiome, and its potential to provide insight into the living health condition

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The microbiome plays many roles in human health, often through the exclusive lens of clinical interest. The inevitable end point for all living hosts, death, has its own altered microbiome configurations. However, little is understood about the ecology and changes of microbial communities after death, or their potential utility for understanding the health condition of the recently living. Here we reveal distinct postmortem microbiomes of human hosts from a large-scale survey of death cases representing a predominantly urban population, and demonstrated these microbiomes reflected antemortem health conditions within 24–48 hours of death. Our results characterized microbial community structure and predicted function from 188 cases representing a cross-section of an industrial-urban population. We found strong niche differentiation of anatomic habitat and microbial community turnover based on topographical distribution. Microbial community stability was documented up to two days after death. Additionally, we observed a positive relationship between cell motility and time since host death. Interestingly, we discovered evidence that microbial biodiversity is a predictor of antemortem host health condition (e.g., heart disease). These findings improve the understanding of postmortem host microbiota dynamics, and provide a robust dataset to test the postmortem microbiome as a tool for assessing health conditions in living populations.

The human body is a host for a network of microorganisms in constant flux for each of the estimated 7.5 billion people on Earth. The composition and role of the human microbiome has been extensively studied to evaluate human health[1–3]. The microbiota of a living host is highly influenced by their environment, presence or absence of disease, development, illicit or prescribed chemical substances, and nutrition[2–5]. These elements are responsible for microbial community heterogeneity within and across host populations over time[6–8]. Yet, there is a limited understanding of the human microbiome after death. Decomposition is a complex biochemical process dominated by predictable patterns of decay caused by enzymatic reactions, and loss of the processes that help maintain cellular integrity[9]. Previous work has documented a dynamic, stochastic community of microorganisms existing on a now primordial resource (e.g., no host immunity) that undergoes dispersal, local diversification, and competitive interactions after host death[10,11]. These definitions of human microbiota spatial and temporal shifts throughout decomposition, however, have resulted from work conducted at anthropological facilities[12,13,14]. These facilities adhere to stringent legal requirements for donor programs (e.g., no communicable diseases or antibiotic resistant infections), and some anthropological research facilities do not accept autopsied bodies. An additional bias is that those most likely to donate their bodies to medical sciences represent older sociodemographics groups (white, male, >65 years old)[15]. Hence, donor programs have encompassed a limited population. There is a critical need to test the robustness and applicability of previous work by expanding postmortem microbiome research to other demographics of our communities[16].

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"…we discovered evidence that microbial biodiversity is a predictor of antemortem host health condition (e.g., heart disease). These findings improve the understanding of postmortem host microbiota dynamics, and provide a robust dataset to test the postmortem microbiome as a tool for assessing health conditions in living populations."

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Research
Microecology—Review

The Human Microbiota in Health and Disease

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ABSTRACT

Trillions of microbes have evolved with and continue to live on and within human beings. A variety of environmental factors can affect intestinal microbial imbalance, which has a close relationship with human health and disease. Here, we focus on the interactions between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases. We also review important advances in techniques associated with microbial research, such as DNA sequencing, metabonomics, and proteomics combined with computation-based bioinformatics. Current research on the human microbiota has become much more sophisticated and more comprehensive. Therefore, we propose that research should focus on the host–microbe interaction and on cause-effect mechanisms, which could pave the way to an understanding of the role of gut microbiota in health and disease, and provide new therapeutic targets and treatment approaches in clinical practice.

1. Introduction

More than 100 trillion symbiotic microorganisms live on and within human beings and play an important role in human health and disease. The human microbiota, especially the gut microbiota, has even been considered to be an "essential organ" [1], carrying approximately 150 times more genes than are found in the entire human genome [2]. Important advances have shown that the gut microbiota is involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity [3–6]. Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota [7,8] (Fig.1).

In recent decades, a tremendous amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease [7,9–23] via several mechanisms. First, the microbiota has the potential to increase energy extraction from food [24], increase nutrient harvest [9,10], and alter appetite signaling [25,26]. The microbiota contains far more versatile metabolic genes than are found in the human genome, and provides humans with unique and specific enzymes and biochemical pathways [9]. In addition, a large proportion of the metabolic microbiotic processes that are beneficial to the host are involved in either nutrient acquisition or xenobiotic processing, including the metabolism of undigested carbohydrates and the biosynthesis of vitamins [10]. Second, the human microbiota also provides a physical barrier, protecting its host against foreign pathogens through competitive exclusion and the production of antimicrobial substances [11–13]. Finally, the microbiota is essential in the development of the intestinal mucosa and immune system of the host [14,16]. For example, germ-free (GF) animals have abnormal numbers of several immune cell types, deficits in local and systemic lymphoid structures, poorly formed spleens and lymph

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"Trillions of microbes have evolved with and continue to live on and within human beings. A variety of environmental factors can affect intestinal microbial imbalance, which has a close relationship with human health and disease. Here, we focus on the interactions between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases."

Therefore, we propose that research should focus on the host–microbe interaction and on cause-effect mechanisms, which could pave the way to an understanding of the role of gut microbiota in health and disease, and provide new therapeutic targets and treatment approaches in clinical practice.

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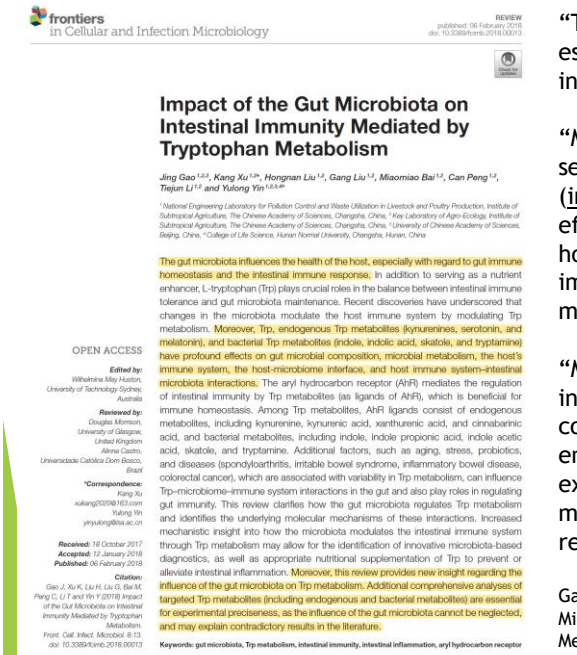

frontiers in Cellular and Infection Microbiology

REVIEW
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Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism

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The gut microbiota influences the health of the host, especially with regard to gut immune homeostasis and the intestinal immune response. In addition to serving as a nutrient enhancer, L-tryptophan (Trp) plays crucial roles in the balance between intestinal immune tolerance and gut microbiota maintenance. Recent discoveries have underscored that changes in the microbiota modulate the host immune system by modulating Trp metabolism. Moreover, Trp, endogenous Trp metabolites (kynurenines, serotonin, and melatonin), and bacterial Trp metabolites (indole, indolic acid, skatole, and tryptamine) have profound effects on gut microbial composition, microbial metabolism, the host's immune system, the host-microbiome interface, and host immune system–intestinal microbiota interactions. The aryl hydrocarbon receptor (AhR) mediates the regulation of intestinal immunity by Trp metabolites (as ligands of AhR), which is beneficial for immune homeostasis. Among Trp metabolites, AhR ligands consist of endogenous metabolites, including kynurenine, kynurenic acid, xanthurenic acid, and cinnabarinic acid, and bacterial metabolites, including indole, indole propionic acid, indole acetic acid, skatole, and tryptamine. Additional factors, such as aging, stress, probiotics, and diseases (spondyloarthritis, irritable bowel syndrome, inflammatory bowel disease, colorectal cancer), which are associated with variability in Trp metabolism, can influence Trp–microbiome–immune system interactions in the gut and also play roles in regulating gut immunity. This review clarifies how the gut microbiota regulates Trp metabolism and identifies the underlying molecular mechanisms of these interactions. Increased mechanistic insight into how the microbiota modulates the intestinal immune system through Trp metabolism may allow for the identification of innovative microbiota-based diagnostics, as well as appropriate nutritional supplementation of Trp to prevent or alleviate intestinal inflammation. Moreover, this review provides new insight regarding the influence of the gut microbiota on Trp metabolism. Additional comprehensive analyses of targeted Trp metabolites (including endogenous and bacterial metabolites) are essential for experimental preciseness, as the influence of the gut microbiota cannot be neglected, and may explain contradictory results in the literature.

Keywords: gut microbiota, Trp metabolism, intestinal immunity, intestinal inflammation, aryl hydrocarbon receptor

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Gao J, Xu K, Liu H, Liu G, Bai M, Peng C, Li T and Yin Y (2018) Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. Front. Cell. Infect. Microbiol. 8:13. doi: 10.3389/fcimb.2018.00013

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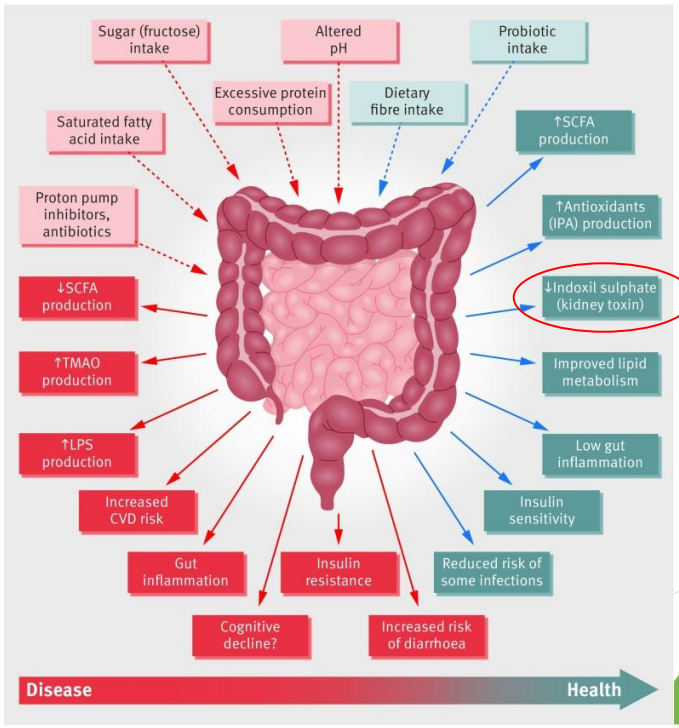

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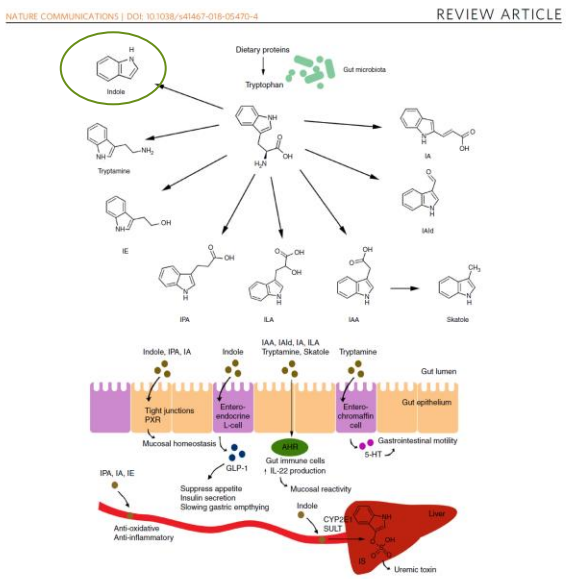

Fig. 1 Mechanisms of action of microbial tryptophan catabolites on host physiology. Degradation of dietary proteins leads to the release of tryptophan, which is converted into various catabolites by the gut microbiota. The tryptophan catabolites include indole, tryptamine, indoleethanol (IE), indolepropionic acid (IPA), indolelactic acid (ILA), indoleacetic acid (IAA), skatole, indolealdehyde (IAId) and indoleacrylic acid (IA) and may affect host physiology in numerous ways. Indole, IPA and IA affect mucosal homeostasis by decreased intestinal permeability possibly mediated by the pregnane X receptor (PXR). Indole induces the release of glucagon-like peptide 1 (GLP-1) in enteroendocrine L-cells. GLP-1 is known to suppress appetite, insulin secretion and to slow gastric emptying. Several tryptophan catabolites act on the aryl hydrocarbon receptor (AHR) found in intestinal immune cells and thereby alter innate and adaptive immune responses in a ligand-specific fashion (e.g. IAId induces increased production of interleukin-22 (IL-22) via AHR activation). Tryptamine induces the release of 5-hydroxytryptamine (5-HT, serotonin) by enterochromaffin cells. 5-HT stimulates gastrointestinal motility by acting on enteric nervous system neurons. Tryptophan catabolites are absorbed through the intestinal epithelium and enter the bloodstream where some (e.g. IPA, IE, IA) have anti-oxidative and anti-inflammatory effects, whereas indoxyl-sulfate (IS), which is produced in the liver from indole by the actions of CYP2E1 and sulfotransferases (SULT), has cytotoxic effects in high concentrations

"Tryptophan catabolites thus affect various physiological processes and may contribute to intestinal and systemic homeostasis in health and disease."

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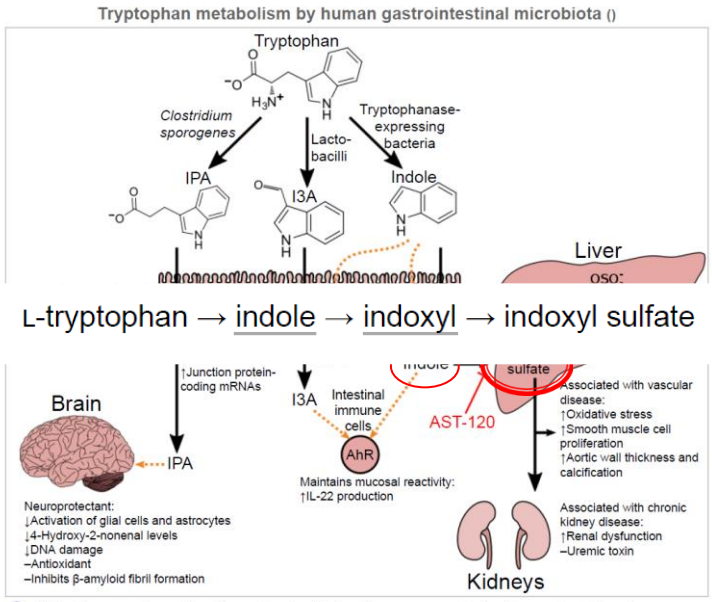

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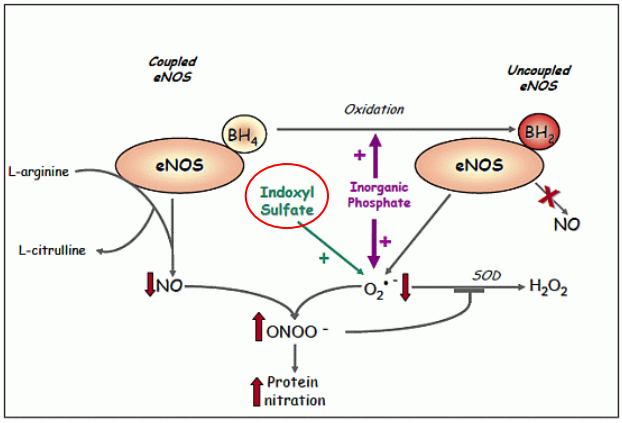

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NO + OO^- → $OONO^-$

Nitric oxide

Super oxide

ROS

Big
Ugly
Loner
Losers
Yearning attention

+

O2

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Cell Reports
Article

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Host Genotype and Gut Microbiome Modulate Insulin Secretion and Diet-Induced Metabolic Phenotypes

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SUMMARY

Genetic variation drives phenotypic diversity and influences the predisposition to metabolic disease. Here, we characterize the metabolic phenotypes of eight genetically distinct inbred mouse strains in response to a high-fat/high-sucrose diet. We found significant variation in diabetes-related phenotypes and gut microbiota composition among the different mouse strains in response to the dietary challenge and identified taxa associated with these traits. Follow-up microbiota transplant experiments showed that altering the composition of the gut microbiota modifies strain-specific susceptibility to diet-induced metabolic disease. Animals harboring microbial communities with enhanced capacity for processing dietary sugars and for generating hydrophobic bile acids showed increased susceptibility to metabolic disease. Notably, differences in glucose-stimulated insulin secretion between different mouse strains were partially recapitulated via gut microbiota transfer. Our results suggest that the gut microbiome contributes to the genetic and phenotypic diversity observed among mouse strains and provide a link between the gut microbiome and insulin secretion.

INTRODUCTION

The intestinal microbiota exerts a profound influence on development, physiology, and health (Clemente et al., 2012; Sommer and Bäckhed, 2013; Tremaroli and Bäckhed, 2012). Although there is substantial interpersonal variation in the composition of the gut microbiota among uninlated healthy subjects, sequencing studies have revealed distal gut community patterns associated with different pathological states, including obesity and diabetes (Ridaura et al., 2013; Qin et al., 2012; Karlsson et al., 2013). Remarkably, alterations in the intestinal microbiota composition have been shown to modulate insulin sensitivity (Vrieze et al., 2010), a key feature in metabolic disease and type 2 diabetes (T2D), and thus play a role in diabetes susceptibility.

Dietary components that are not efficiently absorbed in the proximal intestine reach the distal gut, where they are metabolized by gut microbes. Intestinal microbes impact our health in part by generating numerous metabolites from our diet. Short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, are produced through bacterial fermentation of dietary carbohydrates. SCFAs serve as energy and signaling molecules in the intestine and peripheral organs (den Besten et al., 2013). Specifically, SCFAs are important regulators of both energy and glucose homeostasis (den Besten et al., 2013; Koh et al., 2016). For example, butyrate improves insulin sensitivity (Gao et al., 2009; Hartstra et al., 2015) and T2D patients have reduced levels of butyrate-producing bacteria (Qin et al., 2012). Additionally, acetate modulates insulin secretion from β cells (Priyadarshini et al., 2015; Perry et al., 2016). While primarily associated with metabolic benefits, increased concentrations of butyrate and acetate have been found in the cecum of obese mice, suggesting an increased ability of the microbiome to harvest energy from the diet (Turnbaugh et al., 2006).

Gut microbes also impact host physiology by modifying bile acids (BAs) synthesized by the host (Houten et al., 2006; Kuipers et al., 2014; Ryan et al., 2014; Sayin et al., 2013). In addition to their role in emulsifying lipids, BAs function as hormones through their ability to activate nuclear hormone receptors (Parks et al., 1999) and G-coupled protein receptors (Kawamata et al., 2003). They modulate glucose homeostasis, lipid metabolism, energy expenditure, and intestinal motility (Kuipers et al., 2014). Primary BAs are synthesized from cholesterol in the liver (Russell, 2009), stored in the gallbladder, and secreted into the duodenum upon ingestion of a meal. The gut microbiota catalyzes the production of secondary BAs via deconjugation, dehydrogenation, epimerization, and dehydroxylation of primary BAs

Our results suggest that the gut microbiome contributes to the genetic and phenotypic diversity observed among mouse strains and provide a link between the gut microbiome and insulin secretion.

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What about the Gut-Brain Connection?

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"With leaky gut, damaged cells in your intestines don't produce the enzymes needed for proper digestion. As a result, your body cannot absorb essential nutrients, which can lead to hormone imbalances and a weakened immune system."

USDA, 2016 Flegal KM, Carroll MD, Kit BK, Ogden CL. *JAMA*, 2012 Feb 1;307(5):491-7 Mayo Clinic proceedings, 2012

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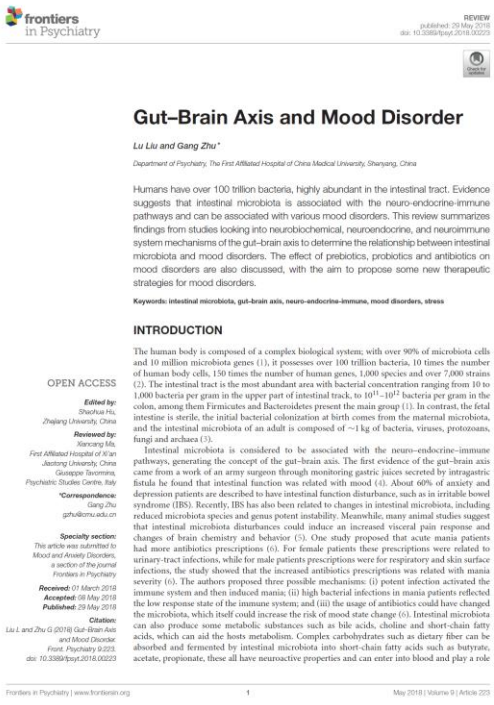

frontiers in Psychiatry

REVIEW

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Gut–Brain Axis and Mood Disorder

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Humans have over 100 trillion bacteria, highly abundant in the intestinal tract. Evidence suggests that intestinal microbiota is associated with the neuro-endocrine-immune pathways and can be associated with various mood disorders. This review summarizes findings from studies looking into neurobiochemical, neuroendocrine, and neuroimmune system mechanisms of the gut–brain axis to determine the relationship between intestinal microbiota and mood disorders. The effect of prebiotics, probiotics and antibiotics on mood disorders are also discussed, with the aim to propose some new therapeutic strategies for mood disorders.

Keywords: intestinal microbiota, gut-brain axis, neuro-endocrine-immune, mood disorders, stress

INTRODUCTION

The human body is composed of a complex biological system; with over 90% of microbiota cells and 10 million microbiota genes (1), it possesses over 100 trillion bacteria, 10 times the number of human body cells, 150 times the number of human genes, 1,000 species and over 7,000 strains (2). The intestinal tract is the most abundant area with bacterial concentration ranging from 10 to 1,000 bacteria per gram in the upper part of intestinal track, to 10^{11}–10^{12} bacteria per gram in the colon, among them Firmicutes and Bacteroidetes present the main group (1). In contrast, the fetal intestine is sterile, the initial bacterial colonization at birth comes from the maternal microbiota, and the intestinal microbiota of an adult is composed of ~1 kg of bacteria, viruses, protozoans, fungi and archaea (3).

Intestinal microbiota is considered to be associated with the neuro–endocrine–immune pathways, generating the concept of the gut–brain axis. The first evidence of the gut–brain axis came from a work of an army surgeon through monitoring gastric juices secreted by intragastric fistula he found that intestinal function was related with mood (4). About 60% of anxiety and depression patients are described to have intestinal function disturbance, such as in irritable bowel syndrome (IBS). Recently, IBS has also been related to changes in intestinal microbiota, including reduced microbiota species and genus potent instability. Meanwhile, many animal studies suggest that intestinal microbiota disturbances could induce an increased visceral pain response and changes of brain chemistry and behavior (5). One study proposed that acute mania patients had more antibiotics prescriptions (6). For female patients these prescriptions were related to urinary-tract infections, while for male patients prescriptions were for respiratory and skin surface infections, the study showed that the increased antibiotics prescriptions was related with mania severity (6). The authors proposed three possible mechanisms: (i) potent infection activated the immune system and then induced mania; (ii) high bacterial infections in mania patients reflected the low response state of the immune system; and (iii) the usage of antibiotics could have changed the microbiota, which itself could increase the risk of mood state change (6). Intestinal microbiota can also produce some metabolic substances such as bile acids, choline and short-chain fatty acids, which can aid the hosts metabolism. Complex carbohydrates such as dietary fiber can be absorbed and fermented by intestinal microbiota into short-chain fatty acids such as butyrate, acetate, propionate, these all have neuroactive properties and can enter into blood and play a role

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"Humans have over 100 trillion bacteria, highly abundant in the intestinal tract. Evidence suggests that intestinal microbiota is associated with the neuro-endocrine-immune pathways and can be associated with various mood disorders. This review summarizes findings from studies looking into neurobiochemical, neuroendocrine, and neuroimmune System mechanisms of the gut-brain axis to determine the relationship between intestinal microbiota and mood disorders. The effect of prebiotics, probiotics and antibiotics on mood disorders are also discussed, with the aim to propose some new therapeutic strategies for mood disorders."

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Prospects & Overviews

Review essays

Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms

Joe Alcock[1], Carlo C. Maley[2][3][4]* and C. Athena Aktipis[2][3][4][5]

Microbes in the gastrointestinal tract are under selective pressure to manipulate host eating behavior to increase their fitness, sometimes at the expense of host fitness. Microbes may do this through two potential strategies: (i) generating cravings for foods that they specialize on or foods that suppress their competitors, or (ii) inducing dysphoria until we eat foods that enhance their fitness. We review several potential mechanisms for microbial control over eating behavior including microbial influence on reward and satiety pathways, production of toxins that alter mood, changes to receptors including taste receptors, and hijacking of the vagus nerve, the neural axis between the gut and the brain. We also review the evidence for alternative explanations for cravings and unhealthy eating behavior. Because microbiota are easily manipulatable by prebiotics, probiotics, antibiotics, fecal transplants, and dietary changes, altering our microbiota offers a tractable approach to otherwise intractable problems of obesity and unhealthy eating.

Keywords:
cravings; evolutionary conflict; host manipulation; microbiome; microbiota; obesity

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Introduction: Evolutionary conflict between host and microbes leads to host manipulation

The struggle to resist cravings for foods that are high in sugar and fat is part of daily life for many people. Unhealthy eating is a major contributor to health problems including obesity [1] as well as sleep apnea, diabetes, heart disease, and cancer [2–4]. Despite negative effects on health and survival, unhealthy eating patterns are often difficult to change. The resistance to change is frequently framed as a matter of "self-control," and it has been suggested that multiple "selves" or cognitive modules exist [5] each vying for control over our eating behavior. Here, we suggest another possibility: that evolutionary conflict between host and microbes in the gut leads microbes to divergent interests over host eating behavior. Gut microbes may manipulate host eating behavior in ways that promote their fitness at the expense of host fitness. Others have hypothesized that microbes may be affecting our eating behavior [6–8], though not in the context of competing fitness interests and evolutionary conflict.

Conflict over resource acquisition and resource allocation can occur as a result of conflict between different genetic interests within an organism. For example, genetic conflict between maternal and paternal genes is hypothesized to play a role in the unusual eating behavior that characterizes the childhood genetic diseases Beckwith–Wiedemann syndrome and Prader–Willi syndrome. These syndromes are characterized by altered appetite and differences in infant suckling that can result from overexpression of genes of paternal or maternal origin, respectively [9, 10]. In parent-of-origin genetic conflict, paternally imprinted genes are thought to drive increased demands for extracting resources from the mother, and maternally imprinted genes tend to resist these effects. Metagenomic conflict between host and microbiome can be considered an extension of this genetic conflict framework, but one that includes other genomes (i.e., microbes in the gut) with genes that affect the physiology and behavior of a host organism, potentially altering host eating behavior in ways that benefit microbe fitness.

"Microbes in the gastrointestinal tract are under selective pressure to manipulate host eating behavior to increase their fitness, sometimes at the expense of host fitness. Microbes may do this through two potential strategies: (i) generating cravings for foods that they specialize on or foods that suppress their competitors, or (ii) inducing dysphoria until we eat foods that enhance their fitness."

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Review

Collective unconscious: How gut microbes shape human behavior

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Psychobiotics

ABSTRACT

The human gut harbors a dynamic and complex microbial ecosystem, consisting of approximately 1 kg of bacteria in the average adult, approximately the weight of the human brain. The evolutionary formation of a complex gut microbiota in mammals has played an important role in enabling brain development and perhaps sophisticated social interaction. Genes within the human gut microbiota, termed the microbiome, significantly outnumber human genes in the body, and are capable of producing a myriad of neuroactive compounds. Gut microbes are part of the unconscious system regulating behavior. Recent investigations indicate that these microbes majorly impact on cognitive function and fundamental behavior patterns, such as social interaction and stress management. In the absence of microbes, underlying neurochemistry is profoundly altered. Studies of gut microbes may play an important role in advancing understanding of disorders of cognitive functioning and social interaction, such as autism.

Humans live in a symbiotic relationship with gut microbes: we provide them with a constant source of nutrition, while in return they help us in a variety of ways including enabling optimal brain development and subsequent functioning (Geurts et al. 2013; Chen et al., 2013). Many human genes are homologs of bacterial genes, mainly derived by descent, but some by gene transfer from bacteria (McFall-Ngai et al., 2013). The word commensal is derived from the Latin term "cum mensa", which means "eating together". It has been postulated that in the absence of bacteria humans would not have developed the current level of cognitive performance (Montiel-Castro et al., 2013) We are fundamentally dependent on a myriad of essential neurochemicals produced by microbes. For example, the brain's serotonergic system, which plays a key role in emotional activity, does not develop appropriately in the absence of microbes (Clarke et al., 2013). Gut microbes are part of the unconscious system regulating behavior. Of major importance for societal functioning is the human trait of sociability. We are social creatures with superior cognitive functioning, which enabled us to become the dominant species on the planet. In rodents, who are raised without any bacteria, one sees altered sociability with clear autistic-like patterns of behavior (Desbonnet et al., 2014). Within an evolutionary framework colonization with crowds of bacteria facilitated mammalian group living in social crowds (Lombardo, 2008; Troyer, 1984), and thus the capacity for environmental dominance. Here we will review the evidence in support of these assertions and the potential role of gut microbes in psychiatry. Unlike many recent reviews published in specialist journals, the current review is aimed at practicing psychiatrists.

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1. Brain–gut–microbiota axis

The general scaffolding of the brain–gut–microbiota axis (BGM) includes the central nervous system (CNS), the neuroendocrine and neuroimmune systems, the sympathetic and parasympathetic arms of the autonomic nervous system (ANS), the enteric nervous system (ENS) and most importantly the intestinal microbiota (Mayer, 2011). These components interact to form a complex reflex network with afferent fibers that project to integrative CNS structures and efferent projections to the smooth muscle (Grenham et al., 2011). Through this bidirectional communication network, signals from the brain can influence the motor, sensory and secretory modalities of the gut and conversely, visceral messages from the gut can influence brain function (Grenham et al., 2011; Montiel-Castro et al., 2013). Less extensively studied, but increasingly appreciated, is the potential impact of the enteric microbiota on brain function (Khanna and Tosh, 2014). The gut microbiota

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"Gut microbes are part of the unconscious system regulating behavior. Recent investigations indicate that these microbes majorly impact on cognitive function and fundamental behavior patterns, such as social interaction and stress management. In the absence of microbes, underlying neurochemistry is profoundly altered."

Journal of Psychiatric Research 63 (2015) 1e9http://dx.doi.org/10.1016/j.jpsychires.2015.02.021

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Brain on Fire Because Gut is on Fire!!!!

Production of inflammatory cytokines

Cross blood-brain barrier (BBB) and activate microglia cells in brain

Brain inflammation/neurodegeneration

Gut-Brain Axis Inflammatory/Neurodegeneration

Enteric nervous system inflammatory/neurodegeneration

Local activation of intestinal microglial cells

INTESTINAL INFLAMMATION

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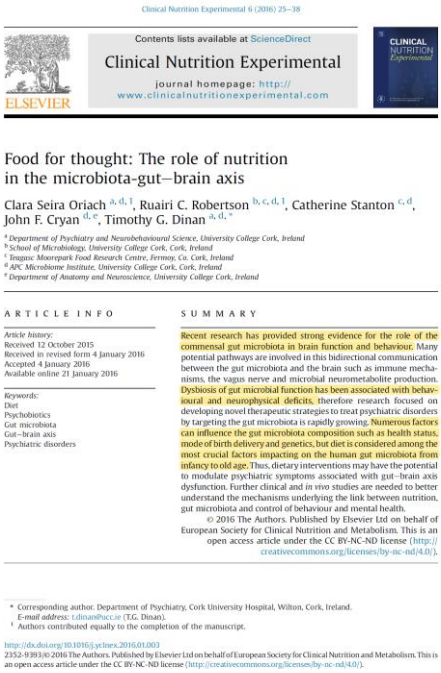

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Food for thought: The role of nutrition in the microbiota-gut–brain axis

Clara Seira Oriach [a, d, 1], Ruairi C. Robertson [b, c, d, 1], Catherine Stanton [c, d], John F. Cryan [d, e], Timothy G. Dinan [a, d, *]

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SUMMARY

Recent research has provided strong evidence for the role of the commensal gut microbiota in brain function and behaviour. Many potential pathways are involved in this bidirectional communication between the gut microbiota and the brain such as immune mechanisms, the vagus nerve and microbial neurometabolite production. Dysbiosis of gut microbial function has been associated with behavioural and neurophysical deficits, therefore research focused on developing novel therapeutic strategies to treat psychiatric disorders by targeting the gut microbiota is rapidly growing. Numerous factors can influence the gut microbiota composition such as health status, mode of birth delivery and genetics, but diet is considered among the most crucial factors impacting on the human gut microbiota from infancy to old age. Thus, dietary interventions may have the potential to modulate psychiatric symptoms associated with gut–brain axis dysfunction. Further clinical and *in vivo* studies are needed to better understand the mechanisms underlying the link between nutrition, gut microbiota and control of behaviour and mental health.

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2352-9393/

"Recent research has provided strong evidence for the role of the commensal gut microbiota in brain function and behaviour."

"Dysbiosis of gut microbial function has been associated with behavioural and neurophysical deficits,"

"Numerous factors can influence the gut microbiota composition such as health status, mode of birth delivery and genetics, but diet is considered among the most crucial factors impacting on the human gut microbiota from infancy to old age."

Clinical Nutrition Experimental 6 (2016) 25e38
http://dx.doi.org/10.1016/j.yclnex.2016.01.003

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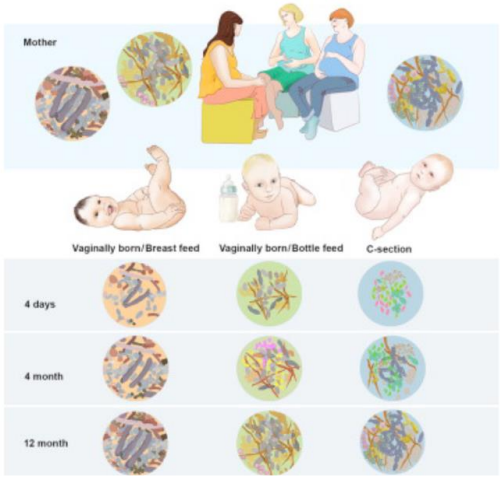
Microbial composition varies according to the original seeding



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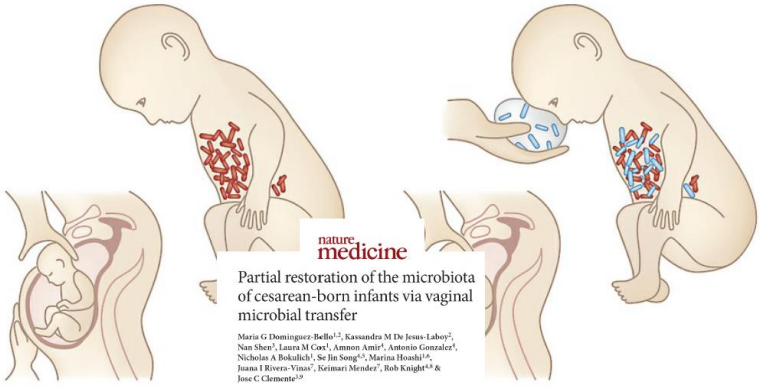
Microbial composition varies according to the original seeding

C-section

C-section + vaginal sponge



nature medicine

Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

Maria G Dominguez-Bello[1,2], Kassandra M De Jesus-Laboy[2], Nan Shen[3], Laura M Cox[1], Amnon Amir[1], Antonio Gonzalez[4], Nicholas A Bokulich[1], Se Jin Song[4,5], Marina Hoashi[1,6], Juana I Rivera-Vinas[7], Keimari Mendez[7], Rob Knight[4,8] & Jose C Clemente[2,9]

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Stress & the gut-brain axis: Regulation by the microbiome

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ABSTRACT

The importance of the gut–brain axis in regulating stress-related responses has long been appreciated. More recently, the microbiota has emerged as a key player in the control of this axis, especially during conditions of stress provoked by real or perceived homeostatic challenge. Diet is one of the most important modifying factors of the microbiota-gut-brain axis. The routes of communication between the microbiota and brain are slowly being unravelled, and include the vagus nerve, gut hormone signaling, the immune system, tryptophan metabolism, and microbial metabolites such as short chain fatty acids. The importance of the early life gut microbiota in shaping later health outcomes also is emerging. Results from preclinical studies indicate that alterations of the early microbial composition by way of antibiotic exposure, lack of breastfeeding, birth by Caesarean section, infection, stress exposure, and other environmental influences - coupled with the influence of host genetics - can result in long-term modulation of stress-related physiology and behaviour. The gut microbiota has been implicated in a variety of stress-related conditions including anxiety, depression and irritable bowel syndrome, although this is largely based on animal studies or correlative analysis in patient populations. Additional research in humans is surely needed to reveal the relative impact and causal contribution of the microbiome to stress-related disorders. In this regard, the concept of psychobiotics is being developed and refined to encompass methods of targeting the microbiota in order to positively impact mental health outcomes. At the 2016 Neurobiology of Stress Workshop in Newport Beach, CA, a group of experts presented the symposium "The Microbiome: Development, Stress, and Disease". This report summarizes and builds upon some of the key concepts in that symposium within the context of how microbiota might influence the neurobiology of stress.

Contents

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"The importance of the gut-brain axis in regulating stress-related responses has long been appreciated. More recently, the microbiota has emerged as a key player in the control of this axis, especially during conditions of stress provoked by real or perceived homeostatic challenge. Diet is one of the most important modifying factors of the microbiota-gut-brain axis."

"…alterations of the early microbial composition by way of antibiotic exposure, lack of breastfeeding, birth by Caesarean section, infection, stress exposure, and other environmental influences -…"

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Neurobiology of Stress 7 (2017) 124e136
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Review article

Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health?

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HIGHLIGHTS

- Interest in how diet influences brain function via the gut microbiome is growing.
- Butyrate can protect the brain and enhance plasticity in neurological disease models.
- Gut microbiota produce butyrate by fermenting carbohydrates in a high fiber diet.
- Hypothesis: A high fiber diet can elevate butyrate to prevent/treat brain disorders.

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ABSTRACT

As interest in the gut microbiome has grown in recent years, attention has turned to the impact of our diet on our brain. The benefits of a high fiber diet in the colon have been well documented in epidemiological studies, but its potential impact on the brain has largely been understudied. Here, we will review evidence that butyrate, a short-chain fatty acid (SCFA) produced by bacterial fermentation of fiber in the colon, can improve brain health. Butyrate has been extensively studied as a histone deacetylase (HDAC) inhibitor but also functions as a ligand for a subset of G protein-coupled receptors and as an energy metabolite. These diverse modes of action make it well suited for solving the wide array of imbalances frequently encountered in neurological disorders. In this review, we will integrate evidence from the disparate fields of gastroenterology and neuroscience to hypothesize that the metabolism of a high fiber diet in the gut can alter gene expression in the brain to prevent neurodegeneration and promote regeneration.

Contents

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frontiers in Psychiatry

REVIEW
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Vagus Nerve as Modulator of the Brain–Gut Axis in Psychiatric and Inflammatory Disorders

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The vagus nerve represents the main component of the parasympathetic nervous system, which oversees a vast array of crucial bodily functions, including control of mood, immune response, digestion, and heart rate. It establishes one of the connections between the brain and the gastrointestinal tract and sends information about the state of the inner organs to the brain *via* afferent fibers. In this review article, we discuss various functions of the vagus nerve which make it an attractive target in treating psychiatric and gastrointestinal disorders. There is preliminary evidence that vagus nerve stimulation is a promising add-on treatment for treatment-refractory depression, posttraumatic stress disorder, and inflammatory bowel disease. Treatments that target the vagus nerve increase the vagal tone and inhibit cytokine production. Both are important mechanism of resiliency. The stimulation of vagal afferent fibers in the gut influences monoaminergic brain systems in the brain stem that play crucial roles in major psychiatric conditions, such as mood and anxiety disorders. In line, there is preliminary evidence for gut bacteria to have beneficial effect on mood and anxiety, partly by affecting the activity of the vagus nerve. Since, the vagal tone is correlated with capacity to regulate stress responses and can be influenced by breathing, its increase through meditation and yoga likely contribute to resilience and the mitigation of mood and anxiety symptoms.

Keywords: depression, PTSD, vagus nerve stimulation, nutrition, probiotics, yoga, meditation, inflammatory bowel disease

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INTRODUCTION

The bidirectional communication between the brain and the gastrointestinal tract, the so-called "brain–gut axis," is based on a complex system, including the vagus nerve, but also sympathetic (e.g., *via* the prevertebral ganglia), endocrine, immune, and humoral links as well as the influence of gut microbiota in order to regulate gastrointestinal homeostasis and to connect emotional and cognitive areas of the brain with gut functions (1). The ENS produces more than 30 neurotransmitters and has more neurons than the spine. Hormones and peptides that the ENS releases into the blood circulation cross the blood–brain barrier (e.g., ghrelin) and can act synergistically with the vagus nerve, for example to regulate food intake and appetite (2). The brain–gut axis is becoming increasingly important as a therapeutic target for gastrointestinal and psychiatric disorders, such as inflammatory bowel disease (IBD) (3), depression (4), and posttraumatic stress disorder (PTSD) (5). The gut is an important control center of the immune system and the vagus nerve has immunomodulatory properties (6). As a result, this nerve plays important roles in the relationship between the gut, the brain, and

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The Gut Microbiome: A New Frontier in Autism Research

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Abstract

The human gut harbors a complex community of microbes that profoundly influence many aspects of growth and development, including development of the nervous system. Advances in high-throughput DNA sequencing methods have led to rapidly expanding knowledge about this gut microbiome. Here, we review fundamental emerging data on the human gut microbiome, with a focus on potential interactions between the microbiome and autism spectrum disorders (ASD) and consider research on atypical patterns of feeding and nutrition in ASD and how they might interact with the microbiome. Finally we selectively survey results from studies in rodents on the impact of the microbiome on neurobehavioral development. The evidence reviewed here suggests that a deeper understanding of the gut microbiome could open up new avenues of research on ASD, including potential novel treatment strategies.

Keywords

Gut microbiome; Nervous system; Behavior; Autism; Autism spectrum disorders; ASDs; Feeding; Nutrition; Dietary intake; Animal studies; Neurobehavioral development; Genetic disorders; Psychiatry

Introduction

We coexist with vast populations of microbial species that make a host out of the human body. It has been estimated that up to 100 trillion microbial cells make a home out of us[1], and likely outnumber human body cells by an order of magnitude[2], leading some to term the human microbiome our "second genome."[3] Increasingly, it has become clear that these constellations of microbial species are a partner in homeostasis, and when the balance is tipped away from the healthy microbiome there can be a negative outcome on human health[4]. This new appreciation has led to formal efforts to identify comprehensively all components of the microbiome. The Human Microbiome Project (HMP) was launched by

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"The evidence reviewed here suggests that a deeper understanding of the gut microbiome could open up new avenues of research on ASD, including potential novel treatment strategies."

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SCIENCE CHINA
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• REVIEW •

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Alzheimer's disease and gut microbiota

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Alzheimer's disease (AD) is a most common neurodegenerative disorder, which associates with impaired cognition. Gut microbiota can modulate host brain function and behavior via microbiota-gut-brain axis, including cognitive behavior. Germ-free animals, antibiotics, probiotics intervention and diet can induce alterations of gut microbiota and gut physiology and also host cognitive behavior, increasing or decreasing risks of AD. The increased permeability of intestine and blood-brain barrier induced by gut microbiota disturbance will increase the incidence of neurodegeneration disorders. Gut microbial metabolites and their effects on host neurochemical changes may increase or decrease the risk of AD. Pathogenic microbes infection will also increase the risk of AD, and meanwhile, the onset of AD support the "hygiene hypothesis". All the results suggest that AD may begin in the gut, and is closely related to the imbalance of gut microbiota. Modulation of gut microbiota through personalized diet or beneficial microbiota intervention will probably become a new treatment for AD.

Alzheimer's disease, gut microbiota, leaky gut, leaky brain, diet, infection, hygiene hypothesis

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INTRODUCTION

Alzheimer's disease (AD), commonly known as senile dementia or cognitive disorder, is a common central nervous system degenerative disease in the elderly. AD is one of the most common form of dementia, accounting for 60%–80% of all dementia (Alzheimer's Association, 2015). It is estimated that about 36 million people lived with dementia worldwide in 2010 and the numbers were expected to double every 20 years. The numbers will reach to 66 million in 2030 and 115 million in 2050 (Prince et al., 2013). With the acceleration of population aging in the world, the incidence of AD rises year by year, which seriously damage the old people's physical and mental health and quality of life, causing severe pain to the patients and bringing heavy burden to family and society. China's population aging also reached an unprecedented level. The 2010 nationwide census data showed that the elderly population accounted for more than 10% of the population in our country. To speculate according to the current incidence, China will have 8–12 million AD patients by 2050. The high incidence and morbidity of AD have become a major problem affecting human health, which aroused widespread concern in government and medical community and become a hotspot and difficulty in the field of neuroscience research.

The neuropathological hallmarks of AD include extracellular β-amyloid (Aβ) senile plaques (SP) and intracellular neurofibrillary tangles (NFT) (Reitz et al., 2011). At present, it is generally considered that the interaction between genetic and environmental factors takes part in AD pathogenesis. Through genome-wide association studies in AD patients, some genomic regions associated with AD were discovered and some susceptibility genes of AD had been identified, mainly involving in the immune reaction, inflammation, cell migration and lipids transport pathways (Lambert et al., 2013). Apolipoprotein E (ApoE) is one of the most common susceptibility genes, with three

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The Gut Microbiota and Alzheimer's Disease

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Abstract: The gut microbiota comprises a complex community of microorganism species that resides in our gastrointestinal ecosystem and whose alterations influence not only various gut disorders but also central nervous system disorders such as Alzheimer's disease (AD). AD, the most common form of dementia, is a neurodegenerative disorder associated with impaired cognition and cerebral accumulation of amyloid-β peptides (Aβ). Most notably, the microbiota-gut-brain axis is a bidirectional communication system that is not fully understood, but includes neural, immune, endocrine, and metabolic pathways. Studies in germ-free animals and in animals exposed to pathogenic microbial infections, antibiotics, probiotics, or fecal microbiota transplantation suggest a role for the gut microbiota in host cognition or AD-related pathogenesis. The increased permeability of the gut and blood-brain barrier induced by microbiota dysbiosis may mediate or affect AD pathogenesis and other neurodegenerative disorders, especially those associated with aging. In addition, bacteria populating the gut microbiota can secrete large amounts of amyloids and lipopolysaccharides, which might contribute to the modulation of signaling pathways and the production of proinflammatory cytokines associated with the pathogenesis of AD. Moreover, imbalances in the gut microbiota can induce inflammation that is associated with the pathogenesis of obesity, type 2 diabetes mellitus, and AD. The purpose of this review is to summarize and discuss the current findings that may elucidate the role of the gut microbiota in the development of AD. Understanding the underlying mechanisms may provide new insights into novel therapeutic strategies for AD.

Keywords: Aging, Alzheimer's disease, amyloid, amyloid beta-peptides, blood-brain barrier, dysbiosis, gut microbiota, lipopolysaccharides, obesity, type 2 diabetes mellitus

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Springer Link

Analysis of Gut Microbiota in Patients with Parkinson's Disease

Bulletin of Experimental Biology and Medicine

April 2017, Volume 162, Issue 6, pp 734–737 | Cite as

Gut microbiota of patients with Parkinson's disease and healthy volunteers was analyzed by the method of high throughput 16S rRNA sequencing of bacterial genomes. In patients with Parkinson's diseases, changes in the content of 9 genera and 15 species of microorganisms were revealed: reduced content of *Dorea*, *Bacteroides*, *Prevotella*, *Faecalibacterium*, *Bacteroides massiliensis*, *Stoquefichus massiliensis*, *Bacteroides coprocola*, *Blautia glucerasea*, *Dorea longicatena*, *Bacteroides dorei*, *Bacteroides plebeus*, *Prevotella copri*, *Coprococcus eutactus*, and *Ruminococcus callidus*, and increased content of *Christensenella*, *Catabacter*, *Lactobacillus*, *Oscillospira*, *Bifidobacterium*, *Christensenella minuta*, *Catabacter hongkongensis*, *Lactobacillus mucosae*, *Ruminococcus bromii*, and *Papillibacter cinnamivorans*. This microbiological pattern of gut microflora can trigger local inflammation followed by aggregation of α-synuclein and generation of Lewy bodies.

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ARTICLE OPEN

Linkages between oral commensal bacteria and atherosclerotic plaques in coronary artery disease patients

Jyoti Chhibber-Goel[1], Varsha Singhal[1], Debaleena Bhowmik[1], Rahul Vivek[1], Neeraj Parakh[2], Balram Bhargava[2] and Amit Sharma[1]

Coronary artery disease is an inflammatory disorder characterized by narrowing of coronary arteries due to atherosclerotic plaque formation. To date, the accumulated epidemiological evidence supports an association between oral bacterial diseases and coronary artery disease, but has failed to prove a causal link between the two. Due to the recent surge in microbial identification and analyses techniques, a number of bacteria have been independently found in atherosclerotic plaque samples from coronary artery disease patients. In this study, we present meta-analysis from published studies that have independently investigated the presence of bacteria within atherosclerotic plaque samples in coronary artery disease patients. Data were collated from 63 studies covering 1791 patients spread over a decade. Our analysis confirms the presence of 23 oral commensal bacteria, either individually or in co-existence, within atherosclerotic plaques in patients undergoing carotid endarterectomy, catheter-based atherectomy, or similar procedures. Of these 23 bacteria, 5 (*Campylobacter rectus*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia*, *Prevotella nigrescens*) are unique to coronary plaques, while the other 18 are additionally present in non-cardiac organs, and associate with over 30 non-cardiac disorders. We have cataloged the wide spectrum of proteins secreted by above atherosclerotic plaque-associated bacteria, and discuss their possible roles during microbial migration via the bloodstream. We also highlight the prevalence of specific poly-microbial communities within atherosclerotic plaques. This work provides a resource whose immediate implication is the necessity to systematically catalog landscapes of atherosclerotic plaque-associated oral commensal bacteria in human patient populations.

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The oral microbiome – an update for oral healthcare professionals

M. Kilian,*[1] I. L. C. Chapple,[2] M. Hannig,[3] P. D. Marsh,[4] V. Meuric,[5] A. M. L. Pedersen,[6] M. S. Tonetti,[7] W. G. Wade[8] and E. Zaura[9]

In brief

Summarises the benefits of a health-promoting oral microbiome for oral and general health.	Provides an overview of coevolution between humans and oral microbes.	Reviews factors associated with dysbiosis and implications for caries and periodontal disease.	Highlights existing strategies to preserve a balanced oral microbiome for practitioners and patients to follow.

For millions of years, our resident microbes have coevolved and coexisted with us in a mostly harmonious symbiotic relationship. We are not distinct entities from our microbiome, but together we form a 'superorganism' or holobiont, with the microbiome playing a significant role in our physiology and health. The mouth houses the second most diverse microbial community in the body, harbouring over 700 species of bacteria that colonise the hard surfaces of teeth and the soft tissues of the oral mucosa. Through recent advances in technology, we have started to unravel the complexities of the oral microbiome and gained new insights into its role during both health and disease. Perturbations of the oral microbiome through modern-day lifestyles can have detrimental consequences for our general and oral health. In dysbiosis, the finely-tuned equilibrium of the oral ecosystem is disrupted, allowing disease-promoting bacteria to manifest and cause conditions such as caries, gingivitis and periodontitis. For practitioners and patients alike, promoting a balanced microbiome is therefore important to effectively maintain or restore oral health. This article aims to give an update on our current knowledge of the oral microbiome in health and disease and to discuss implications for modern-day oral healthcare.

Introduction

Humans, like all complex multicellular eukaryotes, are not autonomous organisms, but biological units that include numerous microbial symbionts and their genomes.[1] The microbes in and on our bodies form a functional organ that is fundamental to our health and physiology (Fig. 1). Together with our symbiotic microbial residents, we form a 'superorganism', or **holobiont**. The microbial component of the human holobiont is substantial, and at least equals the number of our own cells.[2] The community of our microbial residents is referred to as our **microbiome**, a term that was coined by the Nobel prize laureate Joshua Lederberg 'to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health

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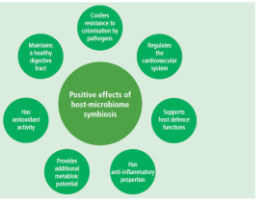
*British Dental Journal 2016; 221: 657-666

Fig. 1 Positive effects of host-microbiome symbiosis



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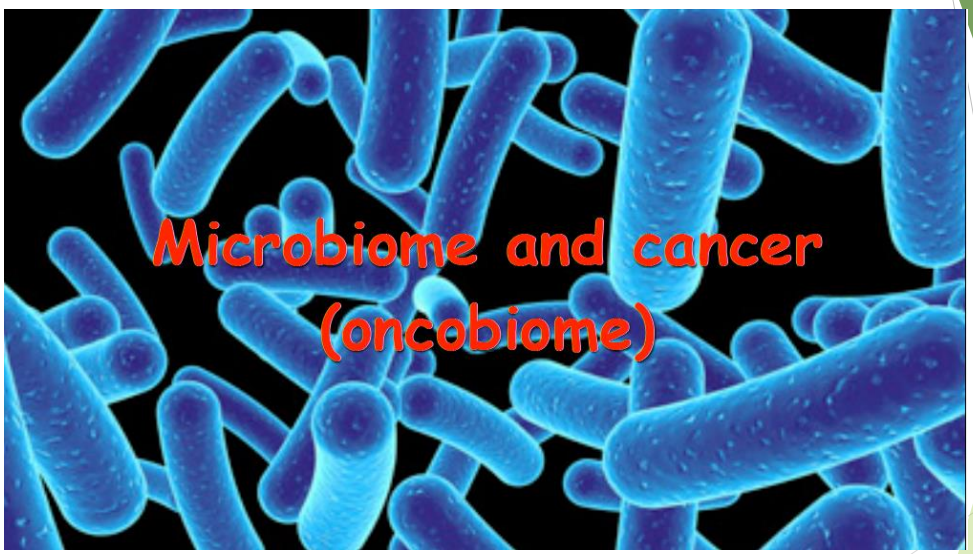

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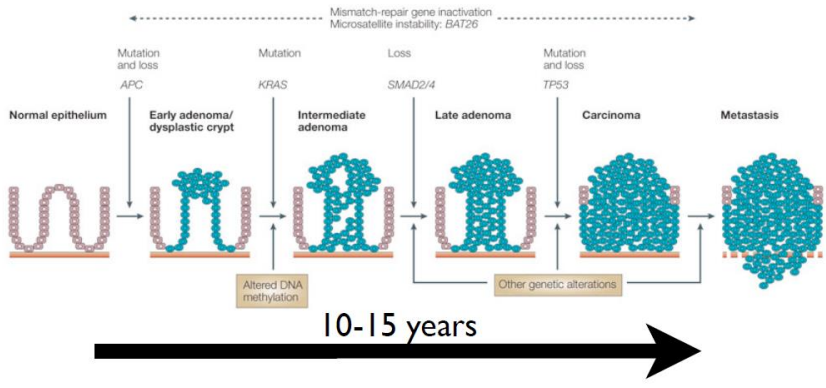
Are intestinal bacteria bystander to the carcinogenic process?



R. Justin Davies, Richard Miller & Nicholas Coleman
Nature Reviews Cancer **5**, 199-209 (March 2005)

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Gut dysbiosis: a potential link between increased cancer risk in ageing and inflammaging.

Biragyn A1, Ferrucci L2.

Abstract
Cancer incidence substantially increases with ageing in both men and women, although the reason for this increase is unknown. In this Series paper, we propose that age-associated changes in gut commensal microbes, otherwise known as the microbiota, facilitate cancer development and growth by compromising immune fitness. Ageing is associated with a reduction in the beneficial commensal microbes, which control the expansion of pathogenic commensals and maintain the integrity of the intestinal barrier through the production of mucus and lipid metabolites, such as short-chain fatty acids. Expansion of gut dysbiosis and leakage of microbial products contributes to the chronic proinflammatory state (inflammaging), which negatively affects the immune system and impairs the removal of mutant and senescent cells, thereby enabling tumour outgrowth. Studies in animal models and the importance of commensals in cancer immunotherapy suggest that this status can be reversible. Thus, interventions that alter the composition of the gut microbiota might reduce inflammaging and rejuvenate immune functions to provide anticancer benefits in frail elderly people.

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doi: 10.1016/S1470-2045(18)30095-0. Epub 2018 Jun 1.

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Clinical Nutrition Experimental 6 (2016) 74–82

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Nutrition in cancer patients with cachexia: A role for the gut microbiota?

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SUMMARY

Cachexia is a multifactorial syndrome that includes muscle wasting and inflammation, and that is associated with chronic underlying diseases, such as cancer, chronic heart failure and chronic kidney disease. Since gut microbes influence host immunity and metabolism, we hypothesized a few years ago that the gut microbiota could be a potential therapeutic target to tackle cancer-related cachexia. In this review, we present evidence from animal and human studies suggesting that the gut microbiota and its crosstalk with the intestine might constitute unexpected targets in the therapeutic management of cancer and related cachexia. Finally, we discuss future research directions and hypotheses to progress in this new promising field, i.e. the role of the gut microbiota in cancer cachexia.

Abbreviations: CHF, chronic heart failure; CKD, chronic kidney disease; LPS, lipopolysaccharides; POS, pectic oligosaccharides; TLR, toll-like receptor.

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Clinical Nutrition Experimental 6 (2016) 74e82
http://dx.doi.org/10.1016/j.yclnex.2015.11.001

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Amirian *et al. Infectious Agents and Cancer* 2013, **8**:42
http://www.infectagentscancer.com/content/8/1/42

INFECTIOUS AGENTS AND CANCER

HYPOTHESIS Open Access

Potential role of gastrointestinal microbiota composition in prostate cancer risk

E Susan Amirian[1,2], Joseph F Petrosino[3,4], Nadim J Ajami[3,4], Yanhong Liu[1,2], Martha P Mims[5] and Michael E Scheurer[1,2*]

Abstract

Background: Among men in the U.S., prostate cancer is the most common cancer and the second leading cause of cancer death. Despite its prevalence, there are few established risk factors for prostate cancer. Some studies have found that intake of certain foods/nutrients may be associated with prostate cancer risk, but few have accounted for how intake and metabolic factors may interact to influence bioavailable nutrient levels and subsequent disease risk.

Presentation of the hypothesis: The composition of the gastrointestinal (GI) microbiome may influence metabolism of dietary compounds and nutrients (e.g., plant phenols, calcium, choline) that may be relevant to prostate cancer risk. We, therefore, propose the hypothesis that GI microbiota may have a markedly different composition among individuals with higher prostate cancer risk. These individuals could have microbial profiles that are conducive to intestinal inflammation and/or are less favorable for the metabolism and uptake of chemopreventive agents.

Testing the hypothesis: Because very little preliminary data exist on this potential association, a case–control study may provide valuable information on this topic. Such a study could evaluate whether the GI microbial profile is markedly different between three groups of individuals: healthy men, those with latent prostate cancer, and those with invasive prostate cancer. Any findings could then be validated in a larger study, designed to collect a series of specimens over time.

Implications of the hypothesis: Given the plethora of information emerging from the Human Microbiome Project, this is an opportune time to explore associations between the microbiome and complex human diseases. Identification of profiles that alter the host's risk for disease may clarify inconsistencies in the literature on dietary factors and cancer risk, and could provide valuable targets for novel cancer prevention strategies.

Keywords: Human microbiome, Metagenome, Prostate cancer, Metabolic process

Background

Prostate cancer is the most common cancer among men in the U.S. [1]. In 2012, approximately 241,740 new diagnoses and 28,170 prostate cancer-related deaths were expected in the U.S. alone (global incidence of 27.9 cases per 100,000) [1,2]. Lifetime risk for prostate cancer is estimated to be 16%, and the median age at diagnosis is 67 years [1].

Despite its prevalence, there are few established risk factors for prostate cancer [3]. According to twin studies, a proportion of cases (10-40%) may be explained by genetic factors [3-5]. However, dietary and lifestyle factors may also influence prostate cancer susceptibility [3,6]. Intake of red meat [7-10], dairy products [11,12], eggs [9,13,14], green tea [15,16], calcium [17-20], lycopene [21-23], selenium [6,24], and fish oil [4,25] have all been examined in relation to prostate cancer risk with relatively inconsistent results. The inconsistency of these findings may partly be due to the use of food intake measures as surrogates for bioavailable micronutrient levels, resulting in some misclassification of nutrient/metabolite exposures [26,27]. Differing levels of nutrient metabolism

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Full list of author information is available at the end of the article

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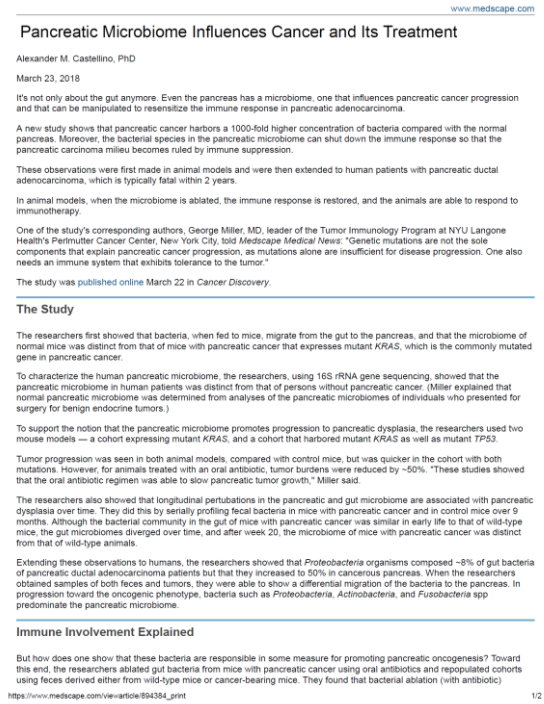

www.medscape.com

Pancreatic Microbiome Influences Cancer and Its Treatment

Alexander M. Castellino, PhD

March 23, 2018

It's not only about the gut anymore. Even the pancreas has a microbiome, one that influences pancreatic cancer progression and that can be manipulated to resensitize the immune response in pancreatic adenocarcinoma.

A new study shows that pancreatic cancer harbors a 1000-fold higher concentration of bacteria compared with the normal pancreas. Moreover, the bacterial species in the pancreatic microbiome can shut down the immune response so that the pancreatic carcinoma milieu becomes ruled by immune suppression.

These observations were first made in animal models and were then extended to human patients with pancreatic ductal adenocarcinoma, which is typically fatal within 2 years.

In animal models, when the microbiome is ablated, the immune response is restored, and the animals are able to respond to immunotherapy.

One of the study's corresponding authors, George Miller, MD, leader of the Tumor Immunology Program at NYU Langone Health's Perlmutter Cancer Center, New York City, told *Medscape Medical News*: "Genetic mutations are not the sole components that explain pancreatic cancer progression, as mutations alone are insufficient for disease progression. One also needs an immune system that exhibits tolerance to the tumor."

The study was published online March 22 in *Cancer Discovery*.

The Study

The researchers first showed that bacteria, when fed to mice, migrate from the gut to the pancreas, and that the microbiome of normal mice was distinct from that of mice with pancreatic cancer that expresses mutant *KRAS*, which is the commonly mutated gene in pancreatic cancer.

To characterize the human pancreatic microbiome, the researchers, using 16S rRNA gene sequencing, showed that the pancreatic microbiome in human patients was distinct from that of persons without pancreatic cancer. (Miller explained that normal pancreatic microbiome was determined from analyses of the pancreatic microbiomes of individuals who presented for surgery for benign endocrine tumors.)

To support the notion that the pancreatic microbiome promotes progression to pancreatic dysplasia, the researchers used two mouse models — a cohort expressing mutant *KRAS*, and a cohort that harbored mutant *KRAS* as well as mutant *TP53*.

Tumor progression was seen in both animal models, compared with control mice, but was quicker in the cohort with both mutations. However, for animals treated with an oral antibiotic, tumor burdens were reduced by ~50%. "These studies showed that the oral antibiotic regimen was able to slow pancreatic tumor growth," Miller said.

The researchers also showed that longitudinal pertubations in the pancreatic and gut microbiome are associated with pancreatic dysplasia over time. They did this by serially profiling fecal bacteria in mice with pancreatic cancer and in control mice over 9 months. Although the bacterial community in the gut of mice with pancreatic cancer was similar in early life to that of wild-type mice, the gut microbiomes diverged over time, and after week 20, the microbiome of mice with pancreatic cancer was distinct from that of wild-type animals.

Extending these observations to humans, the researchers showed that *Proteobacteria* organisms composed ~8% of gut bacteria of pancreatic ductal adenocarcinoma patients but that they increased to 50% in cancerous pancreas. When the researchers obtained samples of both feces and tumors, they were able to show a differential migration of the bacteria to the pancreas. In progression toward the oncogenic phenotype, bacteria such as *Proteobacteria*, *Actinobacteria*, and *Fusobacteria* spp predominate the pancreatic microbiome.

Immune Involvement Explained

But how does one show that these bacteria are responsible in some measure for promoting pancreatic oncogenesis? Toward this end, the researchers ablated gut bacteria from mice with pancreatic cancer using oral antibiotics and repopulated cohorts using feces derived either from wild-type mice or cancer-bearing mice. They found that bacterial ablation (with antibiotic)

https://www.medscape.com/viewarticle/894384_print 1/2

It's not only about the gut anymore. Even the pancreas has a microbiome, one that influences pancreatic cancer progression and that can be manipulated to resensitize the immune response in pancreatic adenocarcinoma.
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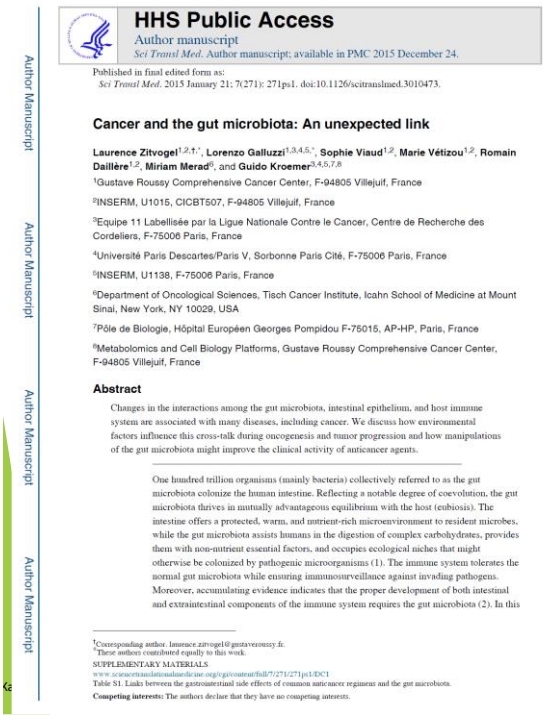

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Cancer and the gut microbiota: An unexpected link

Laurence Zitvogel[1,2,†,*], **Lorenzo Galluzzi**[1,3,4,5,*], **Sophie Viaud**[1,2], **Marie Vétizou**[1,2], **Romain Daillère**[1,2], **Miriam Merad**[6], and **Guido Kroemer**[3,4,5,7,8]

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Abstract

Changes in the interactions among the gut microbiota, intestinal epithelium, and host immune system are associated with many diseases, including cancer. We discuss how environmental factors influence this cross-talk during oncogenesis and tumor progression and how manipulations of the gut microbiota might improve the clinical activity of anticancer agents.

One hundred trillion organisms (mainly bacteria) collectively referred to as the gut microbiota colonize the human intestine. Reflecting a notable degree of coevolution, the gut microbiota thrives in mutually advantageous equilibrium with the host (eubiosis). The intestine offers a protected, warm, and nutrient-rich microenvironment to resident microbes, while the gut microbiota assists humans in the digestion of complex carbohydrates, provides them with non-nutrient essential factors, and occupies ecological niches that might otherwise be colonized by pathogenic microorganisms (1). The immune system tolerates the normal gut microbiota while ensuring immunosurveillance against invading pathogens. Moreover, accumulating evidence indicates that the proper development of both intestinal and extraintestinal components of the immune system requires the gut microbiota (2). In this

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[†]These authors contributed equally to this work.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/7/271/271ps1/DC1

Table S1. Links between the gastrointestinal side effects of common anticancer regimens and the gut microbiota.

Competing interests: The authors declare that they have no competing interests.

"Changes in the interactions among the gut microbiota, intestinal epithelium, and host immune system are associated with many diseases, including cancer. We discuss how environmental factors influence this cross-talk during oncogenesis and tumor progression and how manipulations of the gut microbiota might improve the clinical activity of anticancer agents."

Sci Transl Med. 2015 January 21; 7(271): 271ps1. doi:10.1126/scitranslmed.3010473.

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The Prostate

Volume 73, Issue 3
15 February 2013, Pages 236–241
Original Article

Human papillomavirus and Epstein Barr virus in prostate cancer: Koilocytes indicate potential oncogenic influences of human papillomavirus in prostate cancer[☆]

Authors: Noel J. Whitaker, Wendy K. Glenn, Arisha Sahrudin, Matthew M. Orde, Warick Delprado, James S. Lawson

Abstract

INTRODUCTION

The purpose of this study is to determine if high risk human papillomaviruses (HPV) and Epstein Barr virus (EBV) are both present in the same prostate cancer specimens.

METHODS

We used a range of analytical techniques including in situ polymerase chain reaction (IS-PCR) and standard liquid PCR followed by sequencing of the product to seek to identify HPV and EBV in normal, benign, and malignant prostate tissues.

RESULTS

Both HPV type 18 and EBV gene sequences were identified in a high and approximately equal proportion of normal, benign, and prostate cancer specimens. These sequences were located in the nuclei of prostate epithelial cells. HPV associated koilocytes were identified in 24% of prostate cancer specimens.

CONCLUSIONS

The presence of both HPV and EBV gene sequences in most of the same normal, benign, and malignant prostate specimens is particularly noteworthy because of recent experimental evidence demonstrating that EBV and HPV can collaborate to increase proliferation of cultured cervical cells.

Because the presence of EBV and HPV in normal, benign, and malignant prostate tissues appears to be ubiquitous, it is possible that they are harmless. On the other hand HPV type 18 in particular, has high oncogenic potential and may be associated with some prostate cancers. The identification of HPV associated koilocytes in prostate cancer specimens is an indication of HPV infection and potential oncogenic influences of human papillomavirus in prostate cancer. Prostate 73: 236–241, 2013. © 2012 Wiley Periodicals, Inc.

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Zonulin

Together with the gut-associated lymphoid tissue and the neuroendocrine network, the intestinal epithelial barrier, with its intercellular tight junctions, controls the equilibrium between tolerance and immunity to nonself antigens. Zonulin is the only physiological modulator of intercellular tight junctions described so far that is involved in trafficking of macromolecules and, therefore, intolerance/immune response balance. When the finely tuned zonulin pathway is deregulated in genetically susceptible individuals, both intestinal and extraintestinal autoimmune, inflammatory, and neoplastic disorders can occur."

Fasano A. Zonulin and its regulation of intestinal 1. barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011;91(1):151–175.

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Zonulin

Top causes of increased zonulin and development of leaky gut:

.

- Overgrowth of harmful organisms, like bacteria or yeast in the intestine
  - SIBO = small intestinal bacterial overgrowth
  - Fungal dysbiosis or candida overgrowth
  - Parasite infections

- Gliadin in the diet (gluten containing foods)

- Zonulin signals the body as a protective mechanism.
  - When a dysbiotic or "bad" bacterial organism lands on an epithelia cell in the small intestine, zonulin is released as a way to "open" the tight junctions in order to "dilute" the bacterial produced toxins to rid the body of them.
  - Diarrhea is the response

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Zonulin and "Leaky Gut"

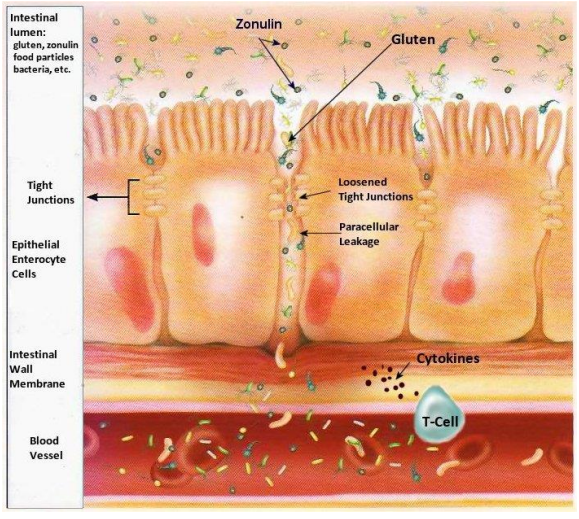

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Table 2. The Major Diseases Associated With Zonulin[a]

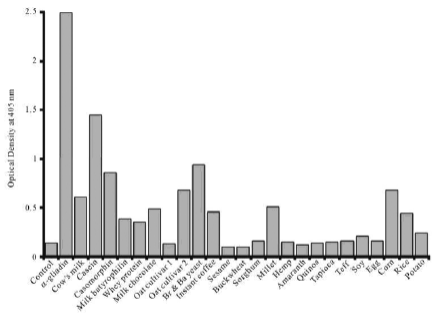
Autoimmune Diseases
- Ankylosing spondylitis
- Asthma
- Celiac disease
- Inflammatory bowel disease
- Multiple sclerosis
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Type 1 diabetes

Diseases of the Nervous System
- Chronic inflammatory demyelinating polyneuropathy
- Schizophrenia

Cancers
- Brain cancer
- Breast cancer
- Glioma
- Lung adenocarcinoma
- Ovarian cancer
- Pancreatic cancer

[a]Modified from Fasano and others.

Figure 4. Cross Reactivity of Antigliadin Antibodies With Other Food Antigens



Reaction of affinity-purified α-gliadin 33-mer polyclonal antibodies to gliadin and different food antigens.

Abbreviations: Br yeast = Brewer's yeast; Ba yeast = Baker's yeast.

Pizzorno J, Zonulin! The Wheat Conundrum Solved (Well, Mostly…) Integrative Medicine, Vol. 12, No. 4, August 2013

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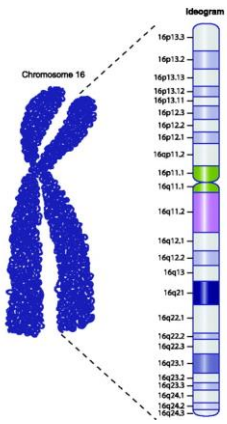
Diseases associated with zonulin and chromosome 16.

Major diseases associated to Zonulin (Pre-HP2)

AUTOIMMUNE DISEASES
- Ankylosing spondylitis.
- Celiac disease
- Inflammatory bowel disease (Cronh's disease)
- Rheumatoid arthritis
- Systemic lupus erythematosus
- Type 1 diabetes

CANCERS
- Brain cancers (gliomas)
- Breast cancer
- Lung adenocarcinoma
- Ovarian cancer
- Pancreatic cancer

DISEASES OF THE NERVOUS SYSTEM
- Chronic inflammatory demyelinating polyneuropathy (CIDP)
- Multiple sclerosis (Autoimmune disease?)
- Schizophrenia (Autoimmune disease?)



Major diseases associated to Chromosome 16

AUTOIMMUNE DISEASES
- Adult polycystic kidney disease
- Inflammatory bowel diseases (NOD2 locus)
- Systemic lupus erythematosus
- Type 1 diabetes
- Rheumatoid arthritis

CANCERS
- Acute nonymphocytic leukemia
- Breast cancer
- Fanconi's anemia
- Lymphoma, diffuse large B-cell
- Myeloid leukemia, acute
- Prostate cancers

DISEASES OF THE NERVOUS SYSTEM
- Batten's disease (juvenile onset neurodegenerative disorder)
- Lou Gehrig's disease
- Leukodystrophy
- Multiple sclerosis
- Autism

Alessio Fasano Physiol Rev 2011;91:151-175

Physiological Reviews

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Zonulin

Treatment Protocol:

"Heal and Seal"

- Four Rs" - remove, repair, restore, and replace
- Reduce histamine foods, often this can lead to an amelioration of symptoms
  - Alcohol
  - Pickled or canned foods – sauerkraut
  - Matured cheeses
  - Smoked meat products – salami, ham, sausages….
  - Shellfish
  - Beans and pulses – chickpeas, soy beans, peanuts
  - Nuts – walnuts, cashew nuts
  - Chocolates and other cocoa based products
  - Vinegar
  - Ready meals
  - Salty snacks, sweets with preservatives and artificial colourings

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Low histamine level foods:

- Fresh meat (cooled, frozen or fresh)
- Freshly caught fish
- Chicken (skinned and fresh)
- Egg yolk
- Fresh fruits – with the exception of strawberries, most fresh fruits are considered to have a low histamine level (also see histamine liberators below)
- Fresh vegetables – with the exception of tomatoes
- Grains – rice noodles, yeast free rye bread, rice crisp bread, oats, puffed rice crackers, millet flour, pasta (spelt and corn based)
- Fresh pasteurized milk and milk products
- Milk substitutes – coconut milk, rice milk
- Cream cheese, butter (without the histamine generating rancidity)
- Most cooking oils – check suitability before use
- Most leafy herbs – check suitability before use
- Most non-citric fruit juices
- Herbal teas

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High histamine level foods:

- Alcohol
- Pickled or canned foods – sauerkrauts
- Matured cheeses
- Smoked meat products – salami, ham, sausages….
- Shellfish
- Beans and pulses – chickpeas, soy beans, peanuts
- Nuts – walnuts, cashew nuts
- Chocolates and other cocoa based products
- Vinegar
- Ready meals
- Salty snacks, sweets with preservatives and artificial colorings

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"Celiac disease has become much more common in the last 50 years, and we don't know why," said Dr Joseph Murray of Mayo Clinic "…Obviously human genes haven't changed, but something has changed in our environment to make this disease more common…."

Why Is Gluten So Tough To Handle?

- There are two unique features to gluten that may partly explain its ability to trigger an immune response. They have a high content of proline in the gluten proteins, that are hard to break down using our natural proteases in the gut lumen.

- The gluten fragments are good substrates for the enzyme TransGlutamase (TG2) converting glutamine residues to glutamate. This increases the ability of the gluten peptides to bind to the genetically inherited molecules HLA-DQ2 or HLA-DQ8.

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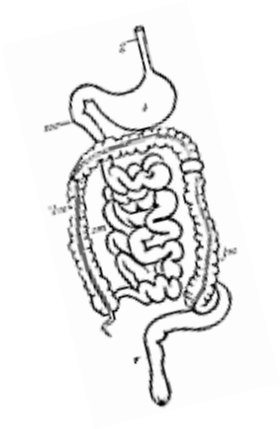
Do You Have The 'Guts' To Be Healthy???



"Death begins in the colon."

Hippocrates

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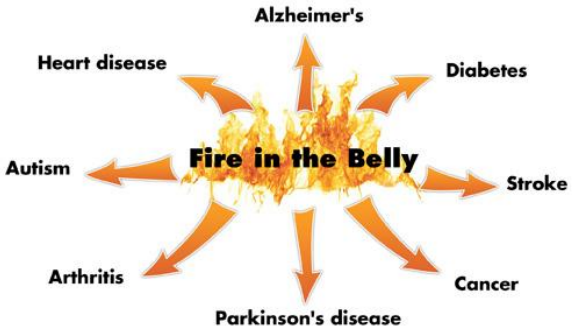

"All diseases start in the intestines"
(Hippocrates)

"The primary seat of insanity is the region of the stomach and intestines."
French psychiatrist Phillipe Pinel (1745– 1826), known as the father of modern psychiatry.

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ORIGINAL RESEARCH ARTICLE–BASIC SCIENCE

The Artificial Sweetener Splenda Promotes Gut *Proteobacteria*, Dysbiosis, and Myeloperoxidase Reactivity in Crohn's Disease–Like Ileitis

Alexander Rodriguez-Palacios, DVM, PhD, Andrew Harding, MD,* Paola Menghini, PhD,* Catherine Himmelman,* Mauricio Retuerto, BSc,† Kourtney P. Nickerson, PhD,¶ Minh Lam, PhD,* Colleen M. Croniger, PhD,‡ Mairi H. McLean, MBChB, PhD,*,*†† Scott K. Durum, PhD,** Theresa T. Pizarro, PhD,§ Mahmoud A. Ghannoum, PhD,† Sanja Ilic, PhD,‡‡ Christine McDonald, PhD,¶ and Fabio Cominelli, MD, PhD*,§§*

Background: Epidemiological studies indicate that the use of artificial sweeteners doubles the risk for Crohn's disease (CD). Herein, we experimentally quantified the impact of 6-week supplementation with a commercial sweetener (Splenda; ingredients sucralose maltodextrin, 1:99, w/w) on both the severity of CD-like ileitis and the intestinal microbiome alterations using SAMP1/YitFc (SAMP) mice.

Methods: Metagenomic shotgun DNA sequencing was first used to characterize the microbiome of ileitis-prone SAMP mice. Then, 16S rRNA microbiome sequencing, quantitative polymerase chain reaction, fluorescent in situ hybridization (FISH), bacterial culture, stereomicroscopy, histology, and myeloperoxidase (MPO) activity analyses were then implemented to compare the microbiome and ileitis phenotype in SAMP with that of control ileitis-free AKR/J mice after Splenda supplementation.

Results: Metagenomics indicated that SAMP mice have a gut microbial phenotype rich in *Bacteroidetes*, and experiments showed that *Helicobacteraceae* did not have an exacerbating effect on ileitis. Splenda did not increase the severity of (stereomicroscopic/histological) ileitis; however, biochemically, ileal MPO activity was increased in SAMP treated with Splenda compared with nonsupplemented mice ($P < 0.022$) and healthy AKR mice. Splenda promoted dysbiosis with expansion of *Proteobacteria* in all mice, and *E. coli* overgrowth with increased bacterial infiltration into the ileal lamina propria of SAMP mice. FISH showed increase *malX* gene–carrying bacterial clusters in the ileu of supplemented SAMP (but not AKR) mice.

Conclusions: Splenda promoted gut *Proteobacteria*, dysbiosis, and biochemical MPO reactivity in a spontaneous model of (*Bacteroidetes*-rich) ileal CD. Our results indicate that although Splenda may promote parallel microbiome alterations in CD-prone and healthy hosts, this did not result in elevated MPO levels in healthy mice, only CD-prone mice. The consumption of sucralose/maltodextrin-containing foods might exacerbate MPO intestinal reactivity only in individuals with a pro-inflammatory predisposition, such as CD.

Key words: Crohn's disease, artificial sweetener, myeloperoxidase activity, *Proteobacteria*, *Bacteroidetes*

INTRODUCTION

Recent self-assessment dietary surveys indicate that ~10% of patients suffering from inflammatory bowel disease (IBD) believe that "sugary foods" worsen the severity of their symptoms and trigger flare-ups.[1,2] Epidemiological studies have correspondingly shown a strong association between the use of artificial sweeteners (AS) and an increased risk for IBD (odds ratio > 2.12; 95% confidence interval [CI], 1.1–4.2),[3,4] but

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Supported by: This work was partially supported by National Institutes of Health Grants DK091222, DK055812, and DK042191 to F.C. and by a Career Development Award from the Crohn's & Colitis Foundation to A.R.P. C.M. received support from grant #1DK082457, and K.P.N. from the Howard Hughes Medical Institute "Med into Grad" Initiative. Special acknowledgement goes to the CCF Imaging Core Leica SP5 Confocal Multi-photon Microscope Shared Instrument Grant 1S10RR026820-01, and the Mouse Models Core and the Histology/Imaging Core of the NIH P30 Silvio O. Conte Cleveland Digestive Disease Research Core Center (DK097948).

Author contributions: A.R.P. and F.C. designed the study. A.H., L.K., C.H., and P.M. assisted with laboratory and mouse experiments. S.K.D. and M.H.M. rederived and provided *Helicobacter*-negative mice. T.T.P. provided mice and samples from alternate mouse facility. B. S.I. and A.R.P. developed and validated the "Parallel Lane Plating" culture strategy. A.R.P., A.H., G.M., M.R., and S.I. led microbiome and microbiology analysis. C.M. and K.P.N. performed qPCR and FISH assays. C.M.C. and L.K. conducted glucose tolerance test assays. F.C., T.T.P., S.I., and M.L. commented on and edited the manuscript. All authors read and approved the manuscript.

Conflicts of interest: All authors declare no competing interests.

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Published online 15 March 2018

Assessing Bowel Toxicity

Indican or Obermeyer's Test

Indications:

- Dysbiosis
- Bowel toxemia
- Candidiasis
- Dysbiosis (lack of good or overgrowth of bad)
- Hypochlorhydria
- Putrefaction – protein maldigestion
- Rancidification – poor fat emulsification
- High protein intake

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Article | Published: 19 March 2018

Extensive impact of non-antibiotic drugs on human gut bacteria

Lisa Maier, Mihaela Pruteanu, Michael Kuhn, Georg Zeller , Anja Telzerow, Exene Erin Anderson, Ana Rita Brochado, Keith Conrad Fernandez, Hitomi Dose, Hirotada Mori, Kiran Raosaheb Patil , Peer Bork & Athanasios Typas

Nature 555, 623–628 (29 March 2018) | Download Citation

Abstract

A few commonly used non-antibiotic drugs have recently been associated with changes in gut microbiome composition, but the extent of this phenomenon is unknown. Here, we screened more than 1,000 marketed drugs against 40 representative gut bacterial strains, and found that 24% of the drugs with human targets, including members of all therapeutic classes, inhibited the growth of at least one strain *in vitro*. Particular classes, such as the chemically diverse antipsychotics, were overrepresented in this group. The effects of human-targeted drugs on gut bacteria are reflected on their antibiotic-like side effects in humans and are concordant with existing human cohort studies. Susceptibility to antibiotics and human-targeted drugs correlates across bacterial species, suggesting common resistance mechanisms, which we verified for some drugs. The potential risk of non-antibiotics promoting antibiotic resistance warrants further exploration. Our results provide a resource for future research on drug–microbiome interactions, opening new paths for side effect control and drug repurposing, and broadening our view of antibiotic resistance.

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EMBL European Molecular Biology Laboratory

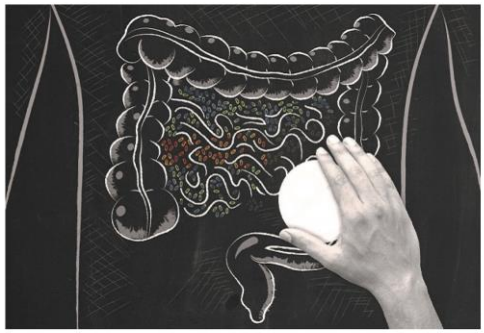
Commonly used drugs affect our gut bacteria

HEIDELBERG, 19 MARCH 2018

Skip to the press release in German

One in four drugs with human targets inhibit the growth of bacteria in the human gut. These drugs cause antibiotic-like side-effects and may promote antibiotic resistance, EMBL researchers report in *Nature* on March 19.

The research team screened over 1000 marketed drugs against 40 representative bacteria from the human gut, and found that more than a quarter of the non-antibiotics (250 out of 923) affect the growth of at least one species in the microbiome. EMBL group leaders Peer Bork, Kiran Patil, Nassos Typas, and Georg Zeller led the work.



25% of the drugs used affect the microbiome!

PLOS ONE

RESEARCH ARTICLE

Altered Gut Microbiota Composition Associated with Eczema in Infants

Huajun Zheng[1☯], Hong Liang[1☯], Yuezhu Wang[2☯], Maohua Miao[1], Tao Shi[3], Fen Yang[1], Enuo Liu[1], Wei Yuan[1*], Zai-Si Ji[1*], De-Kun Li[3]

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Abstract

Eczema is frequently the first manifestation of an atopic diathesis and alteration in the diversity of gut microbiota has been reported in infants with eczema. To identify specific bacterial communities associated with eczema, we conducted a case-control study of 50 infants with eczema (cases) and 51 healthy infants (controls). We performed high-throughput sequencing for V3–V4 hypervariable regions of the 16S rRNA genes from the gut fecal material. A total of 12,386 OTUs (operational taxonomic units) at a 97% similarity level were obtained from the two groups, and we observed a difference in taxa abundance, but not the taxonomic composition, of gut microbiota between the two groups. We identified four genera enriched in healthy infants: *Bifidobacterium*, *Megasphaera*, *Haemophilus* and *Streptococcus*; and five genera enriched in infants with eczema: *Escherichia/Shigella*, *Veillonella*, *Faecalibacterium*, *Lachnospiraceae incertae sedis* and *Clostridium XlVa*. Several species, such as *Faecalibacterium prausnitzii* and *Ruminococcus gnavus*, that are known to be associated with atopy or inflammation, were found to be significantly enriched in infants with eczema. Higher abundance of *Akkermansia muciniphila* in eczematous infants might reduce the integrity of intestinal barrier function and therefore increase the risk of developing eczema. On the other hand, *Bacteroides fragilis* and *Streptococcus salivarius*, which are known for their anti-inflammatory properties, were less abundant in infants with eczema. The observed differences in genera and species between cases and controls in this study may provide insight into the link between the microbiome and eczema risk.

OPEN ACCESS

Citation: Zheng H, Liang H, Wang Y, Miao M, Shi T, Yang F, et al. (2016) Altered Gut Microbiota Composition Associated with Eczema in Infants. PLoS ONE 11(11): e0166026. doi:10.1371/journal.pone.0166026

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Introduction

Eczema is a chronic inflammatory disorder of the skin, which commonly starts during infancy. Its prevalence has been increasing worldwide, and it is more prevalent in affluent societies. It afflicts children mainly in the first year of life (60%) and affects up to 30% of infants [1, 2], while the condition may persist into adulthood. Eczema is frequently the first manifestation of

"The observed differences in genera and species between cases and controls in this study may provide insight into the link between the microbiome and eczema risk".

Zheng H, Liang H, Wang Y, Miao M, Shi T, Yang F, et al. (2016) Altered Gut Microbiota Composition Associated with Eczema in Infants. PLoS ONE 11(11): e0166026. doi:10.1371/journal.pone.0166026

WHAT ARE OPPORTUNISTIC INFECTIONS – ***STEALTH INFECTIONS***?

- The human body carries many "germs" — bacteria, protozoa, fungi, mycoplasma, parasites and viruses. When the immune system is FUNCTIONING OPTIMALLY, it controls these organisms.
- However, when the immune system is weakened or out of balance, these organisms can get out of control and cause major health problems.
- Infections that take advantage of weakness in the immune defenses are called "opportunistic." The phrase "opportunistic infection" is often shortened to "OI."
- They modify Epigenetic Expression!!!!!!!

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Microbiome – Stealth Infections

What are they?

- Viruses
- Bacteria
- Parasites
- Protozoa
- Fungi
- Mycoplasma

These Infections Modify the Immune System causing inflammation, affecting the:

- Brain
- Joints
- Hormones
- Pain
- Virtually anything as we have seen!

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Microbiome – Stealth Infections

Where do these infections live?

- Wherever it is wet and warm
- Liver
- Kidney
- Gums
- Brain

✓ Our job is to find them and create an environment that lets the body heal itself!

✓ Optimize pH– acidic pH depletes O2, virus, bacteria, mycoplasma, bacteria thrive in O2 depleted conditions

✓ Optimize blood sugar regulation

✓ Optimize the Microbiome

You can see how creating an optimal terrain where "Healthy" cells can divide and multiply is so important!!

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Signs of Microbiome Imbalance:

- Digestive issues
  - Gas
  - Bloating
  - Heartburn/acid reflux
  - Diarrhea
  - Constipation
- Mental/brain issues
  - Brain fog
  - OCD
  - Autism
  - Anxiety/depression
- Autoimmune Disease
  - Hashimoto's
  - Grave's disease
  - Rheumatoid arthritis
  - Inflammatory bowel disease
  - Any autoimmune disorder
- Vitamin and Mineral deficiencies
  - D
  - K
  - B12 & B7 (Biotin)
  - Mg
- Stress
- Skin conditions
  - Acne
  - Rosacea
  - Psoriasis
  - Eczema
- Antibiotic use

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PERSPECTIVE | IMMUNOLOGY

How infection can incite sensitivity to food

Elena F. Verdu, Alberto Caminero
+ See all authors and affiliations

Science 07 Apr 2017:
Vol. 356, Issue 6333, pp. 29-30
DOI: 10.1126/science.aan1500

Summary

Immune tolerance to dietary antigens is key to preventing undesirable responses to innocuous antigens ingested with food. On page 44 of this issue, Bouziat *et al.* (*1*) report how viral infection may break oral tolerance to dietary proteins. The findings provide an explanation for the known epidemiological association between viral infections and the onset of food sensitivities, such as celiac disease. The results are of great interest considering the recent increase in prevalence of food allergies and autoimmune disorders, which suggests an unknown environmental risk modifier.

Science
Vol 356, Issue 6333
07 April 2017

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Mechanisms by which gut microorganisms influence food sensitivities

Alberto Caminero, Marlies Meisel, Bana Jabri & Elena F. Verdu✉

Nature Reviews Gastroenterology & Hepatology **16**, 7–18(2019)

Abstract

Finely tuned mechanisms enable the gastrointestinal tract to break down dietary components into nutrients without mounting, in the majority of cases, a dysregulated immune or functional host response. However, adverse reactions to food have been steadily increasing, and evidence suggests that this process is environmental. Adverse food reactions can be divided according to their underlying pathophysiology into food intolerances, when, for instance, there is deficiency of a host enzyme required to digest the food component, and food sensitivities, when immune mechanisms are involved. In this Review, we discuss the clinical and experimental evidence for enteric infections and/or alterations in the gut microbiota in inciting food sensitivity. We focus on mechanisms by which microorganisms might provide direct pro-inflammatory signals to the host promoting breakdown of oral tolerance to food antigens or indirect pathways that involve the metabolism of protein antigens and other dietary components by gut microorganisms. Better understanding of these mechanisms will help in the development of preventive and therapeutic strategies for food sensitivities.

Key points

- The mechanisms underlying the expression of food sensitivities remain unclear; however, several studies demonstrate that gut microorganisms, along with other host predisposing factors, dictate the development of these conditions.
- Gut microorganisms can degrade or modify immunogenic food antigens or allergens, increasing or reducing their immunogenicity.
- Dietary food components that are insufficiently digested by host enzymes become bacterial substrates, leading to the production of metabolites such as short-chain fatty acids, which are involved in gut homeostasis.
- One key factor in the development of food sensitivities is intestinal barrier dysfunction, which can be influenced by gut microorganisms and pathogens through different pathways.
- Mucosal dendritic cells present dietary antigens to naive T helper cells, promoting their differentiation into peripheral T regulatory cells; virus–host interactions abrogate this response, inducing a pathogenic response to antigens.
- Enteric parasites induce T helper 2 cell immunity and protect against food allergy; this contradiction is explained by the observation that parasites induce IL-10, which blocks type 2 immunity.

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RESEARCH

RESEARCH ARTICLE

CELIAC DISEASE

Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease

Romain Bouziat,[1,2]† Reinhard Hinterleitner,[1,2]† Judy J. Brown,[3,4]† Jennifer E. Stencel-Baerenwald,[3,4] Mine Ikizler,[5,7] Toufic Mayassi,[1,2] Marlies Meisel,[1,2] Sangman M. Kim,[1,2] Valentina Discepolo,[1,8] Andrea J. Pruijssers,[9,10] Jordan D. Ernest,[1,2] Jason A. Iskarpatyoti,[4,5] Léa M. M. Costes,[4,7] Ian Lawrence,[1,2] Brad A. Palanski,[6] Mukund Varma,[9,10] Matthew A. Zurenski,[1,2] Solomia Khomandiak,[6,5] Nicole McAllister,[6,4] Pavithra Aravamudhan,[6,5] Karl W. Boehme,[9,11] Fengling Hu,[1] Jamneke N. Samsom,[8] Hans-Christian Reinecker,[8] Sonia S. Kupfer,[1,12] Stefano Guandalini,[13,12] Carol E. Semrad,[1,11] Valérie Abadie,[13] Chaitan Khosla,[6,14,15] Luis B. Barreiro,[16] Ramnik J. Xavier,[6,10,17] Aylwin Ng,[6,10] Terence S. Dermody,[3,4,5,9,18]† Bana Jabri[1,2,11,13,19]†

Viral infections have been proposed to elicit pathological processes leading to the initiation of T helper 1 (T_H1) immunity against dietary gluten and celiac disease (CeD). To test this hypothesis and gain insights into mechanisms underlying virus-induced loss of tolerance to dietary antigens, we developed a viral infection model that makes use of two reovirus strains that infect the intestine but differ in their immunopathological outcomes. Reovirus is an avirulent pathogen that elicits protective immunity, but we discovered that it can nonetheless disrupt intestinal immune homeostasis at inductive and effector sites of oral tolerance by suppressing peripheral regulatory T cell (pT_{reg}) conversion and promoting T_H1 immunity to dietary antigen. Initiation of T_H1 immunity to dietary antigen was dependent on interferon regulatory factor 1 and dissociated from suppression of pT_{reg} conversion, which was mediated by type-1 interferon. Last, our study in humans supports a role for infection with reovirus, a seemingly innocuous virus, in triggering the development of CeD.

Celiac disease (CeD) is a complex immune disorder with an autoimmune component in which genetically susceptible individuals expressing the human leukocyte antigen (HLA) DQ2 or DQ8 molecules display an inflammatory T helper 1 (T_H1) immune response against dietary gluten present in wheat (*1*–*3*). The HLA-DQ2– or HLA-DQ8–restricted T_H1 response against gluten is central to CeD pathogenesis and thought to precede development of villous atrophy (*4*). However, epidemiological and immunological observations support a role for additional genetic and environmental factors in CeD pathogenesis. Similar levels of wheat consumption and expression of CeD-predisposing HLA molecules can be accompanied by striking differences in CeD prevalence (*1*). A remarkable example supporting a role for environmental factors is the high frequency of CeD in Finnish Karelia (>2%), which contrasts with the low incidence of CeD in the adjacent Russian republic of Karelia (0.2%), two neighboring regions harboring genetically similar populations. Furthermore, the incomplete digestion of gluten by intestinal enzymes (*2*, *5*) explains why gluten would be conducive to inducing intestinal T cell responses. However, it does not explain why CeD patients develop a gluten-specific T_H1 response instead of a regulatory immune response, the default intestinal immune reaction to orally ingested protein, characterized by the induction of peripheral regulatory T cells (pT_{reg}) expressing the transcription factor forkhead box P3 (Foxp3) (*6*).

Viral infection experimental model using genetically engineered reoviruses

Despite epidemiological evidence of associations between viral infections and the initiation of CeD (*1*), experimental evidence is lacking. Previous studies have implicated adenovirus, enteroviruses, hepatitis C virus, and rotavirus as triggers of CeD (*7*, *8*). However, little is known about the mechanisms by which viruses evoke the disease. Viruses in the family *Reoviridae* are segmented, double-stranded RNA (dsRNA) viruses that infect humans frequently throughout their lifetime (*9*). Mammalian *Orthoreovirus* (reovirus) strains isolated from humans can infect mice via the oral route and activate innate immune pathways similar to the related rotavirus (*10*, *11*). Two human reovirus isolates, type 1 Lang (T1L) and type 3 Dearing (T3D), differ in replication biology, apoptosis induction, innate immune response activation, cellular tropism, and pathogenesis (*11*). Furthermore, T1L infects the intestine and perturbs intestinal immune homeostasis (*12*, *13*), whereas T3D is incapable of infecting the intestine (*11*). On the basis of the fundamental differences between T1L and T3D, we hypothesized that engineering a T3D reassortant virus capable of intestinal infection would yield two viruses with potentially different effects on tolerance to dietary antigen. Therefore, we recovered a T3D reassortant virus called T3D-RV by introducing the S1 and L2 gene segments of T1L into a T3D genetic background, thus allowing the virus to infect the intestine (fig. S1A) (*12*). Such reassortants arise naturally (*10*, *11*) and can be readily recovered in the laboratory by using reverse genetics (*12*). We first established that the two viruses are similar in their capacity to replicate (fig. S1B) and infect the intestine (fig. S1, C and D). Furthermore, both viruses are cleared (fig. S1E) without inducing intestinal damage (fig. S1F). Although both viruses induced high antireovirus antibody titers, antibody levels observed after T1L infection were significantly higher than those after T3D-RV infection (fig. S1G). However, comparison of the host T cell response in sham- and virus-infected mice revealed that T1L and T3D-RV induced similar T_H1 responses in Peyer's patches (PP) (fig. S1H),

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"Viral infections have been proposed to elicit pathological processes leading to the initiation of T helper 1 (TH1) immunity against dietary gluten and celiac disease (CeD)."

"Last, our study in humans supports a role for infection with reovirus, a seemingly innocuous virus, in triggering the development of CeD."

Bouziat et al., Science 356, 44-50 (2017) 7 April 2017

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Neglected Parasitic Infections in the United States

Most people think of parasitic diseases occurring in poor and developing countries, something they might pick up on an overseas trip. However, parasitic infections still occur in the United States, and in some cases, affect millions of people. Often they can go unnoticed, with few symptoms. But many times these infections cause serious illnesses, including seizures, blindness, heart failure, and even death.

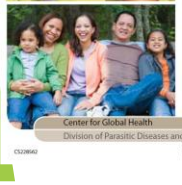
Anyone, regardless of race or economic status, can become infected, although minorities, immigrants, and people living in poor or disadvantaged communities appear to be most at risk. The good news is that most of these infections can be prevented, and many are treatable. However, these infections are often undetected and untreated. Why? Most people do not know they are infected or at risk, or don't have access to appropriate care. And often, health care providers are unfamiliar with these parasitic infections, and may not diagnose or treat them appropriately. We have limited understanding about how many people are infected, or who is most at risk.

There is still a lot we don't know about these infections…but we know enough to act now.

Triatomine bug, which can carry the parasite that causes Chagas disease.

The Five Targeted Infections

CDC has targeted five parasitic infections as priorities for public health action, based on the numbers of people infected, the severity of the illnesses, or our ability to prevent and treat them. These include Chagas disease, neurocysticercosis, toxocariasis, toxoplasmosis, and trichomoniasis.



CDC's Role

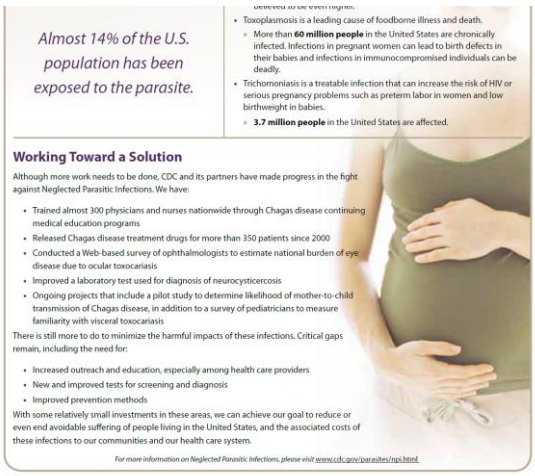
CDC is working to protect people from these health threats by increasing awareness among physicians and the public, synthesizing the existing data to help us better understand these infections, improving diagnostic testing, and for some infections, distributing the needed but otherwise unavailable drugs for treatment.

Center for Global Health
Division of Parasitic Diseases and Malaria

CDC

CS208562 CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.

"Most people think of parasitic diseases occurring in poor and developing countries, something they might pick up on an overseas trip. However, parasitic infections still occur in the United States, and in some cases, affect millions of people. Often they can go unnoticed, with few symptoms. But many times these infections cause serious illnesses, including seizures, blindness, heart failure, and even death."



Almost 14% of the U.S. population has been exposed to the parasite.

believed to be even higher.

- Toxoplasmosis is a leading cause of foodborne illness and death.
  - More than **60 million people** in the United States are chronically infected. Infections in pregnant women can lead to birth defects in their babies and infections in immunocompromised individuals can be deadly.
- Trichomoniasis is a treatable infection that can increase the risk of HIV or serious pregnancy problems such as preterm labor in women and low birthweight in babies.
  - **3.7 million people** in the United States are affected.

Working Toward a Solution

Although more work needs to be done, CDC and its partners have made progress in the fight against Neglected Parasitic Infections. We have:

- Trained almost 300 physicians and nurses nationwide through Chagas disease continuing medical education programs
- Released Chagas disease treatment drugs for more than 350 patients since 2000
- Conducted a Web-based survey of ophthalmologists to estimate national burden of eye disease due to ocular toxocariasis
- Improved a laboratory test used for diagnosis of neurocysticercosis
- Ongoing projects that include a pilot study to determine likelihood of mother-to-child transmission of Chagas disease, in addition to a survey of pediatricians to measure familiarity with visceral toxocariasis

There is still more to do to minimize the harmful impacts of these infections. Critical gaps remain, including the need for:

- Increased outreach and education, especially among health care providers
- New and improved tests for screening and diagnosis
- Improved prevention methods

With some relatively small investments in these areas, we can achieve our goal to reduce or even end avoidable suffering of people living in the United States, and the associated costs of these infections to our communities and our health care system.

For more information on Neglected Parasitic Infections, please visit www.cdc.gov/parasites/npi.html

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Neglected Parasitic Infections: What Every Family Physician Needs to Know

DANA WOODHALL, MD; JEFFREY L. JONES, MD, MPH; PAUL T. CANTEY, MD, MPH; PATRICIA P. WILKINS, PhD; and SUSAN P. MONTGOMERY, DVM, MPH, *Centers for Disease Control and Prevention, Atlanta, Georgia*

Neglected parasitic infections, including Chagas disease, toxocariasis, cysticercosis, and toxoplasmosis, affect millions of persons in the United States. Relatively few resources have been devoted to surveillance, prevention, and treatment of these diseases. Chagas disease primarily affects Latin American immigrants and can cause heart failure and death if not treated. Immediate antiparasitic treatment is indicated for most patients with acute Chagas disease. Treatment is recommended for patients younger than 18 years who have chronic Chagas disease and is generally recommended for adults younger than 50 years who do not have advanced cardiomyopathy; treatment decisions for other patients should be made on an individual basis. Toxocariasis primarily affects children and can cause gastrointestinal, respiratory, and ophthalmologic disease. Treatment options include albendazole and mebendazole. Patients with ocular infection require referral to an ophthalmologist. Neurocysticercosis, a form of cysticercosis, is the most common infectious cause of seizures in some parts of the United States. Initial treatment should focus on symptom control. Humans generally acquire toxoplasmosis by eating undercooked contaminated meat or ingesting things that have been contaminated with cat feces. Congenital infection can result in miscarriage or adverse fetal effects. Treatment is recommended for immunosuppressed persons, pregnant women, and immunocompetent persons with severe symptoms. (*Am Fam Physician*. 2014;89(10):803-811. Copyright © 2014 American Academy of Family Physicians.)

CME This clinical content conforms to AAFP criteria for continuing medical education (CME). See CME Quiz Questions on page 792.

Author disclosure: No relevant financial affiliations.

Neglected parasitic infections, including Chagas disease, toxocariasis, cysticercosis, and toxoplasmosis, can cause severe illness, but limited resources have been devoted to better understanding their impact and burden. Physicians may not be familiar with these infections because their clinical presentation, diagnosis, and treatment are typically not emphasized during medical training. However, it is crucial for family physicians to understand the basic principles of diagnosis and treatment of these diseases. A summary of the key points about epidemiology, clinical manifestations, diagnostic evaluation, and treatment for each disease is presented in *Table 1*.

Chagas Disease

Chagas disease, also known as American trypanosomiasis, is caused by the parasite *Trypanosoma cruzi*. Transmission to humans occurs mainly through contact with insects. Infection occurs when an infected triatomine defecates after a blood meal and the feces, which contains the parasite, is rubbed into the bite wound or mucous membranes (*Figure 1*).[1] Transmission can also occur congenitally or via blood transfusion, organ transplantation, contaminated food, or laboratory exposure. Chagas disease is endemic throughout Mexico and Central and South America, where an estimated 8 to 11 million persons are infected.[2] More than 300,000 persons in the United States are thought to be infected,[2] most of whom acquired the disease in Latin America. However, infected triatomines have been found in the United States, and domestic vector-borne transmission has occurred.[3]

There are two phases of the disease: acute, which lasts for weeks or months after the initial infection, and chronic. Infection is lifelong in the absence of treatment. Clinical manifestations are often mild or absent in the acute phase; swelling around the bite site may be present. If the inoculation site is the conjunctiva, unilateral palpebral edema may occur. Most patients with chronic Chagas disease remain asymptomatic, but 20% to 30% of persons with the infection develop clinical manifestations that can be life-threatening.[4] Cardiac disease, including conduction abnormalities, apical aneurysm,

"Neglected parasitic infections, including Chagas disease, toxocariasis, cysticercosis, and toxoplasmosis, affect millions
of persons in the United States."

"Congenital infection can result in miscarriage or adverse fetal effects. Treatment is recommended for immunosuppressed persons, pregnant women, and immunocompetent persons with severe symptoms."

Am Fam Physician. 2014;89(10):803-811. Copyright © 2014 American Academy of Family Physicians.)

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ScienceDaily

Your source for the latest research news

Scientists unpack how Toxoplasma infection is linked to neurodegenerative disease

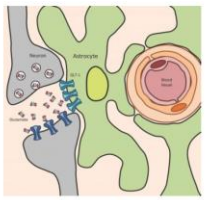
New research focused on glutamate, the most important neurotransmitter in the brain

Date: June 9, 2016

Source: University of California - Riverside

Summary: Toxoplasma gondii, a protozoan parasite, infects a third of the world's population. Working on mice, biomedical scientists report that Toxoplasma infection leads to a disruption of neurotransmitters in the brain and postulates that it triggers neurological disease in those already predisposed to such a disease. The researchers note that Toxoplasma infection leads to a significant increase in glutamate -- the primary and most important neurotransmitter in the brain.

FULL STORY



GLT-1, a glutamate transporter, soaks up glutamate (a neurotransmitter) released by neurons and converts it back into a safer substance.

Credit: Wilson lab, UC Riverside.

Toxoplasma gondii, a protozoan parasite about five microns long, infects a third of the world's population. Ingested via undercooked meat or unwashed vegetables, the parasite infects 15-30 percent of the US population. In France and Brazil, up to 80 percent of the population has the infection.

Particularly dangerous during pregnancy -- infection in pregnant women can cause serious congenital defects and even death of the fetus -- this chronic infection has two components: the unicellular parasite, and inflammation of tissues it causes.

Toxoplasma gondii, a protozoan parasite, infects a third of the world's population.

The researchers note that Toxoplasma infection leads to a significant increase in glutamate
-- the primary and most important neurotransmitter in the brain.

the parasite infects 15-30 percent of the US population.

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Case Reports

Eradication of *Blastocystis hominis* prevents the development of symptomatic Hashimoto's thyroiditis: a case report

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[3] The Public Institution Health Centre of Stolac, Stolac, Bosnia and Herzegovina

Abstract

In this case report we describe a 49 year-old man who presented with chronic urticaria, angioedema and soft stool consistency. During diagnostic examinations Hashimoto's thyroiditis was found even though the patient never had clear symptoms of this disease. *Blastocystis hominis* was isolated through a stool microbiologic examination, implicating that this parasite can cause the development of Hashimoto's thyroiditis and chronic urticaria. After two-weeks treatment with metronidazole the *Blastocystis hominis* was eradicated, then urticaria and angioedema disappeared. During the four years of follow-up, the patient presented without any symptoms, whereas thyroid hormones were normalized and anti-thyroid antibodies declined. For the first time in the literature we show that eradication of *Blastocystis hominis* can prevent the development of both symptomatic Hashimoto's thyroiditis and chronic urticaria.

Key words: *Blastocystis hominis;* urticarial, angioedema; Hashimoto thyroiditis; metronidazole.

J Infect Dev Ctries 2015; 9(7):788-791. doi:10.3855/jidc.4851

(Received 12 February 2014 – Accepted 19 December 2014)

Introduction

Blastocystis hominis is the most common protozoan parasite in humans with incidence between 5-75% depending on the country's level of development [1-3]. Previously, it was considered as a non-pathogenic parasite [4]. However, a number of case reports recently showed that *Blastocystis hominis* infection is associated with chronic urticaria [5-7]. The study of Zuel-Fakkar *et al.* reported that *Blastocystis hominis* was found in 60.6% of patients with urticaria, whereas no parasite was isolated in healthy controls [8]. In general, urticaria is a very common skin disorder that can have immune, non-immune or idiopathic causes [9]. The idiopathic urticaria is accounted for in 75% of all urticaria cases [10]. Skin lesions appearing within a six-week period characterize acute urticaria, whereas chronic urticaria is defined as the presence of urticaria over a longer period of time. The prevalence of chronic urticaria is around one percent in the general population. Around 40% of patients with chronic urticaria have accompanying angioedema, typically affecting face, lips and periorbital region [11]. Chronic urticaria has also been associated with the presence of anti-thyroid antibodies, or autoimmune thyroid disease, such as Hashimoto's thyroiditis with reported prevalence from 12-29% [11-14]. Urticaria is usually treated with oral antihistamines, but in some cases more aggressive treatment with corticosteroids, or cyclosporine is required [15].

It has been demonstrated that *Blastocystis hominis* can cause cutaneous allergies by activation of specific Th2 immune cells producing interleukins IL-3, IL-4, IL-5 and IL-13, which mediate IgE allergic response [2,16]. However, it has not been reported so far that *Blastocystis hominis* is a pathogen that could directly promote the development of autoimmune disease. In this case report we present how the treatment of *Blastocystis hominis* can prevent the development of urticaria and symptomatic Hashimoto's thyroiditis.

Case Report

A previously healthy 49 year-old man presented with urticaria, starting in his forearms and his back that lasted for a few hours and disappeared without treatment. After a few days the same symptoms appeared again, but then he had angioedema of upper lip. This urged him to seek medical help. During the

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Possible Signs/Symptoms associated with Parasites

- Itching – especially around mouth, nose and anus
- Memory problems
- Mood disorders
- Strong cravings for processed and sugary foods
- Recurring yeast infections like Candida
- Anemia or iron deficiency (worms can create enough blood loss to cause anemia or iron
- deficiency)
- Skin ailments such as hives, rashes, weeping eczema, itchy dermatitis, acne, ulcers, sores, lesions, etc.
- Bleeding gums
- Headaches
- Anxiety
- Nervousness
- Teeth grinding and drooling during sleep
- Food allergies/food sensitivities
- Loss of appetite
- Sexual dysfunction
- Chronic fatigue
- Unhealthy food cravings
- Persistent digestive problems (cramps, bloating, gas, etc.)
- Hungry all the time
- Sore/stiff joints
- Breathing problems

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Krogsgaard et al. *Clinical and Translational Gastroenterology* (2018)9:161
DOI 10.1038/s41424-018-0027-2

Clinical and Translational Gastroenterology

ARTICLE Open Access

Characteristics of the bacterial microbiome in association with common intestinal parasites in irritable bowel syndrome

Laura Rindom Krogsgaard[1,2], Lee O 'Brien Andersen[3], Thor Bech Johannesen[3], Anne Line Engsbro[4], Christen Rune Stensvold[3], Henrik Vedel Nielsen[3] and Peter Bytzer[1,2]

Abstract

Objective: A low prevalence of intestinal parasites has been identified in individuals with irritable bowel syndrome (IBS). But potential associations with alterations in the bacterial microbiome remain largely unexplored. We aimed to investigate the relationship between parasites and bacteria in individuals with IBS in order to identify potential trans-kingdom microbial characteristics.

Design: Stool samples were collected from the Danish background population classified into IBS ($n = 119$), unspecific gastrointestinal (GI) symptoms ($n = 114$), and asymptomatic controls ($n = 186$) based on the Rome III criteria for IBS. Bacterial (16S) and eukaryotic (18S) ribosomal DNA was sequenced, and 18S data were merged with data from conventional parasite laboratory tests. The bacterial microbiome was analyzed according to symptom group and parasite colonization status.

Results: Bacterial richness and diversity were similar for IBS and controls but higher in those with unspecific GI symptoms. A higher abundance of Bacteroides and a lower abundance of Faecalibacterium were detected in individuals with IBS and unspecific GI symptoms compared with controls. Principal component analyses indicated differences in bacterial composition related to parasite colonization rather than symptom group. Parasites were detected at the lowest frequency in the IBS group (39%) and in samples dominated by Bacteroides. Higher bacterial richness and diversity were found in parasite-positive samples from controls and those with unspecific GI symptoms but not in individuals with IBS.

Conclusion: Parasite colonization, rather than bacterial composition, differed between individuals with IBS and healthy controls. Parasite colonization was associated to a rich and diverse bacterial microbiome; however, this association was altered in IBS.

Introduction

The intestinal parasites *Dientamoeba fragilis* and *Blastocystis* were previously found to colonize individuals with IBS at a lower frequency compared with asymptomatic controls[1]. This finding indicates that our understanding of the role of some intestinal parasites in gastrointestinal (GI) health and disease is limited. Several studies have reported a difference in the composition of the bacterial microbiome in relation to IBS[2–16], although with inconsistent findings. Organisms from the different kingdoms of the gut microbiome coexist and interact[17], and given the fact that some intestinal parasites, such as *Blastocystis*

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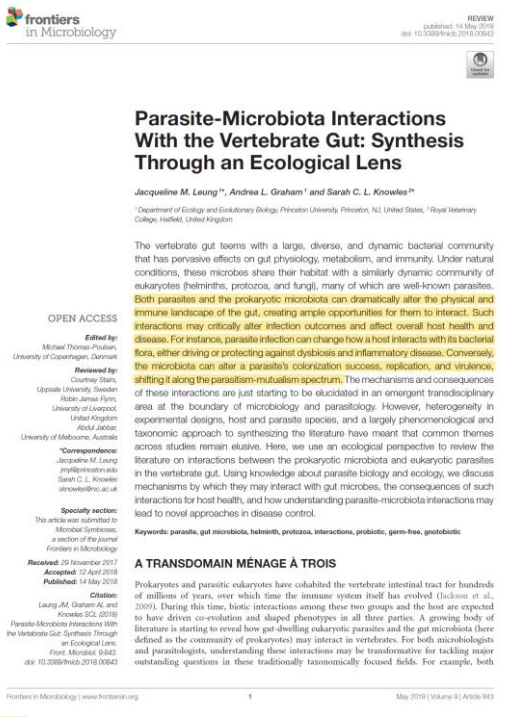
Official journal of the American College of Gastroenterology

"…but potential associations with alterations in the bacterial microbiome remain largely unexplored."

Conclusion: Parasite colonization, rather than bacterial composition, differed between individuals with IBS and healthy controls. Parasite colonization was associated to a rich and diverse bacterial microbiome; however, this association was altered in IBS.

This finding indicates that our understanding of the role of some intestinal parasites in gastrointestinal (GI) health and disease is limited.

Krogsgaard et al. Clinical and Translational Gastroenterology (2018) 9:161
DOI 10.1038/s41424-018-0027-2



frontiers in Microbiology

REVIEW
published: 14 May 2018
doi: 10.3389/fmicb.2018.00843

Parasite-Microbiota Interactions With the Vertebrate Gut: Synthesis Through an Ecological Lens

Jacqueline M. Leung[1]*, Andrea L. Graham*[1] *and Sarah C. L. Knowles*[2]*

[1] Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, United States, [2] Royal Veterinary College, Hatfield, United Kingdom

The vertebrate gut teems with a large, diverse, and dynamic bacterial community that has pervasive effects on gut physiology, metabolism, and immunity. Under natural conditions, these microbes share their habitat with a similarly dynamic community of eukaryotes (helminths, protozoa, and fungi), many of which are well-known parasites. Both parasites and the prokaryotic microbiota can dramatically alter the physical and immune landscape of the gut, creating ample opportunities for them to interact. Such interactions may critically alter infection outcomes and affect overall host health and disease. For instance, parasite infection can change how a host interacts with its bacterial flora, either driving or protecting against dysbiosis and inflammatory disease. Conversely, the microbiota can alter a parasite's colonization success, replication, and virulence, shifting it along the parasitism-mutualism spectrum. The mechanisms and consequences of these interactions are just starting to be elucidated in an emergent transdisciplinary area at the boundary of microbiology and parasitology. However, heterogeneity in experimental designs, host and parasite species, and a largely phenomenological and taxonomic approach to synthesizing the literature have meant that common themes across studies remain elusive. Here, we use an ecological perspective to review the literature on interactions between the prokaryotic microbiota and eukaryotic parasites in the vertebrate gut. Using knowledge about parasite biology and ecology, we discuss mechanisms by which they may interact with gut microbes, the consequences of such interactions for host health, and how understanding parasite-microbiota interactions may lead to novel approaches in disease control.

Keywords: parasite, gut microbiota, helminth, protozoa, interactions, probiotic, germ-free, gnotobiotic

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A TRANSDOMAIN MÉNAGE À TROIS

Prokaryotes and parasitic eukaryotes have cohabited the vertebrate intestinal tract for hundreds of millions of years, over which time the immune system itself has evolved (Jackson et al., 2009). During this time, biotic interactions among these two groups and the host are expected to have driven co-evolution and shaped phenotypes in all three parties. A growing body of literature is starting to reveal how gut-dwelling eukaryotic parasites and the gut microbiota (here defined as the community of prokaryotes) may interact in vertebrates. For both microbiologists and parasitologists, understanding these interactions may be transformative for tackling major outstanding questions in these traditionally taxonomically focused fields. For example, both

"Both parasites and the prokaryotic microbiota can dramatically alter the physical and immune landscape of the gut, creating ample opportunities for them to interact. Such interactions may critically alter infection outcomes and affect overall host health and disease. For instance, parasite infection can change how a host interacts with its bacterial flora, either driving or protecting against dysbiosis and inflammatory disease."

Leung JM, Graham AL and Knowles SCL (2018) Parasite-Microbiota Interactions With the Vertebrate Gut: Synthesis Through an Ecological Lens. Front. Microbiol. 9:843.
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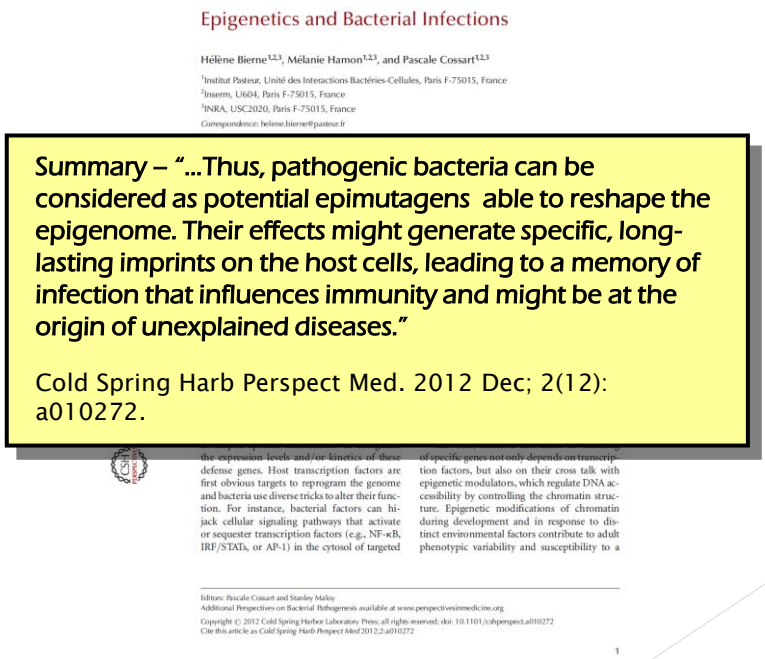

Epigenetics and Bacterial Infections

Hélène Bierne[1,2,3], Mélanie Hamon[1,2,3], and Pascale Cossart[1,2,3]

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Summary – "...Thus, pathogenic bacteria can be considered as potential epimutagens able to reshape the epigenome. Their effects might generate specific, long-lasting imprints on the host cells, leading to a memory of infection that influences immunity and might be at the origin of unexplained diseases."

Cold Spring Harb Perspect Med. 2012 Dec; 2(12): a010272.

the expression levels and/or kinetics of these defense genes. Host transcription factors are first obvious targets to reprogram the genome and bacteria use diverse tricks to alter their function. For instance, bacterial factors can hijack cellular signaling pathways that activate or sequester transcription factors (e.g., NF-κB, IRF/STATs, or AP-1) in the cytosol of targeted

of specific genes not only depends on transcription factors, but also on their cross talk with epigenetic modulators, which regulate DNA accessibility by controlling the chromatin structure. Epigenetic modifications of chromatin during development and in response to distinct environmental factors contribute to adult phenotypic variability and susceptibility to a

Editors: Pascale Cossart and Stanley Maloy
Additional Perspectives on Bacterial Pathogenesis available at www.perspectivesinmedicine.org

Cite this article as *Cold Spring Harb Perspect Med* 2012;2:a010272

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Bacterial Loads

Lyme Disease

Lyme disease is NOT the only bacterium that we are exposed too, or need to be concerned with!!!! Thus, creating another factor tipping the scales away from a healthy balance.

infection in the United States. It occurs in 49 states. More than 90% of the cases occur along the northeastern coast from Maine to Virginia and in Wisconsin, Minnesota, and Michigan. On the West Coast, most cases occur in northern California and Oregon. Lyme disease also occurs in Europe, China, Japan, and the former Soviet Union

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Viral Loads

Stress Flips a Chromatin Switch to Wake Up Latent Virus
Daphne C. Avgousti, Matthew D. Weitzman
[1] Department of Pathology and Laboratory Medicine, University of Pennsylvania

Discovery shows how herpes simplex virus reactivates in neurons to trigger disease
December 9, 2015

When we get cold sores, the reason is likely related to stress. In particular, the neurons in which the herpes simplex virus (HSV) reside, are under stress. For the first time, researchers at the University of North Carolina School of Medicine discovered a cellular mechanism that allows the virus to reactivate. They also found how brain cells are duped into allowing bits of virus to escape the very repressive environment in neurons and cause disease.

HSV is found in about 90 percent of the United States population and leads to cold sores, recurrent eye infections, genital lesions, and in rare cases encephalitis - inflammation of the brain which has a 30 percent mortality rate (70 to 80 percent if left untreated). Its closely related virus, VZV, also causes chicken pox and shingles.

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Viral Loads

Trends Microbiol. 2010 Oct; 18(10): 439–447. doi: 10.1016/j.tim.2010.07.003

Epigenetic reprogramming of host genes in viral and microbial pathogenesis

Konstantinos Paschos and Martin J. Allday
Section of Virology, Division of Infectious Diseases, Faculty of Medicine, Imperial Coll

Abs

One ow epig stablished. The th the rep be inv tle reviews examples of viruses and bacteria known or thought to induce epigenetic changes in host cells, and how this might contribute to disease. The heritable nature of these processes in gene regulation suggests that they could play important roles in chronic diseases associated with microbial persistence; they might also explain so-called 'hit-and-run' phenomena in infectious disease pathogenesis.
PMCID: PMC3089700

Summary – "…This article reviews examples of viruses and bacteria known or thought to induce epigenetic changes in host cells, and how this might contribute to disease"

Trends Microbiol. 2010 Oct; 18(10): 439–447.

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nature genetics

Published: 16 April 2018

Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity

John B. Harley, Xiaoting Chen, Mario Pujato, Daniel Miller, Avery Maddox, Carmy Forney, Albert F. Magnusen, Arthur Lynch, Kashish Chetal, Masashi Yukawa, Artem Barski, Nathan Salomonis, Kenneth M. Kaufman, Leah C. Kottyan & Matthew T. Weirauch

Nature Geneticsvolume 50, pages699–707 (2018)

Abstract:
Explaining the genetics of many diseases is challenging because most associations localize to incompletely characterized regulatory regions. Using new computational methods, we show that transcription factors (TFs) occupy multiple loci associated with individual complex genetic disorders. Application to 213 phenotypes and 1,544 TF binding datasets identified 2,264 relationships between hundreds of TFs and 94 phenotypes, including androgen receptor in prostate cancer and GATA3 in breast cancer. Strikingly, nearly half of systemic lupus erythematosus risk loci are occupied by the Epstein–Barr virus EBNA2 protein and many coclustering human TFs, showing gene–environment interaction. **Similar EBNA2-anchored associations exist in multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, juvenile idiopathic arthritis and celiac disease. Instances of allele-dependent DNA binding and downstream effects on gene expression at plausibly causal variants support genetic mechanisms dependent on EBNA2. Our results nominate mechanisms that operate across risk loci within disease phenotypes, suggesting new models for disease origins**

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PubMed

Format: Abstract

Future Virol. 2019 May;14(5):335-347. doi: 10.2217/fvl-2019-0002. Epub 2019 May 15.

Gut microbial metabolites associated with HIV infection.

Wang Z[1], Qi Q[1].

Author information

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Abstract
HIV infection has been associated with alterations in gut microbiota and related microbial metabolite production. However, the mechanisms of how these functional microbial metabolites may affect HIV immunopathogenesis and comorbidities, such as cardiovascular disease and other metabolic diseases, remain largely unknown. Here we review the current understanding of gut microbiota and related metabolites in the context of HIV infection. We focus on several bacteria-produced metabolites, including tryptophan catabolites, short-chain fatty acids and trimethylamine-N-oxide (TMAO), and discuss their implications in HIV infection and comorbidities. We also prospect future studies using integrative multiomics approaches to better understand host-microbiota-metabolites interactions in HIV infection, and facilitate integrative medicine utilizing the microbiota in HIV infection.

KEYWORDS: HIV; integrative omics; metabolites; metabolomics; microbiota

PMID: 31263508 PMCID: PMC6595475 [Available on 2020-05-01] DOI: 10.2217/fvl-2019-0002

"Here we review the current understanding of gut microbiota and related metabolites in the context of HIV infection. We focus on several bacteria-produced metabolites, including tryptophan catabolites, short-chain fatty acids and trimethylamine-N-oxide (TMAO), and discuss their implications in HIV infection and comorbidities. We also prospect future studies using integrative multiomics approaches to better understand host-microbiota-metabolites interactions in HIV infection, and facilitate integrative medicine utilizing the microbiota in HIV infection."

Future Virol. 2019 May;14(5):335-347. doi: 10.2217/fvl-2019-0002. Epub 2019 May 15.

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DNA Methylation: The Original Anti-Virus Program

POSTED JANUARY 8, 2014

"Security and anti-virus software is a must-have accessory for the internet age, but it turns out that DNA methylation has been protecting us all from retroviral infections for quite a bit longer than any computer program."

A talented research team lead by Richard Meehan from the University of Edinburgh (Scotland) applied HELP-seq analysis and DNA methylation mutants as a model to investigate how retrotransposon activation is selective and context dependent.

"The team scoured the methylome data and made a number of precise novel observations with respect to the specificity of activation; which classes of repeats are activated in mutants and which are not, and the effect of repeat activation in relation to neighboring genes."

Quote from - Richard Meehan from the University of Edinburgh

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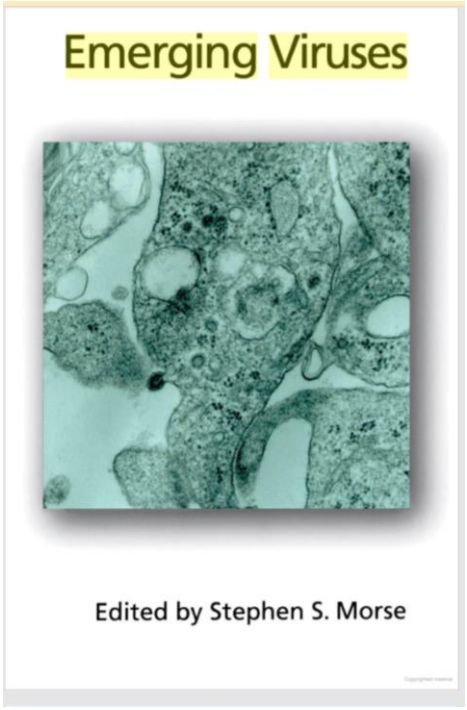

How many different viruses are there on planet Earth?

Twenty years ago Stephen Morse suggested hat there were about one million viruses of vertebrates (he arrived at this calculation by assuming ~20 different viruses in each of the 50,000 vertebrates on the planet). The results of a new study suggest that at least 320,000 different viruses infect mammals.

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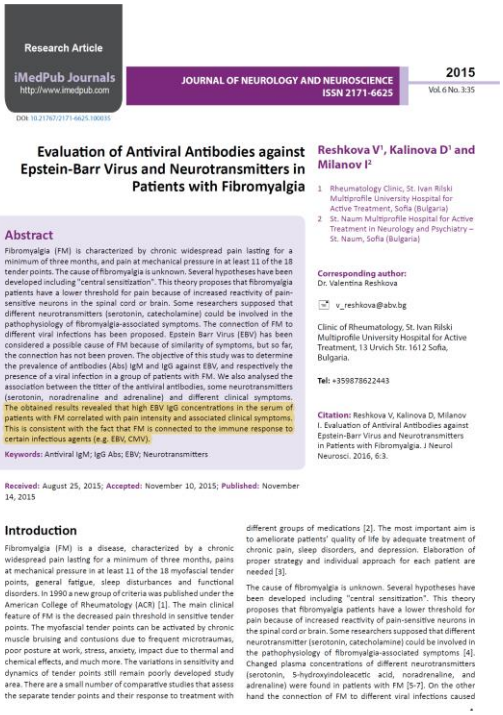

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Evaluation of Antiviral Antibodies against Epstein-Barr Virus and Neurotransmitters in Patients with Fibromyalgia

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Citation: Reshkova V, Kalinova D, Milanov I. Evaluation of Antiviral Antibodies against Epstein-Barr Virus and Neurotransmitters in Patients with Fibromyalgia. J Neurol Neurosci. 2016, 6:3.

Abstract

Fibromyalgia (FM) is characterized by chronic widespread pain lasting for a minimum of three months, and pain at mechanical pressure in at least 11 of the 18 tender points. The cause of fibromyalgia is unknown. Several hypotheses have been developed including "central sensitization". This theory proposes that fibromyalgia patients have a lower threshold for pain because of increased reactivity of pain-sensitive neurons in the spinal cord or brain. Some researchers supposed that different neurotransmitters (serotonin, catecholamine) could be involved in the pathophysiology of fibromyalgia-associated symptoms. The connection of FM to different viral infections has been proposed. Epstein Barr Virus (EBV) has been considered a possible cause of FM because of similarity of symptoms, but so far, the connection has not been proven. The objective of this study was to determine the prevalence of antibodies (Abs) IgM and IgG against EBV, and respectively the presence of a viral infection in a group of patients with FM. We also analysed the association between the titter of the antiviral antibodies, some neurotransmitters (serotonin, noradrenaline and adrenaline) and different clinical symptoms. The obtained results revealed that high EBV IgG concentrations in the serum of patients with FM correlated with pain intensity and associated clinical symptoms. This is consistent with the fact that FM is connected to the immune response to certain infectious agents (e.g. EBV, CMV).

Keywords: Antiviral IgM; IgG Abs; EBV; Neurotransmitters

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Introduction

Fibromyalgia (FM) is a disease, characterized by a chronic widespread pain lasting for a minimum of three months, pains at mechanical pressure in at least 11 of the 18 myofascial tender points, general fatigue, sleep disturbances and functional disorders. In 1990 a new group of criteria was published under the American College of Rheumatology (ACR) [1]. The main clinical feature of FM is the decreased pain threshold in sensitive tender points. The myofascial tender points can be activated by chronic muscle bruising and contusions due to frequent microtraumas, poor posture at work, stress, anxiety, impact due to thermal and chemical effects, and much more. The variations in sensitivity and dynamics of tender points still remain poorly developed study area. There are a small number of comparative studies that assess the separate tender points and their response to treatment with different groups of medications [2]. The most important aim is to ameliorate patients' quality of life by adequate treatment of chronic pain, sleep disorders, and depression. Elaboration of proper strategy and individual approach for each patient are needed [3].

The cause of fibromyalgia is unknown. Several hypotheses have been developed including "central sensitization". This theory proposes that fibromyalgia patients have a lower threshold for pain because of increased reactivity of pain-sensitive neurons in the spinal cord or brain. Some researchers supposed that different neurotransmitter (serotonin, catecholamine) could be involved in the pathophysiology of fibromyalgia-associated symptoms [4]. Changed plasma concentrations of different neurotransmitters (serotonin, 5-hydroxyindoleacetic acid, noradrenaline, and adrenaline) were found in patients with FM [5-7]. On the other hand the connection of FM to different viral infections caused

"The obtained results revealed that high EBV IgG concentrations in the serum of patients with FM correlated with pain intensity and associated clinical symptoms. This is consistent with the fact that FM is connected to the immune response to certain infectious agents (e.g. EBV, CMV)."

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frontiers in Molecular Neuroscience

MINI REVIEW
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Enteroviral Infection: The Forgotten Link to Amyotrophic Lateral Sclerosis?

*Yuan Chao Xue[1,2], Ralph Feuer[3], Neil Cashman[4] and Honglin Luo[1,2]**

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that primarily attacks motor neurons in the brain and spinal cord, leading to progressive paralysis and ultimately death. Currently there is no effective therapy. The majority of ALS cases are sporadic, with no known family history; unfortunately the etiology remains largely unknown. Contribution of Enteroviruses (EVs), a family of positive-stranded RNA viruses including poliovirus, coxsackievirus, echovirus, enterovirus-A71 and enterovirus-D68, to the development of ALS has been suspected as they can target motor neurons, and patients with prior poliomyelitis show a higher risk of motor neuron disease. Multiple efforts have been made to detect enteroviral genome in ALS patient tissues over the past two decades; however the clinical data are controversial and a causal relationship has not yet been established. Recent evidence from *in vitro* and animal studies suggests that enterovirus-induced pathology remarkably resembles the cellular and molecular phenotype of ALS, indicating a possible link between enteroviral infection and ALS pathogenesis. In this review, we summarize the nature of enteroviral infection, including route of infection, cells targeted, and viral persistence within the central nervous system (CNS). We review the molecular mechanisms underlying viral infection and highlight the similarity between viral pathogenesis and the molecular and pathological features of ALS, and finally, discuss the potential role of enteroviral infection in frontotemporal dementia (FTD), a disease that shares common clinical, genetic, and pathological features with ALS, and the significance of anti-viral therapy as an option for the treatment of ALS.

Keywords: amyotrophic lateral sclerosis, enterovirus, TDP-43 pathology, nucleocytoplasmic trafficking, RNA metabolism, autophagy, neuroinflammation

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease marked by progressive degeneration of both upper and lower motor neurons, resulting in paralysis and eventual death within 3–5 years after diagnosis (Brown and Al-Chalabi, 2017; Hardiman et al., 2017; van Es et al., 2017). Despite primarily a motor neuron disease, nearly 50% of ALS patients show cognitive/behavioral impairments, suggesting an involvement of non-motor systems in ALS pathogenesis (Hardiman et al., 2017). The overall prevalence of ALS in Europe and North America is estimated at ~3–5 cases per 100,000 people and increases with age (Brown and Al-Chalabi, 2017).

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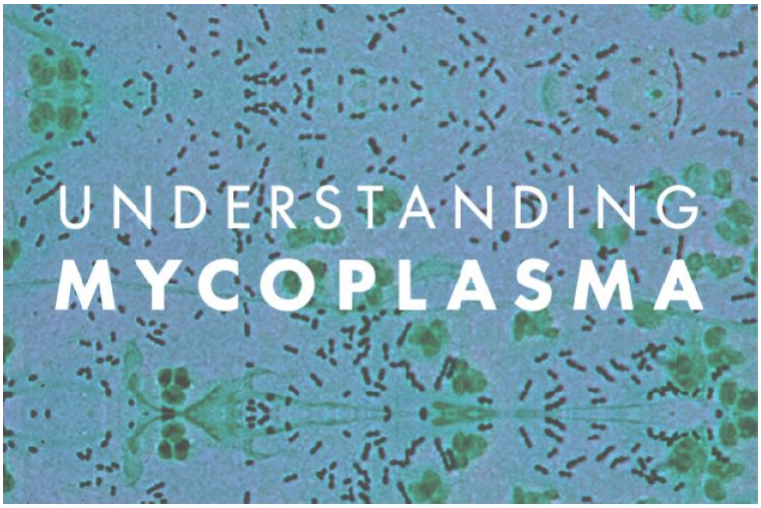

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REVIEW

Fungal stealth technology

Chad A. Rappleye, William E. Goldman

PlumX Metrics

DOI: https://doi.org/10.1016/j.it.2007.10.001

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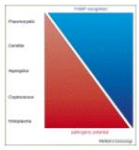
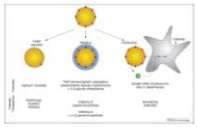
Medically important fungi range from commensal organisms that cause opportunistic infections to primary fungal pathogens that can cause disease in immunocompetent hosts. Host phagocyte-expressed pattern-recognition receptors represent one obstacle to infection, and the extent to which fungal cells can evade detection by host receptors helps shape their pathogenic potential. This review highlights recently defined mechanisms employed by successful fungal pathogens to conceal their immunostimulatory molecular signatures from leukocyte receptors or to disrupt host response signals. Continued improvements in our understanding of these fungal stealth mechanisms should provide new options for future therapeutics to expose these fungal pathogens and limit their virulence capacity.





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How to identify and treat Stealth Infections

- Using Simple tests to alert you that an infection is an underlying issue
  - Indican
  - Romberg's – remember Gary
- Use history and physical findings to determine more precise treatment plan: cold sores, warts, history of mono, recurring yeast infections, etc.
- For example using a CBC with differential
- Total WBC under 5.0– means chronic infection
- Total WBC greater than 8 – could be acute infection
- Lymphocytes closer to Neutrophils suggest viral component

Monocytes over 7* suggest Microbiome imbalances

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How to "assess " Stealth Infections

Polys	55 – 65	40 – 74	Also known as neutrophils, it makes up the majority of the white blood cells. It is the body's first line of defense.
Lymphocytes	25 – 40	14 – 46	The second most abundant white blood cell. They are aggressively antiviral. They manufacture globulins which react with antigens.
Monocytes	3 – 7	4 – 13	Derived from stem cells in the bone marrow. They are primarily phagocytic working outside the blood vessels.
Eosinophils	0 – 3	0 – 7	These white blood cells are elevated in IgE mediated allergies and when there are parasites present.
Basophils	<1	0 – 3	These white blood cells are elevated with toxic allergic reactions.
Sedimentation Rate (ESR)	0 – 8	0 – 30	This test is particularly important in chronic inflammatory disease. It measures how quickly red blood cells settle or coagulate.

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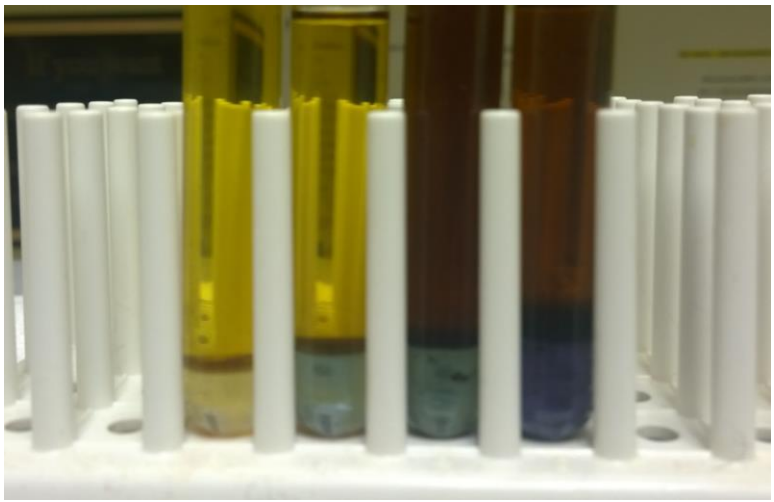
How to "assess " Stealth Infections



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How to Treat Stealth Infections

<u>Bacteria</u> – Concomitantly addressing pH, adrenals, and digestion

- E.N.V (GBLV) homeopathic – Zorex (10 – 15 drops tid)
- Caprin (4 – 6 tid)
- Organic Oregano Oil Blend Emulsified – 3–5 drops in water tid
- ADP (3 – 4 tid)
- NAC (N-Acetyl-L-Cysteine) (1 tid for 1 week, then 1 bid)
- Butyric-Cal-Mag
- Food-Grade Diatomaceous Earth (1/2 – 1 tsp bid) ???

- Considerations:
  - ✓ Bio–Immunozyme
  - ✓ Cytozyme THY
  - ✓ IAG
  - ✓ Kleen Mouth – Zorex

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How to Treat Stealth Infections

Virus – Concomitantly addressing pH, adrenals, and digestion. Generally after bacteria, get the Low-Hanging Fruit.

- Candida homeopathic – Zorex – (10 – 15 drops tid)
- Organic Oregano Oil Blend Emulsified – 3-5 drops in water tid
- ADP (3 – 4 tid)
- NAC (N-Acetyl-L-Cysteine) (1 tid for 1 week, then 1 bid)
- UltraVir-X (1 – 2 tid)
- Olive Leaf (1 bid) – Zorex
- Considerations:
  - ✓ L-Lysine
  - ✓ Bio-Immunozyme
  - ✓ Cytozyme THY
  - ✓ IAG

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How to Treat Stealth Infections

Fungal infections – Concomitantly addressing pH, adrenals, and digestion

- Candida homeopathic – Zorex (10 – 15 drops tid)
- Caprin (4 – 6 tid)
- ADP (3 – 4 tid)
- Organic Oregano Oil Blend Emulsified – 3-5 drops in water tid
- FC-Cidal (1 – 3 tabs tid)
- Olive leaf - Zorex (1 – tid)
- NAC (N-Acetyl-L-Cysteine) (1 tid for 1 week, then 1 bid)
- Considerations:
  - ✓ Bio-Immunozyme
  - ✓ Cytozyme THY
  - ✓ IAG

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How to Treat Stealth Infections

Parasites – Concomitantly addressing pH, adrenals, and digestion. Generally after bacteria and virus; get the Low-Hanging Fruit.

- Organic Oregano Oil Blend Emulsified – 3-5 drops in water tid
- ADP (2 – 3 tid)
- Dysbiocide (2 – 3 bid, 10 days on, 5 days off)
- NAC (N-Acetyl-L-Cysteine) (1 tid for 1 week, then 1 bid)
- Food-Grade Diatomaceous Earth (1/2 – 1 tsp bid)
- Parasite Comp (1/2 – 1 tsp bid with above) – Zorex
- Considerations:
  - ✓ Olive Leaf (1 bid) – Zorex
  - ✓ Bio-Immunozyme
  - ✓ Cytozyme THY

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How to Treat Stealth Infections

Four worms were placed in four separate test tubes:

1st in beer
2nd in wine
3rd in whiskey
4th in mineral water

The next day, the teacher shows the results:

The 1st worm in beer, dead.
The 2nd in wine, dead.
The 3rd in whiskey, dead.
The 4th in mineral water, alive and healthy.

The teacher asks the class:
- What do we learn from this experience?

And a child responds:
- Whoever drinks beer, wine and whiskey, does not have worms.

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Molecular Mimicry

According to Microbiologist Professor Garth Nicholson, founder of the Institute for Molecular Medicine, located in California, stealth type bacterial infections can play a causal role in illnesses such as: chronic fatigue syndrome, fibromyalgia, multiple sclerosis, motor neuron disease, Parkinson's disease, Alzheimer's disease, arthritis, autism and Lyme disease.

"Stealth infections are in general bacterial infections but in some cases can be viral infections. They did inside cells and hide inside cells and can't be seen by the immune system. The most common spelled infections we have studied and found amongst fatiguing neurodegenerative diseases are Chlamydia pneumoniae, Mycoplasma, and Borrelia burgdorferi. These intracellular bacteria have different life forms, some of them are free swimming, some of them are inside cells, some of them are metabolically active in some forms are metabolically inactive. When they are metabolically inactive they are difficult to find. They're genetic signature is not as strong."

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Molecular Mimicry as a Mechanism of Autoimmune Disease

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Abstract

A variety of mechanisms have been suggested as the means by which infections can initiate and/or exacerbate autoimmune diseases. One mechanism is molecular mimicry, where a foreign antigen shares sequence or structural similarities with self-antigens. Molecular mimicry has typically been characterized on an antibody or T cell level. However, structural relatedness between pathogen and self does not account for T cell activation in a number of autoimmune diseases. A proposed mechanism that could have been misinterpreted for molecular mimicry is the expression of dual T cell receptors (TCR) on a single T cell. These T cells have dual reactivity to both foreign and self-antigens leaving the host vulnerable to foreign insults capable of triggering an autoimmune response. In this review, we briefly discuss what is known about molecular mimicry followed by a discussion of the current understanding of dual TCRs. Finally, we discuss three mechanisms, including molecular mimicry, dual TCRs and chimeric TCRs, by which dual reactivity of the T cell may play a role in autoimmune diseases.

Keywords

Molecular mimicry; Autoimmune diseases; Dual T cell receptor; Virus infection; Immunopathology

Chronic autoimmune diseases are the byproduct of the immune system recognizing self-antigens as foreign, which can lead to inflammation and destruction of specific tissues and organs (immunopathology) [1]. The impact of these diseases is global and heterogeneous with over 100 million people afflicted with more than 80 different autoimmune diseases [2]. While the etiology of autoimmune diseases is not fully elucidated, the causes are likely based on a combination of hereditary and environmental factors [3]. Although host genetic background contributes to the induction of an immune response to self, epidemiological and molecular evidence implicates infectious agents (viral and bacterial) as the principal environmental insults responsible for the induction of autoimmune diseases (reviewed in [4–6]). Prolonged proinflammatory responses to infectious have been associated with the initiation and exacerbation of autoimmune diseases (reviewed in [4, 7, 8]). Inflammation is facilitated by proinflammatory cytokines such as type I interferon (IFN), interleukin (IL)-1β, IL-12, IFNγ, IL-17 and tumor necrosis factor (TNF) α (reviewed in [7, 9, 10]). However, these proinflammatory cytokines are critical for clearance of pathogens, suggesting that environmental factors are able to divert the immune response towards immunopathogenesis. Although a number of immune cells are responsible for secreting proinflammatory cytokines, the primary cell types implicated in a vast majority of autoimmune disorders are autoreactive B and T cells, or antibody recognition of self [11]. Although a number of viruses and bacteria have been linked to the initiation of certain autoimmune diseases,

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"A variety of mechanisms have been suggested as the means by which infections can initiate and/or exacerbate autoimmune diseases. One mechanism is molecular mimicry, where a foreign antigen shares sequence or structural similarities with self-antigens."

"A proposed mechanism that could have been misinterpreted for molecular mimicry is the expression of dual T cell receptors (TCR) on a single T cell."

Clin Rev Allergy Immunol. 2012 February ; 42(1): 102–111. doi:10.1007/s12016-011-8294-7.

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4 Major Factors: Epimutagens

- Microbiome – Stealth Infections
- **Acid/Alkaline Balance**
- Blood Sugar Regulation
- Stress

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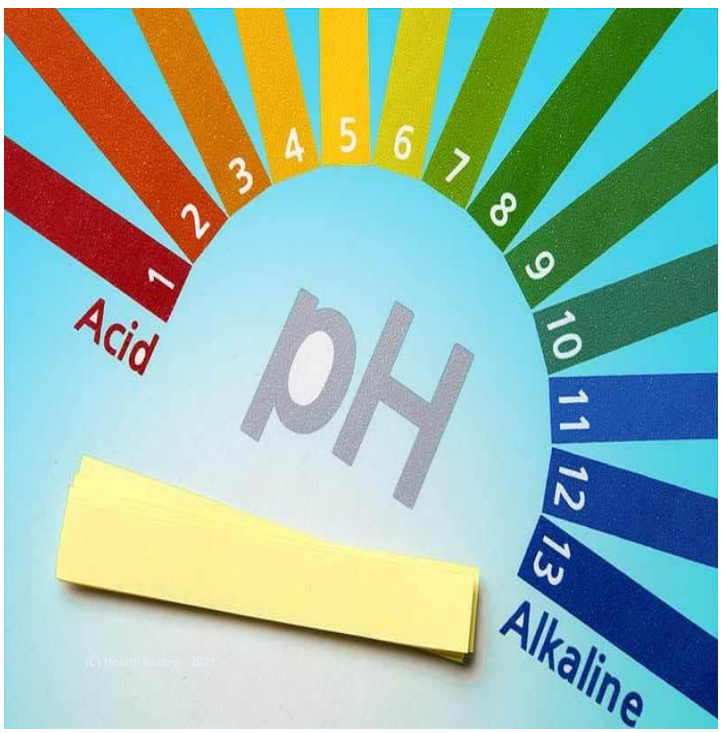

pH And the Acid Alkaline Balancing Act

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The Importance of Acid and Alkaline Balance for Health

Virtually all chronic degenerative diseases including cancer, heart disease, Alzheimer's Disease, arthritis, osteoporosis, kidney and gall stones, and tooth decay are associated with excess acidity in the body (or is it inflammation?).

The body maintains a homeostatic mechanism maintaining a constant pH 7.35 - 7.45 in the blood, this mechanism works by depositing and withdrawing acid and alkaline minerals from other locations including the bones, soft tissues, body fluids and saliva. Therefore, the pH of these other tissues can fluctuate greatly. The pH of saliva and urine offer a window through which we can see the overall pH balance in the body.

Cancer cannot exist in an alkaline environment. All forms of arthritis are associated with excess acidity. Acid in the body dissolves both teeth and bones. Whatever health situation you are faced with, you can monitor your progress toward a proper acid/alkaline balance by testing urine and saliva pH.

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What is pH & Balance

What is pH? It is short for "potential of hydrogen" or the measure of the hydrogen ion concentration of a solution. A lower number on the pH scale indicates more acidic and a higher pH number is indicative of being more alkaline.

Body Chemistry: the body has an acid-alkaline ratio called pH, which is the balance between positively charged ions, (acid forming) and negatively charged ions (alkaline forming). The body continually strives to maintain a balanced blood pH of 7.35-7.45 .

The body depends on mineral reserves, such as calcium, magnesium, potassium, sodium, lithium and rubidium to maintain a balanced pH.

The Standard American Diet tends to lean toward the acidic side of the scale and creates an imbalance in the body. As a result, draws heavily on the mineral reserves that keep the blood pH in balance.

Unfortunately, our modern-day lifestyles cause our buffer systems to struggle to maintain the proper body pH tends to place a strain on the blood pH which opens the door to a hidden danger.

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What are Alkalinity and Acidity?

Water (H_2O) ionizes into hydrogen (H+) and hydroxyl (OH-) ions. When these ions are in equal proportions, the pH is a neutral 7. When there are more H+ ions than OH- ions then the water is said to be acid. If OH- ions outnumber the H+ ions then the water is alkaline.

The pH scale goes from 0 to 14 and is logarithmic, which means that each step is ten times the previous. In other words, a pH of 4.5 is 10 times more acid than 5.5, 100 times more acid than 6.5 and 1,000 times more acid than 7.5.

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Inadequate removal of the acid. The result is an acidic accumulation in the tissue and cells.

Steps to remove excess relative acidosis:

- Bicarbonate buffers in the blood especially NA and K
- When these nutrients are reduced the body turns to Calcium and Magnesium primarily as buffers
- Blow off CO2 as we breathe. That is one reason why exercise is so critical to your patients
- Urinate as much acid as we can without causing harm to the kidneys and urinary tract. Number one deficiency in our diet is good water.
- Feces will eliminate some acidity. Another major deficiency in our diet is fiber. 1900's we would ingest 40-50 grams of fiber. Today we are lucky if we get 15 grams of fiber per day.
- When these systems are taxed, the cells themselves begin to store excess acidity which affects their metabolism and slows down cellular function.

Remember many of the acids are metabolic byproducts of normal metabolism.
(Lactic acid, Co2, NH3 to urea)

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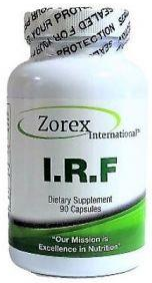
Factors that cause an acid-alkaline imbalance



- **Stress**
- **Environmental toxins –in our food, air and water**
- **Pharmaceuticals**
- **Lack of sleep or sleep apnea**
- **Lack of exercise**
- **Nutritional factors:**
- **Vitamin and mineral depletion – food isn't what it used to be.**
- **Unhealthy (chemically altered) fats and oils – lots in today's junk foods.**
- **Lack of omega-3 oils**
- **Eating too many foods that are acidic. The body will try to raise the pH by using the minerals in the alkaline reserve, mainly sodium from the stomach and calcium from the bones, as well as potassium, magnesium and iron. This is the cause of Osteoporosis and a number of other diseases. Acids buildup in the cells, causing pain, which may be diagnosed as Arthritis, Fibromyalgia, MS, Lupus, etc. An acidic diet creates an acidic oxygen-starved body, and an acidic oxygen starved body suffers from the symptoms listed above.**
- **Synthesized food products that are ironically marketed as healthy alternatives to natural fats and oils.**
- **Slow Intestinal Transit Time from acidic foods that cause constipation that leave toxins accumulating in the colon or any process that deprives the cells of oxygen and other nutrients, including essential minerals.**

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ALKALOSIS

- High alkalinity in the body-can be caused by depleted minerals as well. This condition may slow down digestion of foods and that leads to a toxic bowel and urinary tract problems.
- If a high alkaline condition exists, it is very challenging and dangerous to the body; difficult to resolve.
- One of the primary causes is alkaline drugs.
- Elevated alkaline levels can lead to some of the following issues: autoimmune disease, skin and liver challenges, allergies, constipation, chronic infections, parasites, body odor and bad breath, just to name a few.

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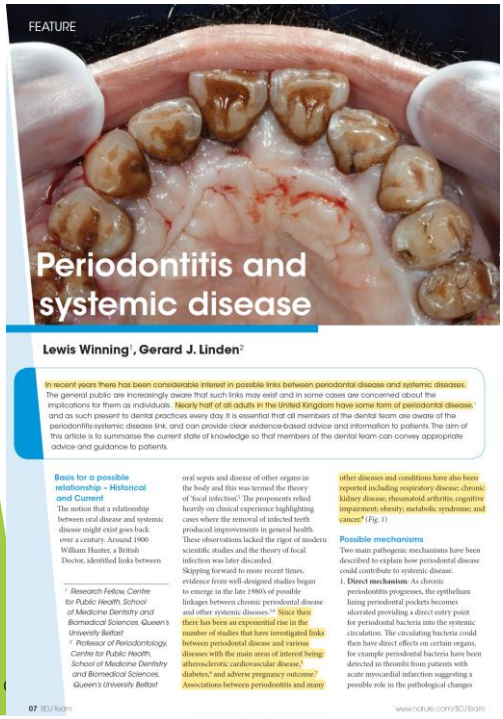

FEATURE

Periodontitis and systemic disease

Lewis Winning[1], Gerard J. Linden[2]

In recent years there has been considerable interest in possible links between periodontal disease and systemic diseases. The general public are increasingly aware that such links may exist and in some cases are concerned about the implications for them as individuals. Nearly half of all adults in the United Kingdom have some form of periodontal disease,[1] and as such present to dental practices every day. It is essential that all members of the dental team are aware of the periodontitis-systemic disease link, and can provide clear evidence-based advice and information to patients. The aim of this article is to summarise the current state of knowledge so that members of the dental team can convey appropriate advice and guidance to patients.

Basis for a possible relationship – Historical and Current

The notion that a relationship between oral disease and systemic disease might exist goes back over a century. Around 1900 William Hunter, a British Doctor, identified links between oral sepsis and disease of other organs in the body and this was termed the theory of 'focal infection'.[2] The proponents relied heavily on clinical experience highlighting cases where the removal of infected teeth produced improvements in general health. These observations lacked the rigor of modern scientific studies and the theory of focal infection was later discarded.

Skipping forward to more recent times, evidence from well-designed studies began to emerge in the late 1980s of possible linkages between chronic periodontal disease and other systemic diseases.[3,4] Since then there has been an exponential rise in the number of studies that have investigated links between periodontal disease and various diseases with the main areas of interest being: atherosclerotic cardiovascular disease,[5] diabetes,[6] and adverse pregnancy outcome.[7] Associations between periodontitis and many other diseases and conditions have also been reported including respiratory disease; chronic kidney disease; rheumatoid arthritis; cognitive impairment; obesity; metabolic syndrome; and cancer.[8] (*Fig. 1*)

Possible mechanisms

Two main pathogenic mechanisms have been described to explain how periodontal disease could contribute to systemic disease.

1. **Direct mechanism**: As chronic periodontitis progresses, the epithelium lining periodontal pockets becomes ulcerated providing a direct entry point for periodontal bacteria into the systemic circulation. The circulating bacteria could then have direct effects on certain organs, for example periodontal bacteria have been detected in thrombi from patients with acute myocardial infarction suggesting a possible role in the pathological changes

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[2] Professor of Periodontology, Centre for Public Health, School of Medicine Dentistry and Biomedical Sciences, Queen's University Belfast

"Since then there has been an exponential rise in the number of studies that have investigated links between periodontal disease and various diseases with the main areas of interest being: atherosclerotic cardiovascular disease, diabetes, and adverse pregnancy outcome.

Associations between periodontitis and many other diseases and conditions have also been reported including respiratory disease; chronic kidney disease; rheumatoid arthritis; cognitive impairment; obesity; metabolic syndrome; and cancer."

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PMCID: PMC3800408
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Salivary pH: A diagnostic biomarker

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Abstract

Go to:

Objectives:

Go to:

Saliva contains a variety of host defense factors. It influences calculus formation and periodontal disease. Different studies have been done to find exact correlation of salivary biomarkers with periodontal disease. With a multitude of biomarkers and complexities in their determination, the salivary pH may be tried to be used as a quick chairside test. The aim of this study was to analyze the pH of saliva and determine its relevance to the severity of periodontal disease.

Study Design:

Go to:

The study population consisted of 300 patients. They were divided into three groups of 100 patients each: Group A had clinically healthy gingiva, Group B who had generalized chronic gingivitis and Group C who had generalized chronic periodontitis. The randomized unstimulated saliva from each patient was collected and pH was tested. Data was analyzed statistically using analysis of variance technique.

Results:

Go to:

The salivary pH was more alkaline for patients with generalized chronic gingivitis as compared with the control group ($P = 0.001$) whereas patients with generalized chronic periodontitis had more acidic pH as compared with the control group ($P = 0.001$).

Conclusion:

Go to:

These results indicate a significant change in the pH depending on the severity of the periodontal condition. The salivary pH shows significant changes and thus relevance to the severity of periodontal disease. Salivary pH may thus be used as a quick chairside diagnostic biomarker.

Keywords: Gingivitis/physiopathology, hydrogen-ion concentration, periodontitis, saliva/physiology

INTRODUCTION

Go to:

Conclusion: "These results indicate a significant change in the pH depending on the severity of the periodontal condition. The salivary pH shows significant changes and thus relevance to the severity of periodontal disease. Salivary pH may thus be used as a quick chairside diagnostic biomarker."

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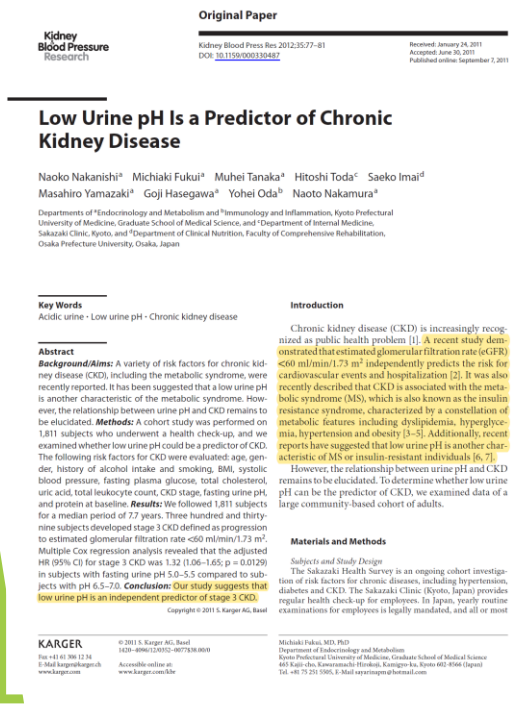

Original Paper

Kidney Blood Pressure Research

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Low Urine pH Is a Predictor of Chronic Kidney Disease

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Key Words
Acidic urine · Low urine pH · Chronic kidney disease

Abstract
Background/Aims: A variety of risk factors for chronic kidney disease (CKD), including the metabolic syndrome, were recently reported. It has been suggested that a low urine pH is another characteristic of the metabolic syndrome. However, the relationship between urine pH and CKD remains to be elucidated. ***Methods:*** A cohort study was performed on 1,811 subjects who underwent a health check-up, and we examined whether low urine pH could be a predictor of CKD. The following risk factors for CKD were evaluated: age, gender, history of alcohol intake and smoking, BMI, systolic blood pressure, fasting plasma glucose, total cholesterol, uric acid, total leukocyte count, CKD stage, fasting urine pH, and protein at baseline. ***Results:*** We followed 1,811 subjects for a median period of 7.7 years. Three hundred and thirty-nine subjects developed stage 3 CKD defined as progression to estimated glomerular filtration rate <60 ml/min/1.73 m^2. Multiple Cox regression analysis revealed that the adjusted HR (95% CI) for stage 3 CKD was 1.32 (1.06–1.65; p = 0.0129) in subjects with fasting urine pH 5.0–5.5 compared to subjects with pH 6.5–7.0. ***Conclusion:*** Our study suggests that low urine pH is an independent predictor of stage 3 CKD.

Introduction

Chronic kidney disease (CKD) is increasingly recognized as public health problem [1]. A recent study demonstrated that estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 independently predicts the risk for cardiovascular events and hospitalization [2]. It was also recently described that CKD is associated with the metabolic syndrome (MS), which is also known as the insulin resistance syndrome, characterized by a constellation of metabolic features including dyslipidemia, hyperglycemia, hypertension and obesity [3–5]. Additionally, recent reports have suggested that low urine pH is another characteristic of MS or insulin-resistant individuals [6, 7].

However, the relationship between urine pH and CKD remains to be elucidated. To determine whether low urine pH can be the predictor of CKD, we examined data of a large community-based cohort of adults.

Materials and Methods

Subjects and Study Design

The Sakazaki Health Survey is an ongoing cohort investigation of risk factors for chronic diseases, including hypertension, diabetes and CKD. The Sakazaki Clinic (Kyoto, Japan) provides regular health check-up for employees. In Japan, yearly routine examinations for employees is legally mandated, and all or most

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Our study suggests that low urine pH is an independent predictor of stage 3 CKD.

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The Journal of Immunology

Oral $NaHCO_3$ Activates a Splenic Anti-Inflammatory Pathway: Evidence That Cholinergic Signals Are Transmitted via Mesothelial Cells

Sarah C. Ray, Babak Baban, Matthew A. Tucker, Alec J. Seaton, Kyu Chul Chang, Elinor C. Mannon, Jingping Sun, Bansari Patel, Katie Wilson, Jacqueline B. Musall, Hiram Ocasio, Debra Irsik, Jessica A. Filosa, Jennifer C. Sullivan, Brendan Marshall, Ryan A. Harris and Paul M. O'Connor

J Immunol May 15, 2018, 200 (10) 3568-3586; DOI: https://doi.org/10.4049/jimmunol.1701605

Abstract

We tested the hypothesis that oral $NaHCO_3$ intake stimulates splenic anti-inflammatory pathways. Following oral $NaHCO_3$ loading, macrophage polarization was shifted from predominantly M1 (inflammatory) to M2 (regulatory) phenotypes, and $FOXP3^+CD4^+$ T-lymphocytes increased in the spleen, blood, and kidneys of rats. Similar anti-inflammatory changes in macrophage polarization were observed in the blood of human subjects following $NaHCO_3$ ingestion. Surprisingly, we found that gentle manipulation to visualize the spleen at midline during surgical laparotomy (sham splenectomy) was sufficient to abolish the response in rats and resulted in hypertrophy/hyperplasia of the capsular mesothelial cells. Thin collagenous connections lined by mesothelial cells were found to connect to the capsular mesothelium. Mesothelial cells in these connections stained positive for the pan-neuronal marker PGP9.5 and acetylcholine esterase and contained many ultrastructural elements, which visually resembled neuronal structures. Both disruption of the fragile mesothelial connections or transection of the vagal nerves resulted in the loss of capsular mesothelial acetylcholine esterase staining and reduced splenic mass. Our data indicate that oral $NaHCO_3$ activates a splenic anti-inflammatory pathway and provides evidence that the signals that mediate this response are transmitted to the spleen via a novel neuronal-like function of mesothelial cells

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The concept of acid/alkaline imbalance as the cause of disease is not new. Howard Hay, M.D., in a 1935 ground-breaking book, *A New Health Era, stated that all disease is caused by autointoxication ('self-poisoning) due to acid accumulation in the body:*

"Now we depart from health in just the proportion to which we have allowed our alkalies to be dissipated by introduction of acid-forming food in too great amount... It may seem strange to say that all disease is the same thing, no mater what its myriad modes of expression, but it is verily so."

"Remember that all disease, all fatigue, all old age, is this same accumulation of the acid end-products of digestion and metabolism, and you have the key to prevention of this trio of afflictions."

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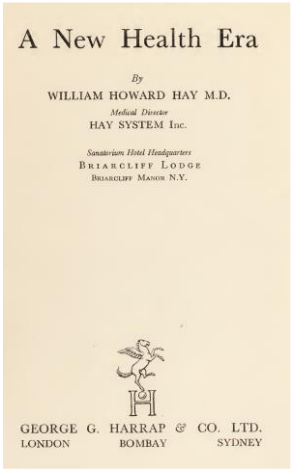
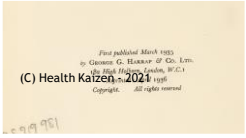

A New Health Era

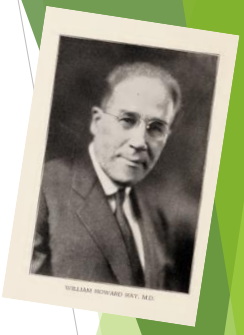
By
WILLIAM HOWARD HAY M.D.
Medical Director
HAY SYSTEM Inc.

Sanatorium Hotel Headquarters
BRIARCLIFF LODGE
BRIARCLIFF MANOR N.Y.

GEORGE G. HARRAP & CO. LTD.
LONDON BOMBAY SYDNEY



"We too often confuse effects with causes, which is not surprising when we consider that all our medical studies have been directed toward pathology, the evidence of disease, not its cause."



"When our medical colleges teach the facts of nutrition as they now teach those of pathology, we shall soon begin to need fewer doctors, fewer nurses, fewer druggists, fewer morticians, and we shall have accumulated a huge potentiality of accomplishment in bounding health."

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Relative acidity can cause osteoporosis, excess free radical damage and insulin dysregulation. Without the correct pH, hormones and enzymes cannot function at their maximum capacity. Oxygen saturated hemoglobin molecules can't release oxygen properly to oxygen starved tissue when the pH is too low.

What grows in an acidic, oxygen depleted environment?

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Assessing a patient's relative Acid/Alkaline Balance

- pH strips by mouth, away from food - under 7.0?
- pH strips for first morning urine - under 6.5?
  - Midday closer to 7.0
- CO2 under 24
- Anion Gap over 13
  - The **anion gap** is the difference between the measured cations (positively charged ions) and the measured **anions**(negatively charged ions) in serum, plasma, or urine.

Blood pH must be maintained at 7.35 – 7.45!!

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Protocols to aid in regulating Acid/Alkaline Balance

The protocols are modified by the patient history and laboratory markers and the changing of these markers. They will change, that's why I like to use in office tests. To test, repeat testing and change therapies on the fly – as patient symptoms and laboratory tests indicate.

- Dietary changes
- Fix digestion
- Alkalizing supplementation
  - ✓ Baking soda (¼ to ½ tsp bid)
  - ✓ Potassium HP with magnesium– Biotics product (1 tsp/day if serum K isn't elevated)
  - ✓ Bio-D Forte –Biotics product (2,000 – 10,000 IU/day – directed by lab)
  - ✓ NitroGreens – Biotics product

Retest – in office and at home with chart

Sodium bicarbonate ingestion prior to training improves mitochondrial adaptations in rats

David J. Bishop,[1,2] Claire Thomas,[3,5] Tom Moore-Morris,[3] Michaël Tonkonogi,[6,7] Kent Sahlin,[7,8] and Jacques Mercier[3,4]

[1]Institute of Sport, Exercise, and Active Living; [2]School of Sport and Exercise Science, Victoria University, Melbourne, Victoria, Australia; [3]Université Montpellier, Unité de Formation et de Recherche (UFR) Médecine, EA4202, [4]Institut National de la Sante et de la Recherche Medicale, ERI 25, F-34000, Montpellier; [5]Université Evry Val d'Essonne, UFR Sciences Fondamentales et Appliquées, Département Sciences et Techniques des Activités Physiques et Sportives, EA3872, U902, F-91025, Evry, France; [6]Lognet Institute of Sport Science, Dalarna University, Falun; [7]The Swedish School of Sport and Health Sciences; and [8]Karolinska Institutet, Stockholm, Sweden

We tested the hypothesis that re- ducing hydrogen ion accumulation during training would result in greater improvements in muscle oxidative capacity and time to exhaustion (TTE). Male Wistar rats were randomly assigned to one of three groups (CON, PLA, and BIC). CON served as a sedentary control, whereas PLA ingested water and BIC ingested sodium bicarbonate 30 min prior to every training session. Training consisted of seven to twelve 2-min intervals performed five times/wk for 5 wk. Following training, TTE was significantly greater in BIC (81.2 ± 24.7 min) compared with PLA (53.5 ± 30.4 min), and TTE for both groups was greater than CON (6.5 ± 2.5 min). Fiber respiration was determined in the soleus (SOL) and extensor digitorum longus (EDL), with either pyruvate (Pyr) or palmitoyl carnitine (PC) as substrates. Compared with CON (14.3 ± 2.6 nmol $O_2 \cdot min^{-1} \cdot mg\ dry\ wt^{-1}$), there was a signifi greater SOL-Pyr state 3 respiration in both PLA (19.6 ± 3.0 nmol $O_2 \cdot min^{-1} \cdot mg\ dry\ wt^{-1}$) and BIC (24.4 ± 2.8 nmol $O_2 \cdot min^{-1} \cdot mg\ dry\ wt^{-1}$), with a significantly greater value in BIC. However, state 3 respiration was significantly lower in the EDL from both trained groups compared with CON. These differences remained significant in the SOL, but not the EDL, when respiration was corrected for citrate synthase activity (an indicator of mitochondrial mass). These novel findings suggest that reducing muscle hydrogen ion accumulation during running training is associated with greater improvements in both mitochondrial mass and mitochondrial respiration in the soleus.

mitochondrial respiration; state 3 respiration; muscle pH; citrate synthase; muscle buffer capacity

MITOCHONDRIA ARE CENTRAL TO THE CONVERSION of energy by oxidizing substrates and generating the cell fuel ATP. During steady-state exercise, the rate of mitochondrial ATP production is closely matched to the rate of ATP hydrolysis, and this demonstrates the existence of efficient cellular mechanisms to control mitochondrial ATP synthesis. However, there is evidence that the maximal rate of mitochondrial ATP production (V_{max}) is in excess of that which is required during exercise with large muscle groups (e.g., 2-legged exercise) (52). Nonetheless, despite this apparent overcapacity, there are further increases in V_{max} with endurance training (14, 53, 59). Although this adaptation will have little influence on whole body maximal oxygen utilization, it will play a major role in reducing metabolic perturbations and increasing time to exhaustion (TTE) during submaximal exercise (18). In contrast, reduced mitochondrial respiration appears to provide an important mechanism that links a low aerobic capacity to the pathogenesis of cardiovascular disease (63) and insulin resistance (33). Regularly performed exercise can result in a rapid increase in the activities of oxidative enzymes (26), mitochondrial density (56), and mitochondrial respiration (14, 49, 53, 54, 60). However, given the importance of mitochondrial respiration for both performance and health, further research is required to determine factors that regulate training-induced changes in mitochondrial respiration. One potential factor, unexplored to date, is the degree of acidosis experienced during training. We have reported recently that reducing H^+ accumulation during training (via pretraining ingestion of sodium bicarbonate, $NaHCO_3$) resulted in greater improvements in both short-term endurance and the lactate threshold in humans (20). Because the lactate threshold has previously been correlated with mitochondrial respiration (28), we hypothesized that this finding may have been due to the positive effects of reducing H^+ accumulation during training on training-induced changes in mitochondrial respiration.

When a molecular view on training adaptation is taken, it is apparent that adaptations to training are the consequence of changes in gene expression that lead to the accumulation of specific proteins. It has been shown that the muscle environment (e.g., low glycogen) is a determining factor for the transcription of some genes in response to training (40). Recent research suggests that cellular pH may also affect the training-induced expression of some genes, in particular, nuclear-encoded genes that have been proposed to regulate mitochondrial biogenesis [e.g., peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)] (5, 38). Such findings further suggest that reducing H^+ accumulation during training may promote mitochondrial adaptations.

Therefore, the present study investigated for the first time the effects of altering muscle pH during training (via $NaHCO_3$ ingestion) on citrate synthase (CS) activity (a marker of mitochondrial mass) and mitochondrial respiration in rat skeletal muscle. Although many previous studies have investigated the effects of training on the function of isolated mitochondria (7, 30, 43), the structure of the mitochondrial membrane and the

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JLB JOURNAL OF LEUKOCYTE BIOLOGY
Inflammation, Extracellular Mediators, & Effector Molecules

Mild acidosis delays neutrophil apoptosis via multiple signaling pathways and acts in concert with inflammatory mediators

Driss El Kebir, Everton de Oliveira Lima dos Santos, Soukaina Mansouri, Meriem Sekheri, János G. Filep

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Abstract

Accumulating evidence indicates development of local extracellular acidosis in inflamed tissues in response to infection and tissue injury. Activation of infiltrating neutrophils contributes to a transient decrease in pH, which, in turn, triggers innate immunity. In this study, we investigated the impact of extracellular acidosis on neutrophil apoptosis, a critical determinant of the outcome of the inflammatory response and analyzed the underlying signaling pathways. Culture of human isolated neutrophils in mildly acidotic conditions (pH 6.5–7.0) resulted in activation of NF-κB; intracellular accumulation of cAMP; and phosphorylation of Akt, ERK, and p38 MAPK; and preservation of Mcl-1 expression. Consequently, extracellular acidosis prevented disruption of mitochondrial transmembrane potential and translocation of cytochrome *c* and apoptosis-inducing factor from the mitochondria to cytoplasm and nuclei, respectively and inhibited caspase-3 activity. Pharmacological inhibition of ERK, PI3K, NF-κB, or PKA partially reversed survival cues by extracellular acidosis and redirected neutrophils to apoptosis. Conversely, dibutyryl cAMP (100–500 μM) delayed apoptosis of neutrophils cultured at pH 7.4. Extracellular acidosis-generated survival cues were additive to the potent prosurvival signals from bacterial DNA, LPS, modified C-reactive protein, and serum amyloid A. Acidosis increased CpG DNA uptake by neutrophils and augmented phosphorylation of ERK and Akt, leading to preservation of Mcl-1 expression. Our results identified extracellular acidosis as a survival signal for neutrophils by suppressing the constitutive apoptotic machinery and suggest that transient decreases in local pH can enhance neutrophil responses to inflammatory stimuli, thereby contributing to amplification or prolongation of the inflammatory response.

"Accumulating evidence indicates development of local extracellular acidosis in inflamed tissues in response to infection and tissue injury. Activation of infiltrating neutrophils contributes to a transient decrease in pH, which, in turn, triggers innate immunity."

"Our results identified extracellular acidosis as a survival signal for neutrophils by suppressing the constitutive apoptotic machinery and suggest that transient decreases in local pH can enhance neutrophil responses to inflammatory stimuli, thereby contributing to amplification or prolongation of the inflammatory response."

El Kebir, D. , Oliveira Lima dos Santos, E. , Mansouri, S. , Sekheri, M. and Filep, J. G. (2017), Mild acidosis delays neutrophil apoptosis via multiple signaling pathways and acts in concert with inflammatory mediators. J Leuk Biol, 102: 1389-1400. doi:10.1189/jlb.3A0117-041R

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Medscape

Background

Metabolic acidosis is an acid-base disorder characterized by a decrease in serum pH that results from either a primary decrease in plasma bicarbonate concentration ($[HCO_3^-]$) or an increase in hydrogen ion concentration ($[H^+]$). [1] It is not a disease but rather a biochemical abnormality. The clinical manifestations of a metabolic acidosis are nonspecific, and its differential diagnoses include common and rare diseases.

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Payen and Haloui Critical Care 2014, 18:664
http://ccforum.com/content/18/6/664

CRITICAL CARE

COMMENTARY

Acid-base status is an important factor for inflammation, but don't forget CO_2!

Didier Payen[1,2*] and Houda Haloui[1,2]

See related research by Zampieri et al., http://ccforum.com/content/18/4/R154

Abstract

Zampieri and colleagues used sophisticated statistical methods to create a picture of acid-base pattern and inflammation relationship in a clinical context. The observed independent relationship between acidosis and albumin concentration and inflammatory pattern opens up a new area for research. It has become clear that, in addition to the characterization of mediators, receptors, and cellular phenotypes, the inflammatory response has to be interpreted in light of acid-base status, albumin concentration, and probably also carbon dioxide level.

Until now, the interplay between acid-base status and inflammation has received little attention, especially in a clinical context. The article by Zampieri and colleagues [1] in a previous issue of *Critical Care* is a pioneering study analyzing the relationship between acidosis variables, inflammatory mediators, and end-organ failures (acute kidney injury and shock). Since the metabolic and inflammatory reactions are simultaneous, the demonstration of interplay that is more than a simultaneous modification remains a difficult challenge. Because of this, the authors used three different statistical methods to separate the confounding factors. First, they developed a generalized linear model using the measured mediator as a dependent variable and components of acid-base status as variables. Second, they performed a multivariate adaptive regression with splines in order to evaluate the association of selected cytokines and acid-base components. Third, they performed a principal component analysis using Simplified Acute Physiology Score 3 as a way of quantifying illness severity in order to assess the independent association of acid-base variables and cytokine levels. The authors found that, in 87 prospective unselected patients, the level of strong anion gap (SIG) was positively associated with TNFα and IL-6, IL-8, and IL-10. A negative association was found between albumin level and TNFα and IL-6, IL-7, IL-8, and IL-10 and IFNγ. The conclusion drawn from these results opens up a new route for research to understand the mechanisms that link acid-base variables, albumin level, and immunological activation.

Such a topic is important and clinically relevant since plasma and interstitial fluid constitute the microenvironment for immune and tissue cells. Acid-base and albumin characteristics may then interfere with the cell response to different signals such as endotoxin. In addition, both fluid resuscitation and capillary leak may largely influence the composition of the cell microenvironment, especially when a crystalloid such as saline or a balanced crystalloid such as Ringer's lactate is used. The role of surrounding cell pH could be seen as a result of metabolic acidosis and carbon dioxide (CO_2) level, an aspect that was not investigated in the study [2,3]. Given the picture presented in this article, some approaches might be tested to clarify the mechanisms involved in immune modifications induced by acid-base changes. First, immune cells should be drawn from septic patients that have been incubated in the septic plasma or drawn after replacement of septic plasma by healthy plasma; both acid-base conditions or albumin concentration can then be modified to test their impact on immune cells phenotype. This might help to clarify how the pH, the SIG, and albumin concentration change the immune cell phenotypes. Second, similar experiments with healthy cells incubated in plasma from acutely injured patients could be performed to demonstrate the role of physicochemical plasma patterns. Mediators and cell functions then could be evaluated in different acid-base conditions. Until now, few data on alkalosis have been reported in terms of immunity, and the essential information comes from acidosis situations. One author of the study was part

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BioMed Central

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Payen and Haloui Critical Care 2014, 18:664
http://ccforum.com/content/18/6/664

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The only things required by the body to be healthy are:

- Air
- Light
- Water
- Vitamins
- Minerals
- Amino acids
- Essential fatty acids
- Enzymes
- Phytochemicals
- Exercise

Remember –

You cannot NOT Poison the Body Healthy!!!

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FOOD AND CHEMICAL EFFECTS ON ALKALINE/ACID BODY CHEMCIAL BALANCE

Most Alkaline	*More Alkaline*	*Low Alkaline*	*Lowest Alkaline*	**Food Category**	*Lowest Acid*	*Low Acid*	*More Acid*	*Most Acid*
Baking soda	Spices/cinnamon	Herbs (most)		**Seasonings**	Curry	Vanilla	Nutmeg	Jam/jelly
Lime Nectarine Persimmon Raspberry Watermelon Tangerine Mango Lemon	Grapefruit Cantaloupe Honeydew Mango Dewberry Papaya Dates Figs	Pear Apple Avocado Blackberry Cherry Peach Grape	Orange Apricot Banana Blueberry Pineapple Raisin, currant Strawberry	**Fruit**	Guava Dried fruit	Plum Prune Tomato	Cranberry Pomegranate	
Asparagus Lentil Yam Onion Daikon Taro root Sea vegetable Burdock Raw spinach	Kohlrabi Parsnip Garlic Kale Parsley Endive Mustard greens Ginger root Broccoli Sweet potato	Potato Bell pepper Mushroom Cauliflower Eggplant Pumpkin Collard greens	Brussels sprouts Beet Chive Okra Turnip greens Squash Lettuce Potato skins	**Vegetables Beans Legumes**	Spinach Fava beans Kidney beans String beans Chutney Rhubarb	Tofu Pinto beans White beans Navy beans Adzki beans Lima beans Chard Potatoes without skins	Green pea Peanut Snow pea Legumes (other) Carrots Chickpea	Soybean Carob
Pumpkin seeds Olive oil	Poppy seeds Flaxseed oil	Primrose oil Cod liver oil Almonds Sprouts	Avocado oil Seeds (most) Coconut oil Olive oil Chestnuts	**Nuts / Seeds Sprouts Oils**	Pumpkin seed oil Grape seed oil Sunflower oil Pine nuts Canola oil	Almond oil Sesame oil Safflower oil	Pistachio Pecan	Hazelnut Walnut Brazil nut
			Oats Quinoa Wild rice	**Grains Cereals**	Millet Kasha Triticale Amaranth Brown rice	Buckwheat Wheat Spelt Semolina Teff	Corn Rye Oat bran	White flour Barley
				Fowl	Wild duck	Goose Turkey	Chicken	Pheasant
				Meat Fish Shellfish	Venison Coldwater fish	Lamb Elk Shellfish	Pork Veal Mussels/squid	Beef Shellfish
		Quail eggs	Duck eggs	**Eggs**	Chicken eggs			
	Breastmilk			**Dairy**	Cream Yogurt	Goats milk Aged cheese Soy cheese	Casein Fresh cheese Rondeau	Processed cheese Ice cream conal
		Green tea	Ginger tea	**Beverages**		Black tea	Coffee	Beer Soft drinks
Stevia	Molasses	Rice syrup Maple syrup	Sucanat	**Sweeteners**	Processed honey		Saccharin	Sugar Cocoa
		Apple cider		**Vinegar**	Rice vinegar	Balsamic vinegar		White vinegar

How do we assess the Biological Terrain?

Salivary pH Challenge

Reagents needed:
Real lemon juice and water

Directions:
•Prepare the acidifying solution by mixing 1 Tbl of water and 1 TBL of lemon juice together.

•Have patient collect saliva in their mouth and dip the first strip of pH paper in their mouth. Record this as their baseline number.

•Have the patient drink the lemon juice/water mix, wait one minute and remeasure he patient's pH.

•Repeat this for 5 minutes measuring the pH every minute. Care must be used to measure the times consistently as this is a short timed test.

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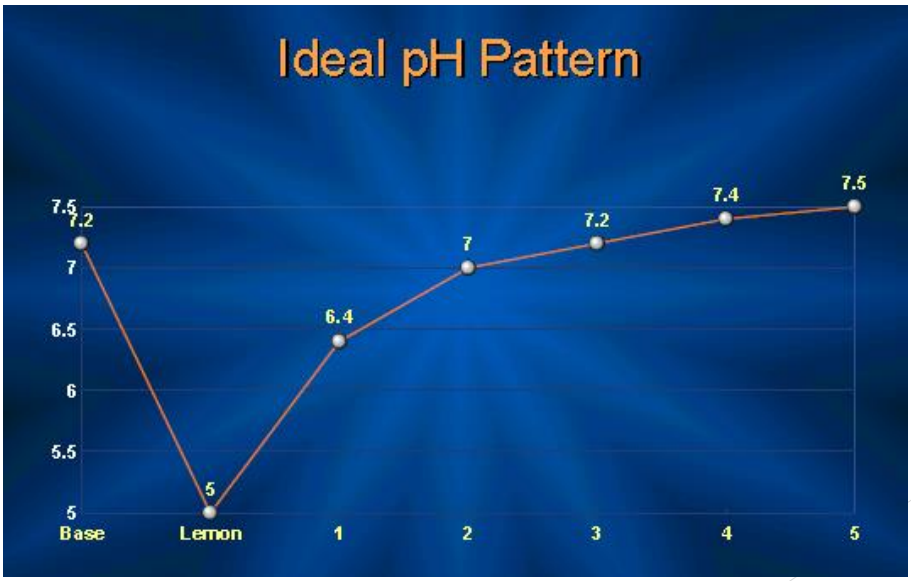
Salivary pH Challenge



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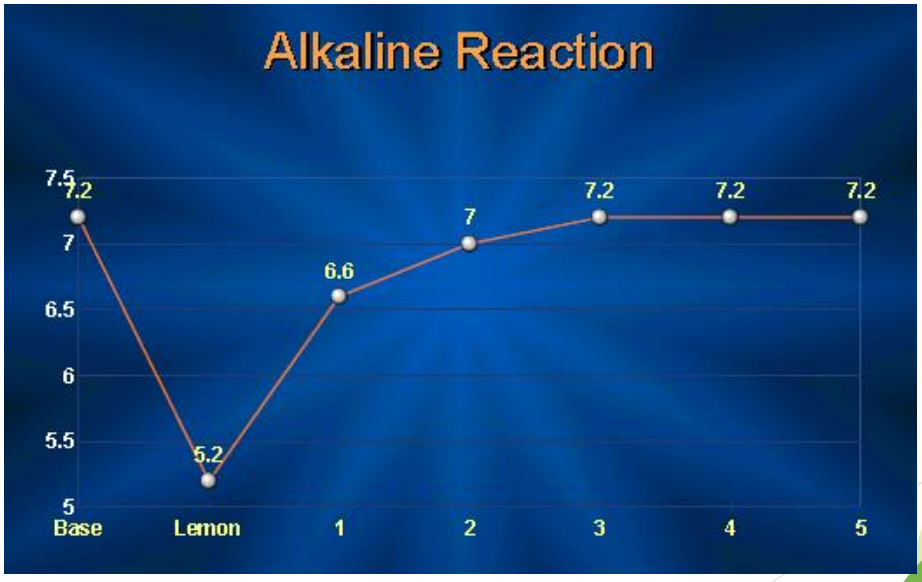

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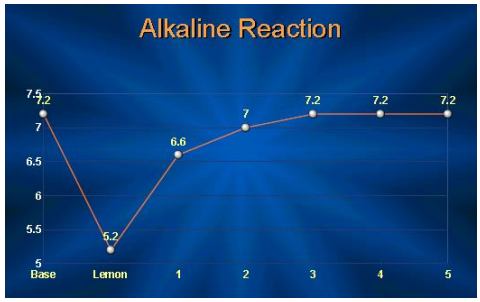

Considerations:

Think low pancreatic enzymes or liver congestion:

- 8X Pancreatin - Zorex
- Intenzyme Forte
- Bromelain plus CLA
- Beta TCP
- B6-Plus*

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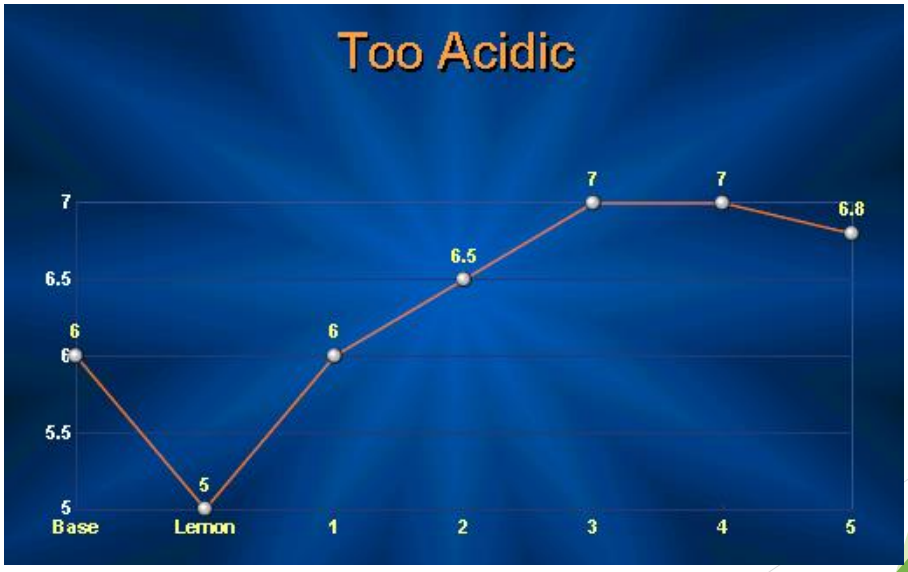
Salivary pH Challenge



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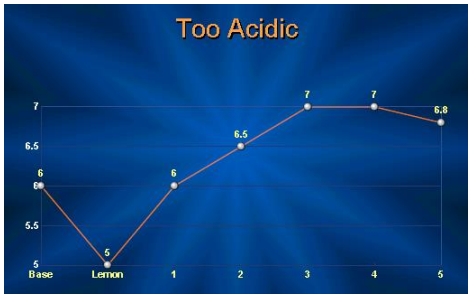

Considerations:

Think low stomach acid

- •HCl Complete - Zorex
- •HydroZyme
- •HCL –Plus
- •Betaine Plus HP

- •Nitro-Greens
- •Change Diet
- •Vitamin C flush
- • Baking soda bath

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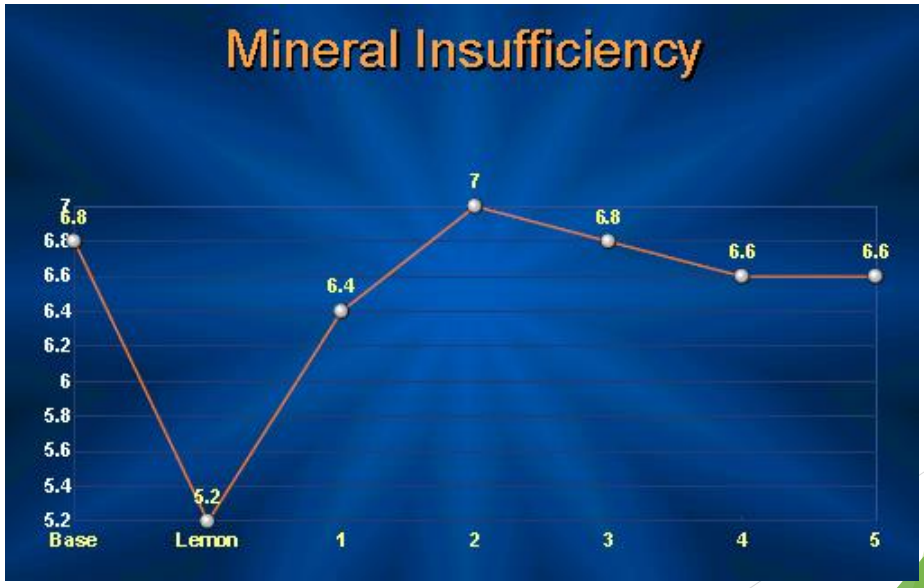
Salivary pH Challenge



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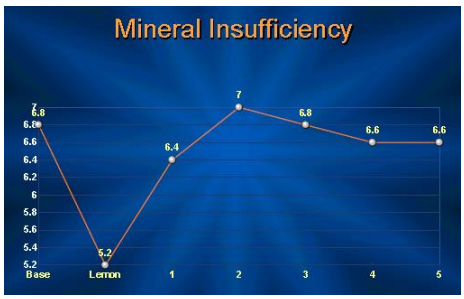
Salivary pH Challenge



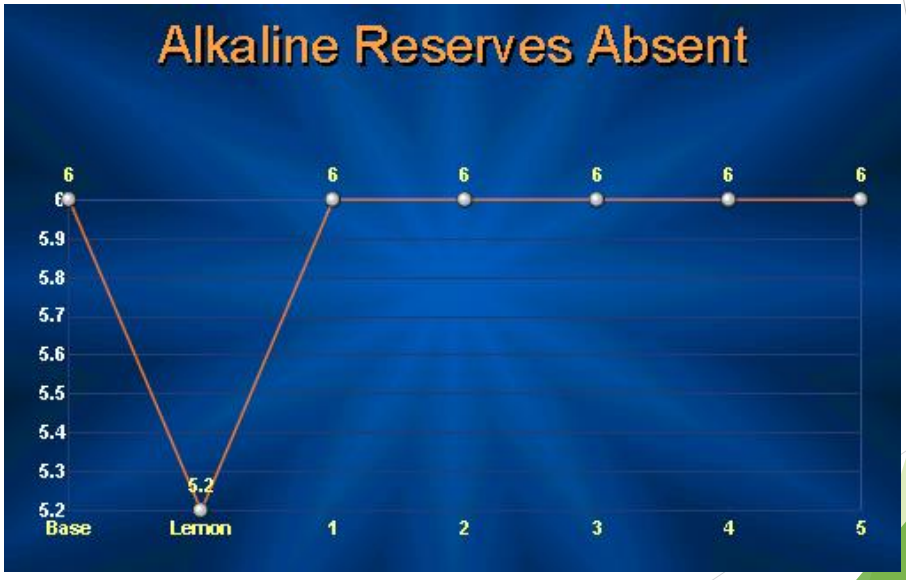
Considerations:

Represents lack of mineral reserves

- •Multi Mins
- •Nitro Greens
- •EFA's

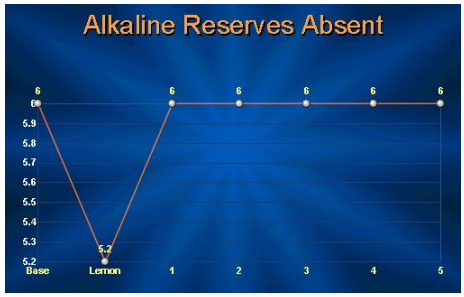
246

Salivary pH Challenge



247

Salivary pH Challenge



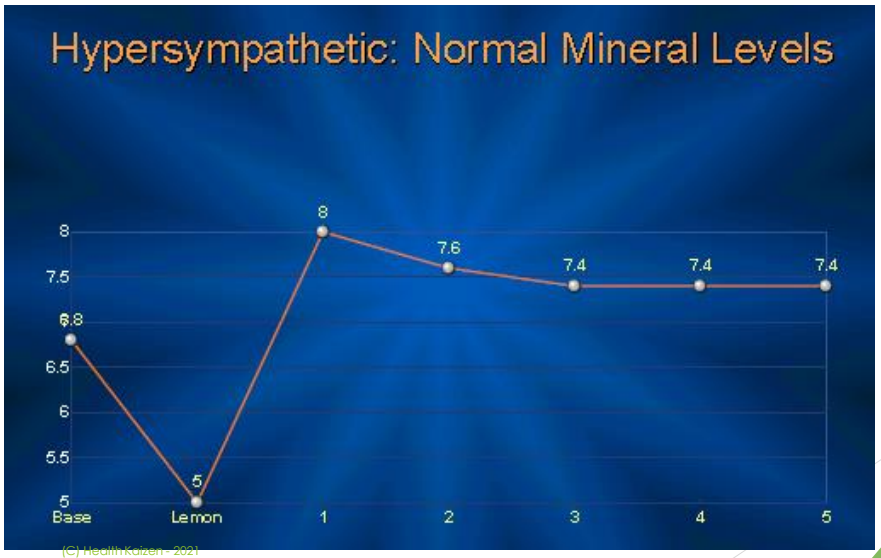
Considerations:

Represents cell rigidity and lack of alkaline reserves

- Nitro Greens
- Sunflax Complex – Zorex
- EFA's
- Multi Mins

248

Salivary pH Challenge



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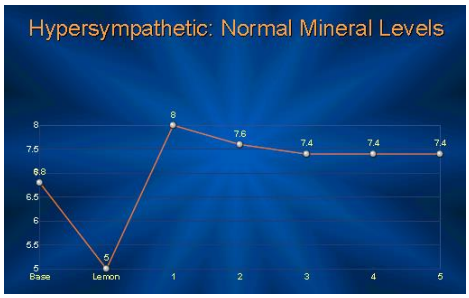
Salivary pH Challenge

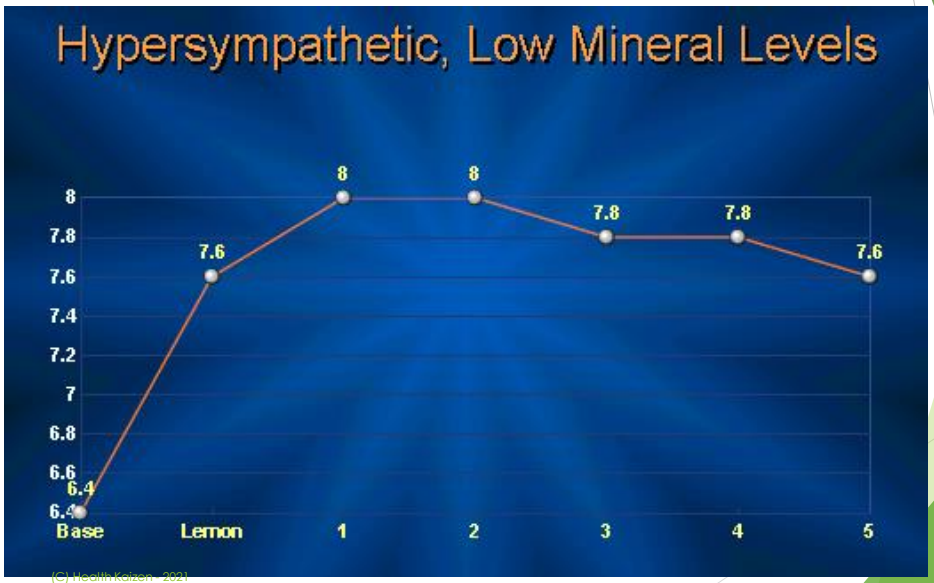


Considerations:

Think Adrenal dysfunction

250

Salivary pH Challenge



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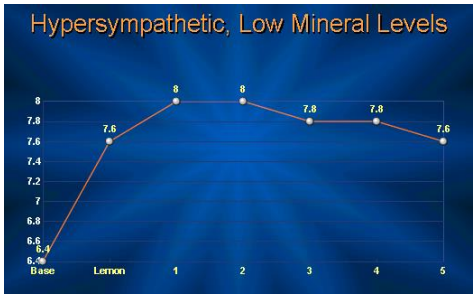
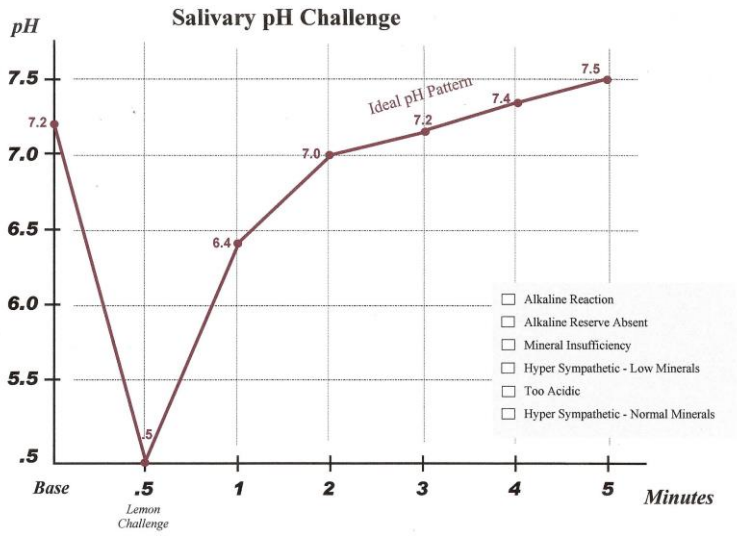
Salivary pH Challenge



Considerations:

Think Adrenal dysfunction

252



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Sodium bicarbonate ingestion prior to training improves mitochondrial adaptations in rats

David J. Bishop,[1,2] Claire Thomas,[3,5] Tom Moore-Morris,[3] Michall Tonkonogi,[6,7] Kent Sahlin,[7,8] and Jacques Mercier[3,4]

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We tested the hypothesis that re- ducing hydrogen ion accumulation during training would result in greater improvements in muscle oxidative capacity and time to exhaustion (TTE). Male Wistar rats were randomly assigned to one of three groups (CON, PLA, and BIC). CON served as a sedentary control, whereas PLA ingested water and BIC ingested sodium bicarbonate 30 min prior to every training session. Training consisted of seven to twelve 2-min intervals performed five times/wk for 5 wk. Following training, TTE was significantly greater in BIC (81.2 ± 24.7 min) compared with PLA (53.5 ± 30.4 min), and TTE for both groups was greater than CON (6.5 ± 2.5 min). Fiber respiration was determined in the soleus (SOL) and extensor digitorum longus (EDL), with either pyruvate (Pyr) or palmitoyl carnitine (PC) as substrates. Compared with CON (14.3 ± 2.6 nmol $O_2 \cdot min^{-1} \cdot mg\ dry\ wt^{-1}$), there was a signifi greater SOL-Pyr state 3 respiration in both PLA (19.6 ± 3.0 nmol $O_2 \cdot min^{-1} \cdot mg\ dry\ wt^{-1}$) and BIC (24.4 ± 2.8 nmol $O_2 \cdot min^{-1} \cdot mg\ dry\ wt^{-1}$), with a significantly greater value in BIC. However, state 3 respiration was significantly lower in the EDL from both trained groups compared with CON. These differences remained significant in the SOL, but not the EDL, when respiration was corrected for citrate synthase activity (an indicator of mitochondrial mass). These novel findings suggest that reducing muscle hydrogen ion accumulation during running training is associated with greater improvements in both mitochondrial mass and mitochondrial respiration in the soleus.

mitochondrial respiration; state 3 respiration; muscle pH; citrate synthase; muscle buffer capacity

MITOCHONDRIA ARE CENTRAL TO THE CONVERSION of energy by oxidizing substrates and generating the cell fuel ATP. During steady-state exercise, the rate of mitochondrial ATP production is closely matched to the rate of ATP hydrolysis, and this demonstrates the existence of efficient cellular mechanisms to control mitochondrial ATP synthesis. However, there is evidence that the maximal rate of mitochondrial ATP production (V_{max}) is in excess of that which is required during exercise with large muscle groups (e.g., 2-legged exercise) (52). Nonetheless, despite this apparent overcapacity, there are further increases in V_{max} with endurance training (14, 53, 59). Although this adaptation will have little influence on whole body maximal oxygen utilization, it will play a major role in reducing metabolic perturbations and increasing time to exhaustion (TTE) during submaximal exercise (18). In contrast, reduced mitochondrial respiration appears to provide an important mechanism that links a low aerobic capacity to the pathogenesis of cardiovascular disease (63) and insulin resistance (33). Regularly performed exercise can result in a rapid increase in the activities of oxidative enzymes (26), mitochondrial density (56), and mitochondrial respiration (14, 49, 53, 54, 60). However, given the importance of mitochondrial respiration for both performance and health, further research is required to determine factors that regulate training-induced changes in mitochondrial respiration. One potential factor, unexplored to date, is the degree of acidosis experienced during training. We have reported recently that reducing H^+ accumulation during training (via pretraining ingestion of sodium bicarbonate, $NaHCO_3$) resulted in greater improvements in both short-term endurance and the lactate threshold in humans (20). Because the lactate threshold has previously been correlated with mitochondrial respiration (28), we hypothesized that this finding may have been due to the positive effects of reducing H^+ accumulation during training on training-induced changes in mitochondrial respiration.

When a molecular view on training adaptation is taken, it is apparent that adaptations to training are the consequence of changes in gene expression that lead to the accumulation of specific proteins. It has been shown that the muscle environment (e.g., low glycogen) is a determining factor for the transcription of some genes in response to training (40). Recent research suggests that cellular pH may also affect the training-induced expression of some genes, in particular, nuclear-encoded genes that have been proposed to regulate mitochondrial biogenesis [e.g., peroxisome proliferator-activated receptor–γ coactivator-1α. (PGC-1α.)] (5, 38). Such findings further suggest that reducing H^+ accumulation during training may promote mitochondrial adaptations.

Therefore, the present study investigated for the first time the effects of altering muscle pH during training (via $NaHCO_3$ ingestion) on citrate synthase (CS) activity (a marker of mitochondrial mass) and mitochondrial respiration in rat skeletal muscle. Although many previous studies have investigated the effects of training on the function of isolated mitochondria (7, 30, 43), the structure of the mitochondrial membrane and the

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4 Major Factors: Epimutagens
- Microbiome – Stealth Infections
- Acid/Alkaline Balance
- **Blood Sugar Regulation**
- Stress

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How important is healthy blood sugar regulation?

Obtaining and maintaining proper blood sugar metabolism is essential for health!!!! Protracted unhealthy blood sugar has significant effects upon: Blood vessels, eyes, nerves, kidneys and pancreas. It also affects energy levels, blood pressure, cholesterol, triglycerides, overall cardiovascular health, body shape to name a few.

It is estimated that his many as 50% of Americans age 20 years or older may be "prediabetic" sometimes referred to as "insulin resistance." If this condition goes unrecognized and no lifestyle or dietary changes are made, it is quite likely that they will move on to the next stage of diabetes.

No doubt obesity, excess sweets and refined or processed foods, and lack of exercise are major contributors to poor blood sugar metabolism.

Recognizing this "pre-diabetic state" is vitally important to the long-term health of your patients!

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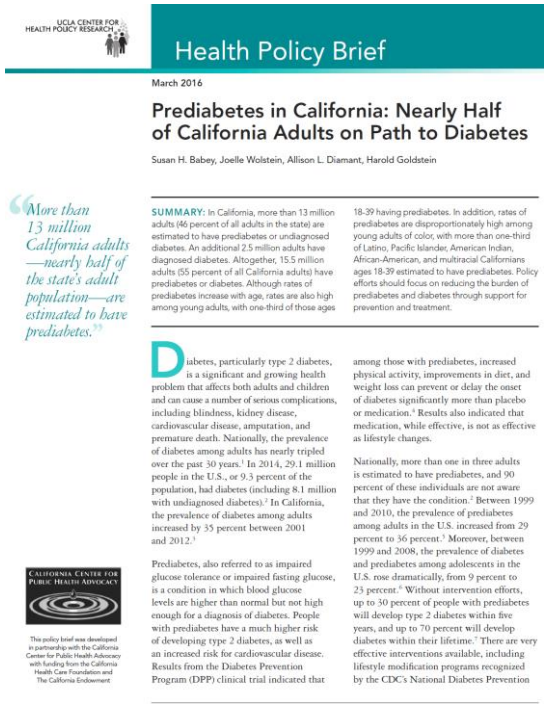

UCLA CENTER FOR HEALTH POLICY RESEARCH

Health Policy Brief

March 2016

Prediabetes in California: Nearly Half of California Adults on Path to Diabetes

Susan H. Babey, Joelle Wolstein, Allison L. Diamant, Harold Goldstein

"More than 13 million California adults —nearly half of the state's adult population—are estimated to have prediabetes."

SUMMARY: In California, more than 13 million adults (46 percent of all adults in the state) are estimated to have prediabetes or undiagnosed diabetes. An additional 2.5 million adults have diagnosed diabetes. Altogether, 15.5 million adults (55 percent of all California adults) have prediabetes or diabetes. Although rates of prediabetes increase with age, rates are also high among young adults, with one-third of those ages 18-39 having prediabetes. In addition, rates of prediabetes are disproportionately high among young adults of color, with more than one-third of Latino, Pacific Islander, American Indian, African-American, and multiracial Californians ages 18-39 estimated to have prediabetes. Policy efforts should focus on reducing the burden of prediabetes and diabetes through support for prevention and treatment.

Diabetes, particularly type 2 diabetes, is a significant and growing health problem that affects both adults and children and can cause a number of serious complications, including blindness, kidney disease, cardiovascular disease, amputation, and premature death. Nationally, the prevalence of diabetes among adults has nearly tripled over the past 30 years.[1] In 2014, 29.1 million people in the U.S., or 9.3 percent of the population, had diabetes (including 8.1 million with undiagnosed diabetes).[2] In California, the prevalence of diabetes among adults increased by 35 percent between 2001 and 2012.[3]

Prediabetes, also referred to as impaired glucose tolerance or impaired fasting glucose, is a condition in which blood glucose levels are higher than normal but not high enough for a diagnosis of diabetes. People with prediabetes have a much higher risk of developing type 2 diabetes, as well as an increased risk for cardiovascular disease. Results from the Diabetes Prevention Program (DPP) clinical trial indicated that among those with prediabetes, increased physical activity, improvements in diet, and weight loss can prevent or delay the onset of diabetes significantly more than placebo or medication.[4] Results also indicated that medication, while effective, is not as effective as lifestyle changes.

Nationally, more than one in three adults is estimated to have prediabetes, and 90 percent of these individuals are not aware that they have the condition.[2] Between 1999 and 2010, the prevalence of prediabetes among adults in the U.S. increased from 29 percent to 36 percent.[5] Moreover, between 1999 and 2008, the prevalence of diabetes and prediabetes among adolescents in the U.S. rose dramatically, from 9 percent to 23 percent.[6] Without intervention efforts, up to 30 percent of people with prediabetes will develop type 2 diabetes within five years, and up to 70 percent will develop diabetes within their lifetime.[7] There are very effective interventions available, including lifestyle modification programs recognized by the CDC's National Diabetes Prevention

CALIFORNIA CENTER FOR PUBLIC HEALTH ADVOCACY

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UCLA Center For Health Policy Research

March 2016

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Does Inflammation Trigger Insulin Resistance and Diabetes?

It's not just obesity--more evidence links inflammation with type 2 diabetes
Nov 18, 2009 |By Melinda Wenner

Nearly 21 million Americans suffer from type 2 diabetes, and every year 800,000 more are diagnosed. Considering the growing numbers, scientists are trying to fit together the disease's disparate puzzle pieces. People who acquire it are typically obese, suffer from chronic inflammation and are resistant to insulin, the hormone that removes sugar from the blood and stores it as energy. For years no one has known exactly how the three characteristics are related, if at all. But a handful of recent studies suggest that they are inextricably linked through the actions of specific inflammatory immune cells and a master genetic switch—and the hope is that an understanding of the relations could open the door to new therapeutic opportunities.

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Glycation as an Inflammatory Pathway

Advanced glycation end products (AGEs) are a complex group of compounds formed when sugar reacts with amino acids. Glycation is one of the major molecular mechanisms whereby damage accrues in your body, which leads to disease and aging

This can occur both in the food you eat, and inside your body.

AGEs may be implicated in the development of the chronic degenerative diseases associated with aging, including but not limited to:

- Cardiovascular disease
- Alzheimer's disease – "Type 3 Diabetes"
- Diabetes
- Cancer

A number of studies have shown that restricting the consumption of AGEs can lead to an increased lifespan in animal models.

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Cell Reports
Article

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Host Genotype and Gut Microbiome Modulate Insulin Secretion and Diet-Induced Metabolic Phenotypes

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http://dx.doi.org/10.1016/j.celrep.2017.01.062

SUMMARY

Genetic variation drives phenotypic diversity and influences the predisposition to metabolic disease. Here, we characterize the metabolic phenotypes of eight genetically distinct inbred mouse strains in response to a high-fat/high-sucrose diet. We found significant variation in diabetes-related phenotypes and gut microbiota composition among the different mouse strains in response to the dietary challenge and identified taxa associated with these traits. Follow-up microbiota transplant experiments showed that altering the composition of the gut microbiota modifies strain-specific susceptibility to diet-induced metabolic disease. Animals harboring microbial communities with enhanced capacity for processing dietary sugars and for generating hydrophobic bile acids showed increased susceptibility to metabolic disease. Notably, differences in glucose-stimulated insulin secretion between different mouse strains were partially recapitulated via gut microbiota transfer. Our results suggest that the gut microbiome contributes to the genetic and phenotypic diversity observed among mouse strains and provide a link between the gut microbiome and insulin secretion.

INTRODUCTION

The intestinal microbiota exerts a profound influence on development, physiology, and health (Clemente et al., 2012; Sommer and Bäckhed, 2013; Tremaroli and Bäckhed, 2012). Although there is substantial interpersonal variation in the composition of the gut microbiota among unrelated healthy subjects, sequencing studies have revealed distal gut community patterns associated with different pathological states, including obesity and diabetes (Ridaura et al., 2013; Qin et al., 2012; Karlsson et al., 2013). Remarkably, alterations in the intestinal microbiota composition have been shown to modulate insulin sensitivity (Vrieze et al., 2010), a key feature in metabolic disease and type 2 diabetes (T2D), and thus play a role in diabetes susceptibility.

Dietary components that are not efficiently absorbed in the proximal intestine reach the distal gut, where they are metabolized by gut microbes. Intestinal microbes impact our health in part by generating numerous metabolites from our diet. Short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, are produced through bacterial fermentation of dietary carbohydrates. SCFAs serve as energy and signaling molecules in the intestine and peripheral organs (den Besten et al., 2013). Specifically, SCFAs are important regulators of both energy and glucose homeostasis (den Besten et al., 2013; Koh et al., 2016). For example, butyrate improves insulin sensitivity (Gao et al., 2009; Hartstra et al., 2015) and T2D patients have reduced levels of butyrate-producing bacteria (Qin et al., 2012). Additionally, acetate modulates insulin secretion from β cells (Priyadarshini et al., 2015; Perry et al., 2016). While primarily associated with metabolic benefits, increased concentrations of butyrate and acetate have been found in the cecum of obese mice, suggesting an increased ability of the microbiome to harvest energy from the diet (Turnbaugh et al., 2006).

Gut microbes also impact host physiology by modifying bile acids (BAs) synthesized by the host (Houten et al., 2006; Kuipers et al., 2014; Ryan et al., 2014; Sayin et al., 2013). In addition to their role in emulsifying lipids, BAs function as hormones through their ability to activate nuclear hormone receptors (Parks et al., 1999) and G-coupled protein receptors (Kawamata et al., 2003). They modulate glucose homeostasis, lipid metabolism, energy expenditure, and intestinal motility (Kuipers et al., 2014). Primary BAs are synthesized from cholesterol in the liver (Russell, 2009), stored in the gallbladder, and secreted into the duodenum upon ingestion of a meal. The gut microbiota catalyzes the production of secondary BAs via deconjugation, dehydrogenation, epimerization, and dehydroxylation of primary BAs

Our results suggest that the gut microbiome contributes to the genetic and phenotypic diversity observed among mouse strains and provide a link between the gut microbiome and insulin secretion.

Cell Reports 18, 1739–1750, February 14, 2017

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Raygan et al. Diabetol Metab Syndr (2018) 10:51
https://doi.org/10.1186/s13098-018-0353-2

Diabetology & Metabolic Syndrome

RESEARCH Open Access

The effects of probiotic supplementation on metabolic status in type 2 diabetic patients with coronary heart disease

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Abstract

Background: This study was conducted to evaluate the effects of probiotic supplementation on metabolic profiles in diabetic patients with coronary heart disease (CHD).

Methods: This randomized, double-blind, placebo-controlled trial was performed among 60 diabetic patients with CHD, aged 40–85 years at a cardiology clinic in Kashan, Iran, from October 2017 through January 2018. Patients were randomly divided into two groups to take either probiotic supplements (n = 30) or placebo (n = 30) for 12 weeks. Fasting blood samples were taken at the beginning of the study and after the 12-week intervention to determine related markers.

Results: After 12-week intervention, probiotic supplementation significantly decreased fasting plasma glucose (β − 20.02 mg/dL; 95% CI − 33.86, − 6.17; $P = 0.005$), insulin (β − 2.09 μIU/mL; 95% CI − 3.77, − 0.41; $P = 0.01$), insulin resistance (β − 0.50; 95% CI − 0.96, − 0.03; $P = 0.03$) and total-/HDL-cholesterol ratio (β − 0.27; 95% CI − 0.52, − 0.03; $P = 0.02$), and significantly increased insulin sensitivity (β 0.008; 95% CI 0.001, 0.01; $P = 0.02$) and HDL-cholesterol levels (β 2.52 mg/dL; 95% CI 0.04, 5.00; $P = 0.04$) compared with the placebo. Moreover, probiotic supplementation led to a significant reduction in serum high sensitivity C-reactive protein (β − 0.88 mg/L; 95% CI − 1.39, − 0.38; $P = 0.001$), and a significant elevation in total antioxidant capacity (β 108.44 mmol/L; 95% CI 47.61, 169.27; $P = 0.001$) and total glutathione levels (β 45.15 μmol/L; 95% CI 5.82, 84.47; $P = 0.02$) compared with the placebo. Probiotic supplementation did not affect other metabolic profiles.

Conclusions: Overall, we found that probiotic supplementation for 12 weeks had beneficial effects on glycemic control, HDL-cholesterol, total-/HDL-cholesterol ratio, biomarkers of inflammation and oxidative stress in diabetic patients with CHD.

Trial registration Clinical trial registration number http://www.irct.ir: IRCT2017082733941N5

Keywords: Probiotic, Coronary heart disease, Metabolic status, Type 2 diabetes mellitus

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Full list of author information is available at the end of the article

Conclusions: "Overall, we found that probiotic supplementation for 12 weeks had beneficial effects on glycemic control, HDL-cholesterol, total-/HDL-cholesterol ratio, biomarkers of inflammation and oxidative stress in diabetic patients with CHD."

Raygan *et al. Diabetol Metab Syndr (2018) 10:51*
https://doi.org/10.1186/s13098-018-0353-2

(C) Health

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Vitamin D Supplementation, Glycemic Control, and Insulin Resistance in Prediabetics: A Meta-Analysis

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Diabetes prevention is a public health priority. Vitamin D supplementation may help prevent the development of diabetes in persons at increased risk. We performed a meta-analysis of controlled clinical trials that assessed glycemic outcome measures among adults at risk for type 2 diabetes, including prediabetes, overweight, or obesity. We searched PUBMED/ MEDLINE, CINAHL, and Google Scholar databases for trials published prior to April 2017. Placebo-controlled clinical trials with random allocation to vitamin D with or without calcium supplementation were selected. Data collection included country, study design, inclusion criteria, sample size, form, and dose of vitamin D, supplementation interval, control group, duration, participant characteristics, comorbidities, baseline and follow-up serum 25-hydroxyvitamin D [25(OH)D] concentration, and available outcome measures [glycosylated hemoglobin (HbA1c), fasting plasma glucose, plasma glucose after 2-hour oral glucose tolerance test, and homeostatic model assessment of insulin resistance (HOMA-IR)]. Data synthesis was conducted using random-effect models (PROSPERO registration no. CRD42017055326). Twenty-eight trials, representing 3848 participants, met the eligibility criteria. Compared with the control group, vitamin D supplementation significantly reduced HbA1c level by −0.48% (95% CI, −0.79 to −0.18), fasting plasma glucose level by −0.46 mmol/L (95% CI, −0.74 to −0.19), and HOMA-IR level by −0.39 (95% CI, −0.68 to −0.11). Subgroup analysis revealed that the effects of vitamin D supplementation on different glycemic measures were influenced by age, calcium coadministration, vitamin D deficiency, serum 25(OH)D level after supplementation, and duration of supplementation. Vitamin D supplementation and improved vitamin D status improved glycemic measures and insulin sensitivity and may be useful as part of a preventive strategy for type 2 diabetes.

Freeform/Key Words: 25-hydroxyvitamin D, cholecalciferol, diabetes, hemoglobin A1c, prediabetes, vitamin D

Every 3 minutes, a Canadian is diagnosed with type 2 diabetes or prediabetes [1]. Currently, more than 5.7 million Canadians have prediabetes [1]. Prediabetes refers to impaired fasting glucose or impaired glucose tolerance, with fasting blood glucose levels above normal but not elevated enough to be diagnosed as type 2 diabetes mellitus [2]. People with prediabetes are at a 50% higher risk of developing type 2 diabetes [3, 4]. Yet, even if these people at high risk do not progress to type 2 diabetes, prediabetics are still prone to some of the long-term

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 2HPG, plasma glucose after 2-hour oral glucose tolerance test; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; IQR, interquartile range; PTH, parathyroid hormone; RCT, randomized controlled trial; SE, standard error.

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doi: 10.1210/js.2017-00472 | Journal of the Endocrine Society | 687–709

"Compared with the control group, vitamin D supplementation significantly reduced HbA1c level by -0.48% (95% CI, -0.79 to -0.18), fasting plasma glucose level by -0.46 mmol/L (95% CI, -0.74 to -0.19), and HOMA-IR level by -0.39 (95% CI, -0.68 to -0.11)."

"Vitamin D supplementation and improved vitamin D status improved glycemic measures and insulin sensitivity and may be useful as part of a preventive strategy for type 2 diabetes."

July 2018 | Vol. 2, Iss. 7, Journal of the Endocrine Society 687-709
doi: 10.1210/js.2017-00472

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The Definition of Diabetes Mellitus

The Webster's Deluxe Unabridged Dictionary Second Edition defines diabetes mellitus as:

- ***Diabetes mellitus*** *is a chronic form of disease characterized by excess of sugar in the blood and urine, hunger, thirst, and gradual loss of weight: also called sugar diabetes.*

Wikipedia incorporates a more scientific description

- ***Diabetes mellitus*** *is a disorder of carbohydrate metabolism. It is a disease characterized by persistent hyperglycemia (high blood sugar levels). It is a metabolic disease that requires medical diagnosis, treatment and lifestyle changes.*

Medical Practice

- *The presence of abnormally elevated glycogenated hemoglobin is diagnostic of diabetes mellitus*

The World Publishing Company- William Collins Publishers, Inc. 1979

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Hyperglycemia

Diabetes

- Type 1
- Type 2

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The Cause of Diabetes Mellitus:

- The ancient Egyptians, Chinese, Macedonians (Hippocrates) and Romans (Aretaeus of Cappadocian) recognized this disease to be one of excessive urine (polyuria), leading to wasting and death. Today, we scientifically characterize this disease by its specific anomaly of carbohydrate metabolism
- Type I: Inability to make insulin
- Type II: Resistance to the insulin made

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The Causes of Diabetes Mellitus:

- Type I: Insulin Deficiency is characterized by:
  - Destruction of the pancreatic islet cells by some infection or autoimmune reaction
  - Typically occurring in a child or adolescent
- Type II: Insulin Resistance is characterized by:
  - Resistance to insulin at the cellular level with the initial excessive insulin production being unable to clear glucose from the blood stream.
- Pre-Diabetes: " Insulin Resistance", "Metabolic Syndrome" is a pre-diabetic condition with excessive insulin production. The glycogenated hemoglobin may be in normal range.

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SUGAR HANDLING

- Adrenals
- Liver
- Pancreas

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What are the Diagnostic Tests for Diabetes Mellitus?

- Screening tests
  - Urine: presence of glucose
  - Blood: elevated glucose level
- Diagnostic tests
  - Blood: elevated fasting serum glucose
  - Insulin: elevated fasting insulin level
  - Red Cell: elevated glycogenated hemoglobin measured directly or as Hemoglobin A1c
- Comprehensive testing
  - Glucose Tolerance Test with Insulin levels

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The Failure of Traditional Medicine

Traditionally trained physicians have been taught to treat Blood Sugar Dysregulation based on numbers alone.

DIABETES MELLITUS GIVES US A CHANCE TO THROW AWAY THE PROTOCOLS AND TREAT THE 'CAUSE'.

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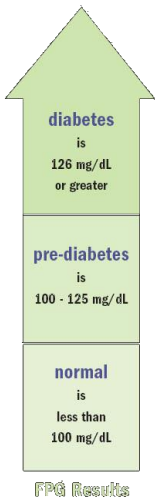
Diagnosing diabetes



Fasting plasma glucose test (FPG) results

Diabetes	126 mg/dl or greater
Pre-diabetes	100 mg/dl to 125 mg/dl
Normal	85-100 mg/dl

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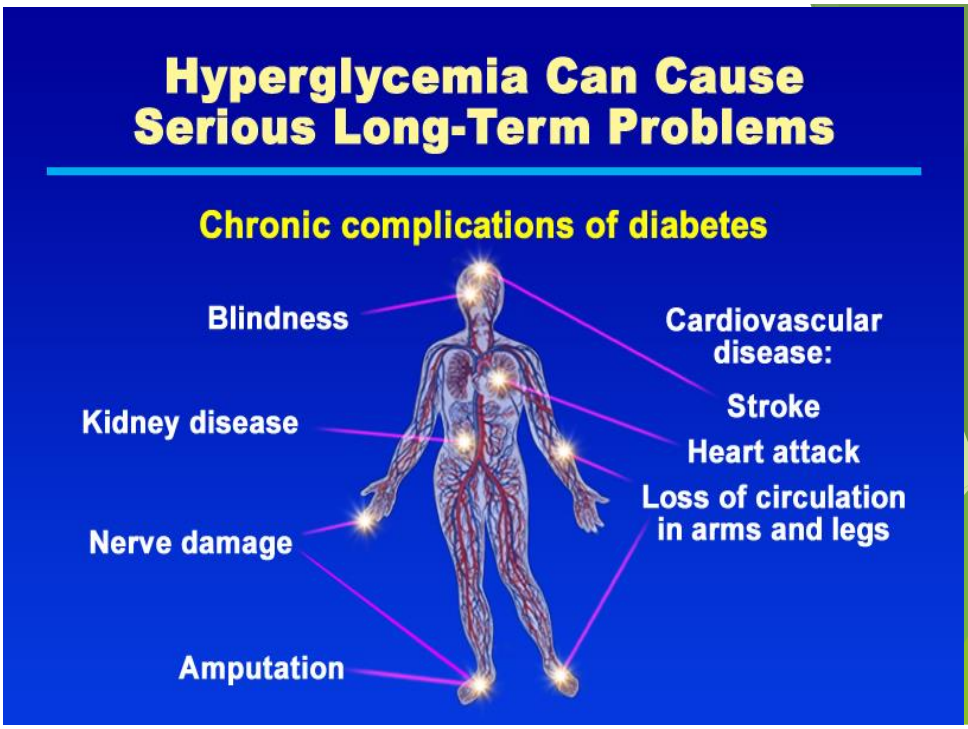

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SERUM GLUCOSE

INCREASED (hyperglycemia)	DECREASED (hypoglycemia)
Diabetes-triglycerides Hgb A1c increased	Fasting hypoglycemia
Thiamine insufficiency	Liver distraction
Disinsulinism (Syndrome-X/Metabolic syndrome)	Hyperinsulinism
Both hyperthyroidism and hypothyroidism	Addison's disease
Infections	Adrenal hypofunction
Chronic nephritis	Pregnancy
Hyperpituitarism	Pancreatic cancer or pancreatitis
Adrenal hyperfunction	Polycystic ovary disease
Cushing's disease	Improper regulation of insulin with IDDM

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SERUM GLUCOSE

INCREASED	DECREASED
Alpha lipoic acid	Niacin
Liver, adrenal, pancreas, pituitary extract	Chromium
B-B vitamins(B1)	Magnesium
Vitamin E	Omega-3 and 6 fatty acids
Zinc, chromium	Liver, pituitary, adrenal, pancreas extract
Magnesium	Paleo- Mediterranean diet
CoQ10	
Paleo-Mediterranean diet – keto based	

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When & How Often Should Testing be done?

- **On insulin:**
  4 times per day.
- **Not on insulin:**
  2 times per day.

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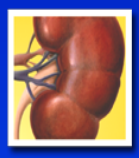
Good News for Type 1 Diabetes

Keeping blood glucose in target range reduces:

Kidney disease as much as 56%



Nerve damage as much as 60%

Eye disease as much as 76%

Diabetes Control and Complications Trial

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Good News for Type 2 Diabetes

Keeping A1c in target range reduces:

Heart attack as much as 16%

Eye damage as much as 21%

Kidney disease as much as 34%

United Kingdom Prospective Diabetes Study

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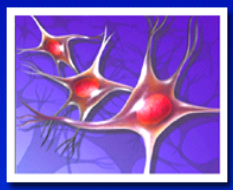
Diabetes Can Lead to Nerve and Small Blood Vessel Damage

Nerve damage (neuropathy)



Can cause problems in:

- Feet and hands
- Heart and circulation
- Stomach, bladder, and sex organs

Small blood vessel damage (microvascular complications)

Can cause:

- Blindness
- Kidney disease

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Type 1 Triggers

- Viral infection
- PARASITES
- Vaccines
- Low levels of vitamin D
- Cow's milk
- Increased insulin demand
- Zonulin

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Type 2 Pre-Disposing Factors:

- Pancreatic, pituitary, thyroid, adrenal and/or liver dysfunction
- Trace mineral deficiency
- Carbohydrate sensitivity (Metabolic Syndrome)
- Carrying too much excess body fat
- Having high blood pressure or cholesterol
- Having a close family member with type 2 diabetes
- Having previously had gestational diabetes
- Medications:
  - ✓ Statins
  - ✓ Corticosteroids
  - ✓ Thiazides (Diuretics)
  - ✓ Beta-blockers

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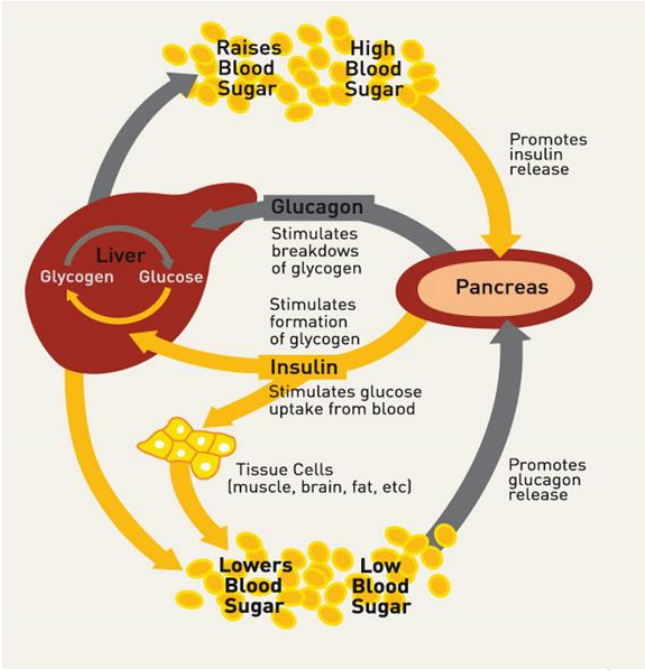

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THE ROLES OF INSULIN AND GLUCAGON

INSULIN	GLUCAGON
✓ lowers elevated blood sugar	✓ raises low blood sugar
✓ shifts metabolism into storage mode	✓ shifts metabolism into burning mode
✓ converts glucose and protein to fat	✓ converts protein and fat to glucose
✓ converts dietary fat to storage	✓ converts dietary fats to ketones and sends them the tissues for energy
✓ removes fat from blood and transports it into fat cells	✓ releases fat from fat cells into the blood for use by tissues as energy
✓ increases the body's production of cholesterol	✓ decreases the body's production of cholesterol
✓ makes the kidneys retain excess fluid	✓ makes the kidneys release excess fluid
✓ stimulates the growth of arterial smooth muscle cells	✓ stimulates the regression of arterial smooth muscle cells
✓ stimulates the use of glucose for energy	✓ stimulates the use of fat for energy

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Hypoglycemia Pre-Disposing Factors:

- Liver/biliary dysfunction
- Endocrine hypofunction (Adrenal, pancreas, pituitary and/or thyroid)
- Hypochlorhydria
- High carbohydrate diets

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Hypoglycemic Symptoms

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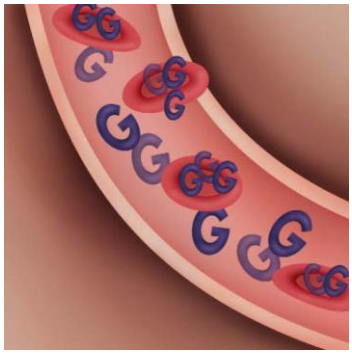
HbA1c: the blood test with a memory



HbA1c in bloodstream.

What is HbA1c?

Hemoglobin is a protein that makes your red blood cells red-colored.

When hemoglobin picks up glucose from your bloodstream, the hemoglobin becomes **glycosylated.**

Glycosylated hemoglobin is HbA1c. The HbA1c test measures the percentage of HbA1c in your blood — a number that corresponds to your average blood glucose for the previous 3 months.

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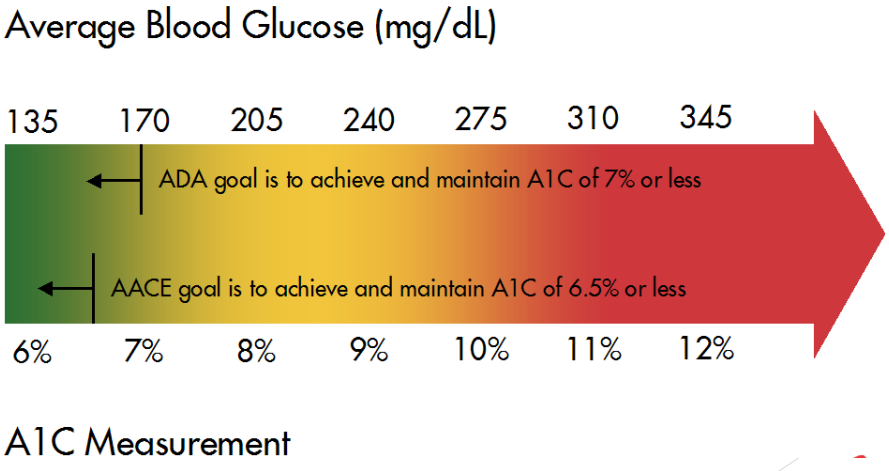
A1C and daily blood glucose go hand in hand



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Metabolic Syndrome

According to the American Heart Association and the National Heart, Lung, and Blood Institute, metabolic syndrome is present if you have three or more of the following signs:

- Blood pressure equal to or higher than 130/85 mmHg
- Fasting blood sugar (glucose) equal to or higher than 100 mg/dL
- Large waist circumference (length around the waist):
  - Men – 40 inches or more
  - Women – 35 inches or more
- Low HDL cholesterol:
  - Men – under 40 mg/dL
  - Women – under 50 mg/dL
- Triglycerides equal to or higher than 150 mg/dL

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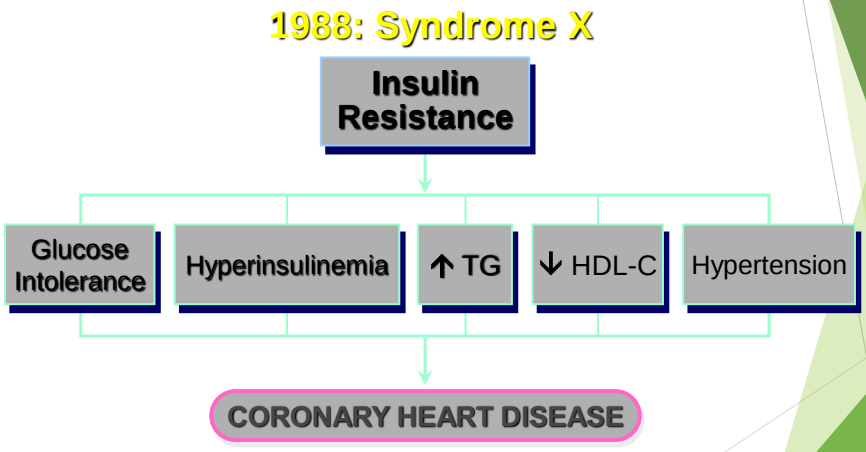
The Metabolic Syndrome: *Historical Perspective*



Reaven G. Diabetes. 1988;37:1565-1607

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The metabolic profile in patients with skin tags.

Sari R[1], Akman A, Alpsoy E, Balci MK.

Author information

Abstract

Although skin tags are associated with diabetes mellitus, insulin resistance, hypertension, obesity, atherogenic lipid profile, no data in the literature show that the presence of skin tags is associated with serum high-sensitive C-reactive protein, uric acid, free fatty acid and leptin level. The purpose of this study was to evaluate the frequency of hypertension, dyslipidemia, insulin resistance and obesity in patients with skin tags and to compare patients with skin tags and normal healthy subjects for insulin resistance, serum lipids, insulin, glucose, leptin, high-sensitive C-reactive protein, free fatty acid levels. We evaluated 113 patients with skin tags and 31 healthy subjects. The two groups were compared with respect to BMI, lipid profile, blood pressure, insulin resistance, serum lipids, insulin, glucose, leptin, high-sensitive C-reactive protein, free fatty acid and homeostatic model assessment of insulin resistance (HOMA-IR). Total 53.9 and 33.6% of patients with skin tags were overweight and obese, respectively. The frequency of hypertension 30.1%, dyslipidemia 59.3% and insulin resistance 21.2% were detected. HOMA-IR ($P < 0.001$) and serum glucose ($P < 0.001$), insulin ($P = 0.002$), high-sensitive C-reactive protein ($P = 0.001$), uric acid ($P = 0.001$), free fatty acid ($P = 0.002$), HbA1c ($P < 0.001$), total cholesterol ($P = 0.018$), LDL-cholesterol ($P = 0.023$), and triglyceride levels ($P = 0.001$) were higher in patients with skin tags than control group. Overweight and/or obesity, dyslipidemia, hypertension, insulin resistance and elevated high-sensitive C-reactive protein are seen in patients with skin tags. Skin tags may be a marker of increased risk of atherosclerosis and cardiovascular disease.

PMID: 20033751 DOI: 10.1007/s10238-009-0086-5

Clin Exp Med. 2010 Sep;10(3):1937. Epub 2009 Dec 24. doi: 10.1007/s1023800900865.

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Associated with Skin Tags:

- **Aging**
- **Human Papillomavirus Infection**
- **Diabetes**
- **Obesity**
- **Friction**
- **Pregnancy**
- **Hyperinsulinemia**
- **Sex steroid imbalance**
- **Polycystic Ovary Syndrome**
- **Birt-Hogg-Dube syndrome**
  - ✓ Although this condition doesn't cause skin tags in adults, children are particularly prone to their development in this state. The disease itself is pretty rare, but in most of the cases where it is the cause, the parents mistake the skin tags for child warts and don't take the issue seriously.
  - ✓ This illness reduces the immunity of the lungs and skin, increasing the chance of tumors and various types of cancer. A particular signature of the disease includes the appearance of skin tags on the child's neck, face and upper chest

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Facts on skin tags:

- ❑ Skin tags are benign tumors of the skin.
- ❑ Some people are more susceptible to skin tags than others.
- ❑ Skin tags commonly occur in creases or folds of the skin.
- ❑ Obesity and diabetes may increase the risk of skin tags developing.
- ❑ Skin tags are typically removed for aesthetic and cosmetic reasons.
- ❑ Methods of skin tag removal include excision and cryotherapy.
- ❑ There are some over-the-counter solutions available for skin tags.
- ❑ There is no evidence to suggest that removing a skin tag causes more to develop.

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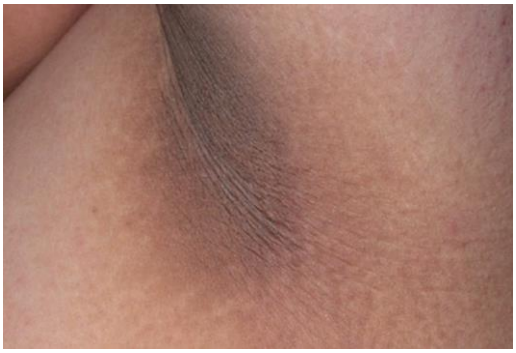
Acanthosis Nigricans



Acanthosis nigricans skin patches occur when epidermal skin cells begin to reproduce rapidly. This abnormal skin cell growth is most commonly triggered by high levels of insulin in the blood. In rare cases, the increase in skin cells may be caused by medications, cancer, or other medical conditions

Other potential conditions:

- stomach cancer, or gastric adenocarcinoma
- adrenal gland disorders, such as Addison's disease
- disorders of the pituitary gland
- low levels of thyroid hormones
- high doses of niacin

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Triglycerides	<100 Levels greater than 60% of total cholesterol should be addressed especially if HDL are 40 or below.	optimal
Fasting Insulin	10 IU /ml or below Over 10 IU/ml	optimal high
HBGA1C or Glycated Hemoglobin	5.4 or less percent 5.6 - 5.8 5.9 - 6.9 7.0 or higher	optimal acceptable high at risk of diabetic complications
Glucose	70 - 85 mg/dl 85 - 100 mg /dl 100 plus	optimal high indicative of diabetes
Anion Gap	(Sodium +Potassium) – (CO2 +Cloride) = Anion Gap; if that number is 14 or over and the CO2 is low (under 24) consider a thiamine deficiency, and supplement with a phosphoralated form like **Bio-3B-G**. Low B1 is often the cause of elevated glucose.	
Insulin Resistance Calculation	(Fasting Insulin X Fasting Glucose) Divide that number by 405. If that calculation is greater than 1.8 you have insulin resistance.	
Leptin	4 - 6 ng/dl Up to 9 ng /dl 10 plus ng/dl	optimal acceptable high

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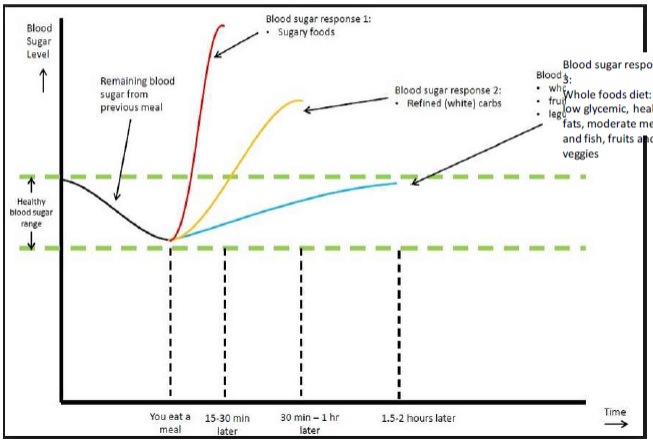
Blood sugar responses to various foods



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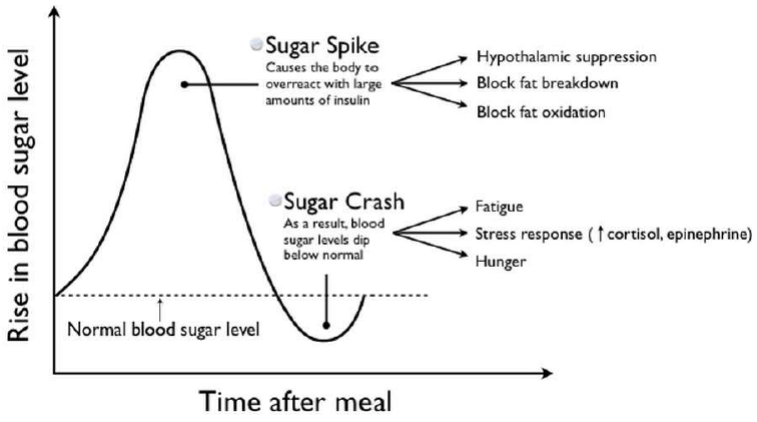
Carb Consumption and Adrenal Stress



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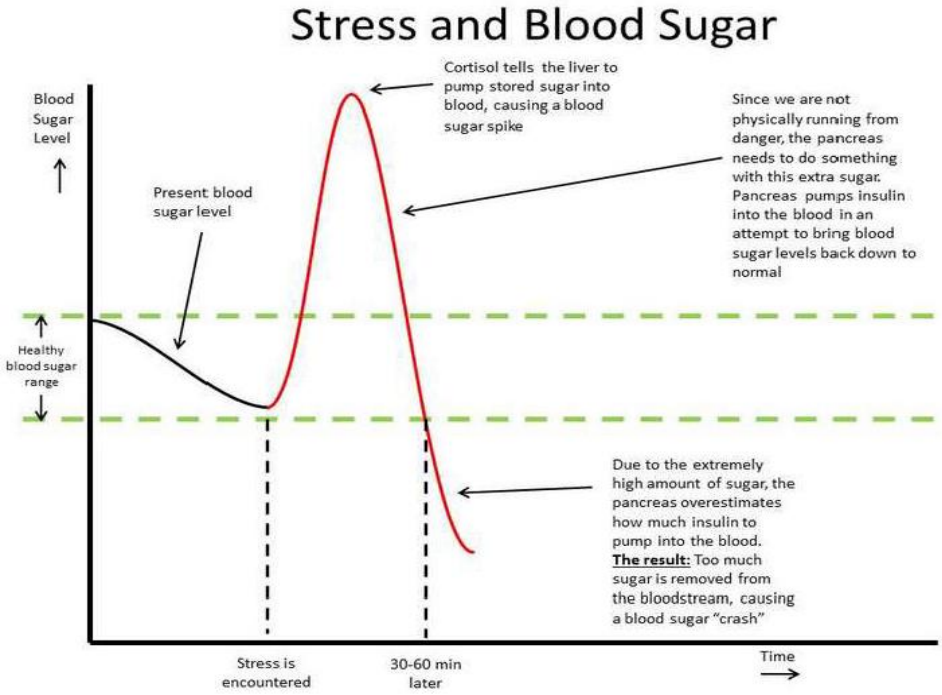

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How to assess "Pre-Diabetic" blood sugar dysregulation?

Obvious ways include:

- Serum Glucose (85 – 100)
- Hemoglobin A1C (<5.4)
- Insulin (<10) ???
- Urine strips
- Triglycerides (70 – 100)
- Physical findings:
  - Body type – "Apple-shaped"
  - **Skin tags**
  - **Acanthosis Nigricans**

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Chang et al. Journal of Biomedical Science 2011, 18:47
http://www.jbiomedsci.com/content/18/1/47

NSC
The cost of publication in Journal of Biomedical Science is borne by the National Science Council, Taiwan

JBS JOURNAL OF BIOMEDICAL SCIENCE

RESEARCH Open Access

Resveratrol retards progression of diabetic nephropathy through modulations of oxidative

Our findings suggest that RSV (resveratrol) protects against oxidative stress, exhibits concurrent proinflammation and anti-inflammation and up-regulates AMPK expression and activation, which may contribute to its beneficial effects on the early stage of DN.

Chang et al. Journal of Biomedical Science, 2011, 18:47

diabetic kidneys.

Conclusions: Our findings suggest that RSV protects against oxidative stress, exhibits concurrent proinflammation and anti-inflammation, and up-regulates AMPK expression and activation, which may contribute to its beneficial effects on the early stage of DN.

Introduction

Diabetes mellitus (DM), mainly characterized by recurrent hyperglycemia, had become one of the chronic disorders derived from insulin deficiency or resistance in the developed countries. As the high blood glucose level in diabetes persisted and progressed without appropriate medical care, relative secondary disorders involving atherosclerosis, retinopathy, nephropathy, neuropathy, stroke, and foot ulcer would individually develop with an insidious onset, which could eventually be life-threatening. Diabetic nephropathy (DN), the second most prevalent diabetes-associated complication inferior to cardiovascular disorders, impaired the renal function of DM patients and therefore cost appreciable medical labor and resource for DN management annually. Histologically featured by thickening of basement membrane, expansion and nodular aggregation of mesangial matrix (the Kimmelstiel-Wilson lesions) and sclerosis in glomeruli, DN could be multifactorial in the pathogenesis. In these risk factors, hyperglycemia was currently regarded as one of the leading causes in the progression of DN. Accumulating evidence also suggested the development of DN was associated with the activation of several stress-sensitive signal pathways, including nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) [1-4]. Additionally, it was reported that

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Full list of author information is available at the end of the article

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4 Major Factors: Epimutagens
- Microbiome – Stealth Infections
- Acid/Alkaline Balance
- Blood Sugar Regulation
- **Stress**

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Stress Is Stressful!

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Stress - What is it?

Definition of *stress*

- **1:** constraining force or influence: such as
  - **a:** a force exerted when one body or body part presses on, pulls on, pushes against, or tends to compress or twist another body or body part *especially* **:** the intensity of this mutual force commonly expressed in pounds per square inch
  - **b:** the deformation caused in a body by such a force
  - **c:** a physical, chemical, or emotional factor that causes bodily or mental tension and may be a factor in disease causation
  - **d:** a state resulting from a stress *especially* **:** one of bodily or mental tension resulting from factors that tend to alter an existent equilibrium
- Downloaded January 12, 2021 - https://www.merriam-webster.com/dictionary/stress

Physical challenges to the integrity of an organism provoke responses to counteract those threats. (Claude Bernard, 1865)

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What is Stress?

The "stress response" is the nonspecific response of the body to any demand put upon it.

(Selye, The Stress of Life, 1956)

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"Stress is the cause of at least 95% of all illness and disease. The remaining 5 percent is genetic and was caused, you guessed it, by stress somewhere in the ancestry of that person."

– Bruce Lipton PhD

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Psychosom Med. 2018 ; 80(2): 141–153. doi:10.1097/PSY.0000000000000545.

Psychological Stress and Mitochondria: A Systematic Review

Martin Picard, PhD and **Bruce S. McEwen, PhD**
Department of Psychiatry, Division of Behavioral Medicine (Picard), Department of Neurology, The H. Houston Merritt Center, Columbia Translational Neuroscience Initiative (Picard), and Columbia Aging Center (Picard), Columbia University; and Laboratory for Neuroendocrinology (McEwen), The Rockefeller University, New York, New York

Abstract

Objective—Mitochondria are multifunctional life-sustaining organelles that represent a potential intersection point between psychosocial experiences and biological stress responses. This article provides a systematic review of the effects of psychological stress on mitochondrial structure and function.

Methods—A systematic review of the literature investigating the effects of psychological stress on mitochondrial function was conducted. The review focused on experimentally controlled studies allowing us to draw causal inference about the effect of induced psychological stress on mitochondria.

Results—A total of 23 studies met the inclusion criteria. All studies involved male laboratory animals, and most demonstrated that acute and chronic stressors influenced specific facets of mitochondrial function, particularly within the brain. Nineteen studies showed significant adverse effects of psychological stress on mitochondria and four found increases in function or size after stress. In humans, only six observational studies were available, none with experimental designs, and most only measured biological markers that do not directly reflect mitochondrial function, such as mitochondrial DNA copy number.

Conclusions—Overall, evidence supports the notion that acute and chronic stressors influence various aspects of mitochondrial biology, and that chronic stress exposure can lead to molecular and functional recalibrations among mitochondria. Limitations of current animal and human studies are discussed. Maladaptive mitochondrial changes that characterize this subcellular state of stress are termed mitochondrial allostatic load. Prospective studies with sensitive measures of specific mitochondrial outcomes will be needed to establish the link between psychosocial stressors, emotional states, the resulting neuroendocrine and immune processes, and mitochondrial energetics relevant to mind-body research in humans.

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The authors have no conflict of interest to report.

"Nineteen studies showed significant adverse effects of psychological stress on mitochondria and four found increases in function or size after stress."

"Overall, evidence supports the notion that acute and chronic stressors influence various aspects of mitochondrial biology, and that chronic stress exposure can lead to molecular and functional recalibrations among mitochondria."

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Psychological Stress and Mitochondria: A Conceptual Framework

Martin Picard, PhD[1,2,3] and **Bruce S. McEwen, PhD**[4]
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[2]Department of Neurology, The H. Houston Merritt Center, Columbia Translational Neuroscience Initiative, Columbia University Medical Center, New York, NY 10032, USA
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Abstract

BACKGROUND—The integration of biological, psychological, and social factors in medicine has benefited from increasingly precise stress response biomarkers. Mitochondria, a sub-cellular organelle with its own genome, produce the energy required for life and generate signals that enable stress adaptation. An emerging concept proposes that mitochondria sense, integrate, and transduce psychosocial and behavioral factors into cellular and molecular modifications. Mitochondrial signaling might in turn contribute to the biological embedding of psychological states.

METHODS—A narrative literature review was conducted to evaluate evidence supporting this model implicating mitochondria in the stress response, and its implementation in behavioral and psychosomatic medicine.

RESULTS—Chronically, psychological stress induce metabolic and neuroendocrine mediators that cause structural and functional recalibrations of mitochondria, which constitutes mitochondrial allostatic load (MAL). Clinically, primary mitochondrial defects affect the brain, endocrine, and immune systems that play a role in psychosomatic processes, suggesting a shared underlying mechanistic basis. Mitochondrial function and dysfunction also contribute to systemic physiological regulation through the release of mitokines and other metabolites. At the cellular level, mitochondrial signaling influences gene expression and epigenetic modifications, and modulates the rate of cellular aging.

CONCLUSIONS—This evidence suggests that MAL represents a potential sub-cellular mechanism for transducing psychosocial experiences and the resulting emotional responses – both adverse and positive – into clinically meaningful biological and physiological changes. The associated article in this issue of *Psychosomatic Medicine* presents a systematic review of the

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The authors have no conflict of interest to report.

'Integrating mitochondria into biobehavioral and psychosomatic research opens new possibilities to investigate how psychosocial factors influence human health and well-being across the lifespan."

Psychosom Med. 2018 ; 80(2): 126–140. doi:10.1097/PSY.0000000000000544.

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The art of medicine

The stress of life: a modern complaint?

In a series of apocalyptic novels published shortly before his death, the British writer J G Ballard (1930-2009) imagined the potential impact of progressively advanced modern societies on human behaviour. Struggling to cope with new forms of work and wealth and with the expansion of leisure, the disaffected middle-class characters that inhabit *Cocaine Nights* (1996), *Super-Cannes* (2000), and *Millennium People* (2003) seek release from the stress of life by turning to violence, sexual licence, and carefully calculated forms of madness. According to Ballard's dystopian vision, the frustration, insecurity, and loneliness of modern lives are only capable of generating communities oppressed by social unrest, political instability, immorality, and injustice.

Although imaginative and controversial, Ballard's fictional portrayals of a species under stress captured an emergent reality. In 2000, the British Health and Safety Executive (HSE) reported an increase in occupational stress between 1990 and 1995, 4 years later, the Whitehall II study highlighted the role of stress in shaping sickness patterns among civil servants. In 2009, the HSE estimated that in the UK 13·5 million working days were lost to stress each year and that the annual cost of work-related stress was in the region of £4 billion. Concerns about the socioeconomic impact of work-place stress have been accentuated by claims that increases in hypertension, heart disease, and depression might also be related to the stress of modern lives. According to the American biologist Robert M Sapolsky, some chronic diseases may be explained in terms of the neuroendocrine disturbances generated by attempts to cope with the stress of rapid social, cultural, and technological change. Although a certain degree of stress is accepted as necessary for performance and productivity, some commentators suggest that unmitigated stress seems to be threatening the health and happiness of western populations in particular.

Although we may like to believe that we are more stressed than our predecessors, complaints about the stress and strain of life have a long history. Even Ballard's idiosyncratic prophecies have their precursors. In the 1970s, the left-wing American writer Alvin Toffler argued that post-war populations were suffering from "future shock", a state caused by "the shattering stress and disorientation that we induce in individuals by subjecting them to too much change in too short a time". The inhabitants of modern "throw-away society", he insisted, were struggling to adapt to the "unwanted tempo" of life manifest in the transience of people and places, the speed of technological innovation, and the surfeit of choice in consumables, education, and the media. In the eyes of Toffler and many of his contemporaries, the inability to cope with change was responsible not only for epidemics of heart disease, obesity, anxiety, depression, and suicide, but also for escalating rates of aggression and crime, the demise of sexual standards, and the instability of international relations.

Toffler's opinions gained some credibility from scientific and clinical studies of stress. In the 1930s and 1940s, the Hungarian-born scientist Hans Selye (1907–82) had suggested that some chronic diseases were the result of faulty adaptation to stress, or what he referred to as the "general adaptation syndrome". Shortly after World War 2, Selye developed a more comprehensive account of human pathology by insisting that failures or irregularities in the hypothalamic-pituitary-adrenal axis were responsible for the inability to cope with stress. Selye's descriptions of the biochemical mechanisms of stress reactions and his vigorous attempts to popularise the language of stress initiated further scientific studies of neuroendocrine pathways, leading eventually to the identification of hypothalamic releasing factors, and also encouraged some doctors and patients to explain patterns of health and illness in terms of the escalating stress of life.

Selye's laboratory investigations were mirrored by psychological studies of stress. The role of stress in shaping individual behaviour had become particularly apparent during World War 2, when British, American, and Canadian air-force authorities had blamed the poor performance of some pilots on "flying stress". In the decades after the war, a number of researchers pursued the psychology of stress in more detail. In his studies of appraisal and coping, for example, the American psychologist Richard Lazarus (1922–2002) argued that psychological stress reactions, which he regarded as directly analogous to the physiological mechanisms revealed by Selye, were shaped by people's perceptions. Around the same time, two American psychiatrists, Thomas H Holmes (1918-88) and Richard H Rahe (b. 1936), developed the Social Readjustment Rating Scale, which attempted to quantify stressful life events such as bereavement, divorce, and illness and to provide doctors with a provisional scheme for predicting disease onset.

Accounts of stress developed after World War 2 were themselves not entirely original, but were heavily dependent on interwar studies of the way in which sociopolitical instability and deepening economic recession were precipitating new forms of nerve strain and rising levels of sickness absence and social unrest. In 1937, the British cardiologist Lord Horder (1871–1955) argued that "the stress of modern life" was a product of the "monotony and drabness" of work, a lack of exercise and sleep, an "increasing sense of international insecurity", and the "anxiety connected with the competition of living". In *The Lancet* 4 years earlier, Walter Langdon Brown (1870–1946), Regius

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Professor of Physic at Cambridge, had already suggested that the proliferation of functional disorders, caused by emotional disturbances operating on the autonomic nervous system, could be explained in terms of a failure to adjust "to conditions which are changing so rapidly".

According to some interwar clinicians, prolonged stress led not only to functional nervous diseases, but also to organic conditions. In 1925, the Chicago psychiatrist William S Sadler (1875–1969) suggested that it was the "tension, the incessant drive of American life, the excited strain of the American temperament" that was responsible for rising mortality rates from high blood pressure and diseases of the heart and kidneys. Human beings, he argued, had not yet adapted to the "stress of a civilization which counts on the airplane and the wireless as commonplaces". Of course, for Sadler the prevalence of stress-related conditions served to establish America's social and technological superiority, an example of hubris also captured by the term "Americanitis", which was popularised by the Harvard psychologist William James (1842–1910) with reference to his own nerve strain.

James's insistence on a link between stress and psychological disturbances was in turn based on earlier studies of insanity and nervousness. In 1890, the English psychiatrist Charles Arthur Mercier (1852–1919) had argued that insanity was a "function of two variables": heredity and stress. For Mercier, stresses ranged from internal physiological disturbances associated with puberty and pregnancy through to factors such as overwork, marital problems, insomnia, and head injuries. Other clinicians echoed his approach, pointing at the same time to the association between stress-induced insanity and social progress. According to the American physician William A White (1870-1937), insanity could be initiated by the "stresses incident to active competition" in the industrial world.

Perhaps the most persistent late Victorian version of a connection between advanced societies and stress was embedded in the concept of neurasthenia, a term popularised in the 1860s by the American neurologist George M Beard (1839–83) and widely adopted by European physicians and their patients. In several books on neurasthenia, or what he referred to as "American nervousness", Beard explained the growing prevalence of nervous fatigue in terms of the pressures of modern life. In a passage that betrayed a multitude of anxieties about rapid technological and cultural change, he argued that nervousness could be traced to the principal features of "modern civilization", namely "steam-power, the periodical press, the telegraph, the sciences, and the mental activity of women". As in many later pronouncements on the consequences of failing to adapt to accelerating social progress, stress and nervousness were thought to be more common among the affluent western middle classes.

Late 19th-century doctors and their patients also believed that stress could generate or exacerbate physical illness.

Hans Selye at the University of Montreal's Institute for Experimental Medicine and Surgery in October, 1950

Clinicians sometimes explained the development of cancer, diabetes, and thyroid disease, or the appearance and severity of influenza, in terms of the debilitating effects of overwork and over-worry. The emotional stresses and strains of bereavement, domestic difficulties, financial problems, and the pace of life were all regarded as plausible triggers of pathology. In 1872, an article in *The Times* suggested that rising death rates from heart disease were the "unavoidable result of the great mental strain and hurried excitement" generated by steam and electricity, over-crowded communities, and the relentless and exhausting struggle for existence. Contemporary belief in the capacity for stress to produce both mental and physical disease was so strong, according to the prominent Cambridge physician T Clifford Allbutt (1836–1925), that many people regarded the 19th century as "a century of stress".

Even a cursory historical survey suggests that it would be presumptuous to assume that we are more stressed, or indeed more preoccupied with stress, than our predecessors. As Selye pointed out in 1980, when we proclaim ours as an "age of stress", we tend to ignore the traumas and dangers faced by earlier societies and the fact that inhabitants of those societies equally regarded themselves as stressed. Indeed, past populations were no less stressed by warfare, epidemic disease, unemployment, and poverty than their modern counterparts are. Since at least the mid-19th century, narratives of distress have been bound together not only by mutual understandings and shared experiences of stress, but also by the apocalyptic fear that stress is the inevitable result of the psychological pressures generated by the unfettered growth of industrial and technological capitalism.

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Further reading

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www.thelancet.com
Vol 383 January 25, 2014

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What is Stress? (cont.)

- "Diseases of Adaptation" (Selye, 1956)
- Consistent triad of tissue damage in animals exposed to prolonged stress:
  - **Decrease in size of thymus gland and lymph nodes**
  - **Gastric ulceration**
  - **Enlargement and discoloration of the adrenal glands**

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Triad of stress according to Hans Seyle

1. Adrenal cortex enlargement
   - Altered cortisol levels
   - Altered DHEA levels
2. Lymphatic atrophy
   - Immune system suppression
   - Chronic illness
3. Ulcers
   - Due to decreased stomach acid
   - Due to decreased mucous neck cell secretion

Seyle referred to the condition - "Just being sick"

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"It is not stress that kills us; it is our reaction to it."

~ Hans Selye

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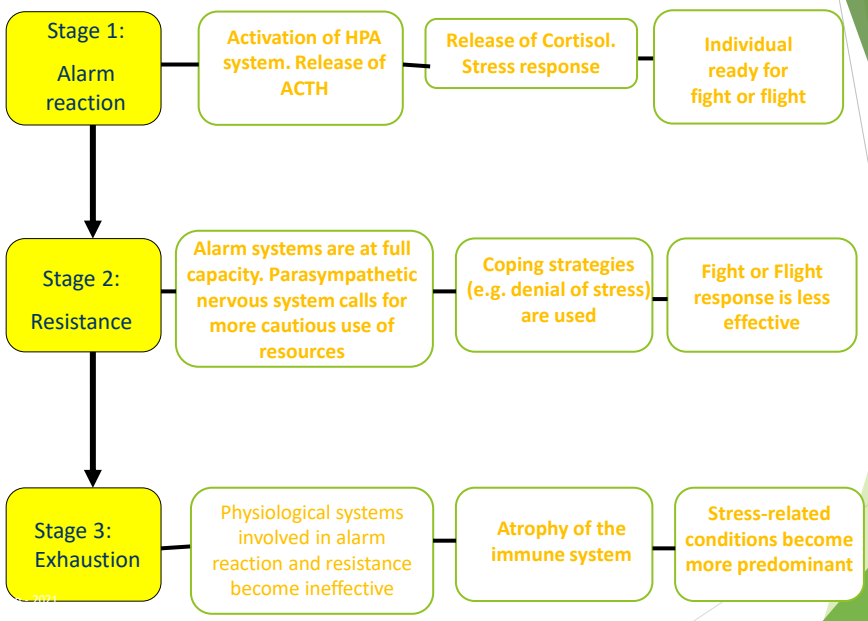

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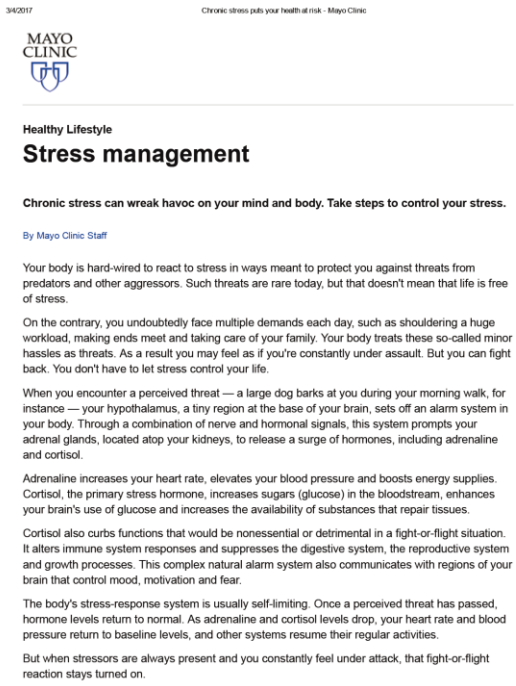

3/4/2017 Chronic stress puts your health at risk - Mayo Clinic

MAYO CLINIC

Healthy Lifestyle

Stress management

Chronic stress can wreak havoc on your mind and body. Take steps to control your stress.

By Mayo Clinic Staff

Your body is hard-wired to react to stress in ways meant to protect you against threats from predators and other aggressors. Such threats are rare today, but that doesn't mean that life is free of stress.

On the contrary, you undoubtedly face multiple demands each day, such as shouldering a huge workload, making ends meet and taking care of your family. Your body treats these so-called minor hassles as threats. As a result you may feel as if you're constantly under assault. But you can fight back. You don't have to let stress control your life.

When you encounter a perceived threat — a large dog barks at you during your morning walk, for instance — your hypothalamus, a tiny region at the base of your brain, sets off an alarm system in your body. Through a combination of nerve and hormonal signals, this system prompts your adrenal glands, located atop your kidneys, to release a surge of hormones, including adrenaline and cortisol.

Adrenaline increases your heart rate, elevates your blood pressure and boosts energy supplies. Cortisol, the primary stress hormone, increases sugars (glucose) in the bloodstream, enhances your brain's use of glucose and increases the availability of substances that repair tissues.

Cortisol also curbs functions that would be nonessential or detrimental in a fight-or-flight situation. It alters immune system responses and suppresses the digestive system, the reproductive system and growth processes. This complex natural alarm system also communicates with regions of your brain that control mood, motivation and fear.

The body's stress-response system is usually self-limiting. Once a perceived threat has passed, hormone levels return to normal. As adrenaline and cortisol levels drop, your heart rate and blood pressure return to baseline levels, and other systems resume their regular activities.

But when stressors are always present and you constantly feel under attack, that fight-or-flight reaction stays turned on.

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Stress

10 Health Problems Related to Stress That You Can Fix

By R. Morgan Griffin
WebMD Feature
Reviewed by Joseph Goldberg, MD

10 Health Problems Related to Stress

What are some of the most significant health problems related to stress? Here's a sampling.

- **Heart disease.**
- **Asthma.**
- **Obesity.**
- **Diabetes.**
- **Headaches.**
- **Depression and anxiety.**
- **Gastrointestinal problems.**
- **Alzheimer's disease..**
- **Accelerated aging.** Stress seemed to accelerate aging about 9 to 17 additional years.
- **Premature death.** It found that caregivers had a 63% higher rate of death than people their age who were not caregivers.

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Stress

Stress negatively affects health on a number of different levels. Our bodies react to stress by producing certain hormones.

These hormones, in particular cortisol, have a number of ill effects upon the body: Accelerated aging, depression, chronic fatigue syndrome, immune system dysfunction, sleep disorders, obesity, high blood pressure, osteoporosis, and decreased memory.

Learning strategies to deal with the emotional, chemical and physical effects of stress is part of a well-balanced health care approach.

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"A highly important change has occurred in the incidence of disease in our country . . . serious infections, formerly extensive and disastrous, have markedly decreased or almost disappeared, . . . meanwhile conditions involving strain in the nervous system have been greatly augmented"
("The role of emotion in disease")

Annals of Internal Medicine [redacted]

Code name – STRESS!!

314

"With our present partial knowledge of the function of the endocrine chain of glands, it appears as though the suprarenals were the first to show signs of fatigue, for the simple reason that they seem to have most of the work to do in the auto-protective functions."

(McNulty, J., New York Medical Journal, 1921, XCIII, pg. 288)

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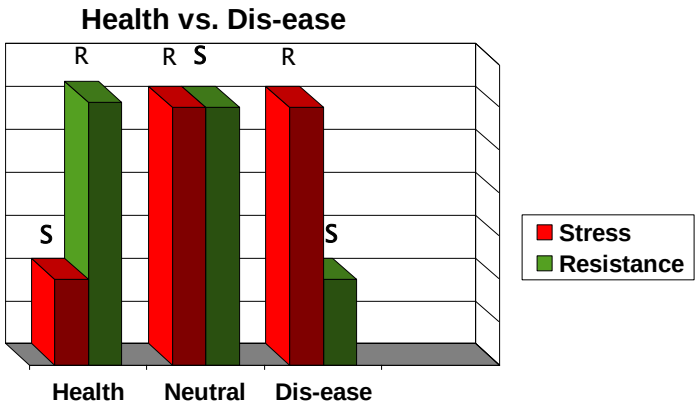
What happens when stress to the individual is greater than the individual's resistance?

- Dis-ease!



316

If I could live my life over again, I would devote it to proving that germs seek their natural habitat, diseased tissue – rather than being the cause of the diseased tissue.

~ Rudolph Virchow

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Common Signs of Altered Adrenal Function

- Weakness, tiredness, and/or fatigue
- Intolerance to bright lights – absolutely needs sunglasses
- Get light-headed if stand up quickly
- Salt craving
- Weight loss/gain
- Loss of scalp hair
- Excess facial and/or body hair (females)
- Constipation
- Diarrhea
- Muscle or joint pains
- Gastrointestinal symptoms
- Altered blood pressure
- Frequent urination

318

The Denial and Cover–up

It has been my clinical experience that approximately 99% of my patients are experiencing some form of adrenal dysfunction; either hypo or hyper functioning.

Our patients deny they have problems as they cover them up and consider it normal. They cover-up their dysfunction with:

- Caffeine
  - Coffee
  - Energy drinks
  - Pop
  - Tea
- Sugar-laden snacks

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Stressors come in many shapes

- Physical stress - such as overwork, lack of sleep, or plain old just overdoing it.
- Chemical stress -from environmental exposures, diets high in refined carbohydrates, food allergies/sensitivities, or imbalances caused by interactions with other endocrine glands such as the thyroid.
- Thermal stress -over-heating or over-chilling of the body
- Emotional and mental stress

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Identifying Adrenal Dysfunction

- **History**
- **Symptom Survey**
- **Physical examination**
- **Laboratory findings**
  - Urine
  - Saliva
  - Blood chemistry findings

321

What does adrenal dysfunction look like on a symptom survey?

Hyperadrenia

- Cannot fall asleep
- Blood pressure increased
- Perspire easily, even with little exertion
- Wakeup tired even with 'normal' sleep
- Tend to be 'keyed' up, trouble calming down
- Feel 'wired' or jittery after drinking coffee
- Clench or grind teeth
- Headaches
- Hot flashes
- Hair growth on face or body (question to females)
- Masculine tendencies (question to females)

322

What does adrenal dysfunction look like on a symptom survey?

Hypoadrenia

- Cannot stay asleep
- Afternoon headache
- Dizziness when standing up quickly
- Blood pressure low
- Crave salt
- Chronic fatigue/get drowsy
- Afternoon yawning/fatigue
- Weakness/dizziness
- Weakness after colds/slow recovery
- Muscular and nervous exhaustion
- Subject to colds, asthma, bronchitis (respiratory disorders)
- Allergies and/or hives
- Difficulty maintaining manipulative correction

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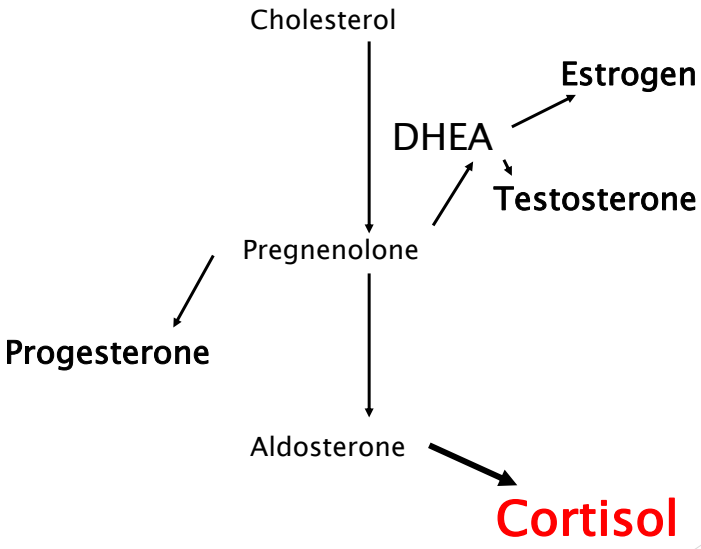
THE CORTISOL STEAL



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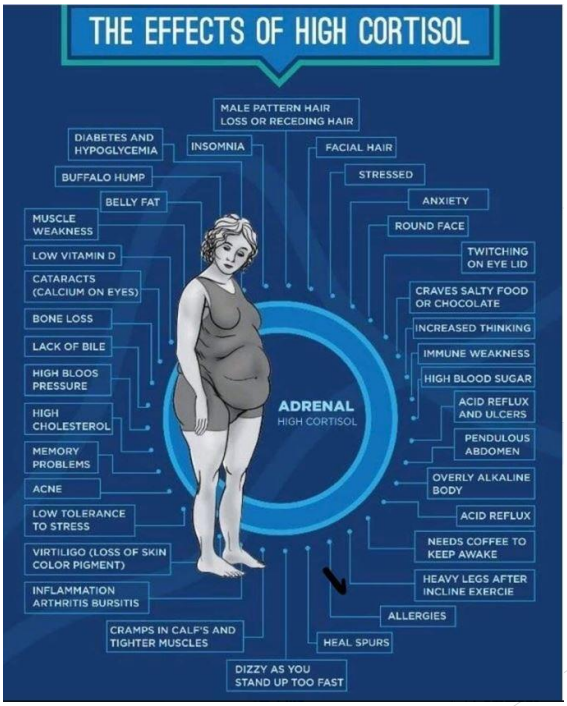

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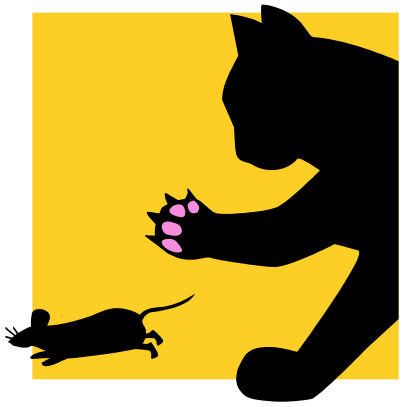
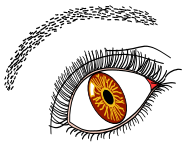
Stress - A Game of Cat and Mouse

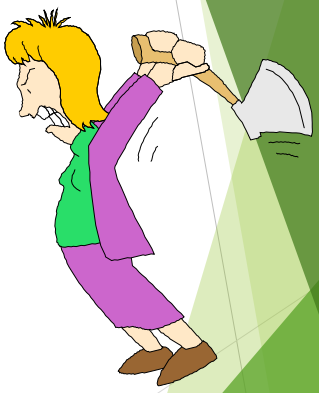


326

The effects of stress on vision

Stress hormones cause the pupils to dilate – therefore bright lights bother your eyes and you need to wear sunglasses whenever it is bright out.





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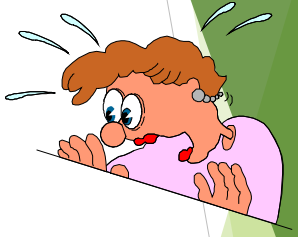
The effects of stress on saliva

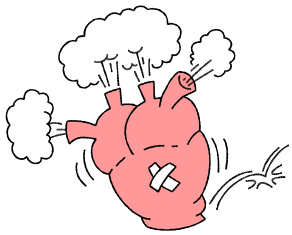
Stress hormones therefore causes inhibition of the salivary glands, your mouth is always dry or you feel the need to chew gum all of the time.



328

The effects of stress on hearts

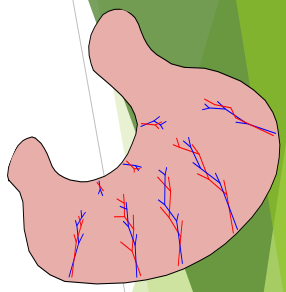
Stress hormones cause the elevation of blood pressure and sclerosis (hardening) of the coronary arteries.



329

The effects of stress on your stomach

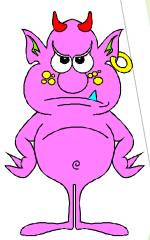
Stress hormones cause a decrease in the secretion of stomach acid and a reduction in the production of mucus protecting the stomach lining.

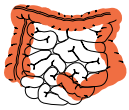


330

The effect of stress on the rest of your digestive tract

Stress hormones cause a decrease in the contractions of the small intestine and an increase in the contractions of the large intestine





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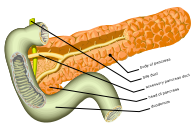
How does stress affect blood sugar?

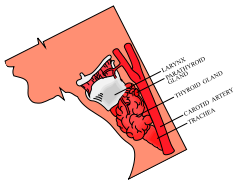
Stress hormones cause an increase in insulin resistance which then makes the cells less receptive to insulin.

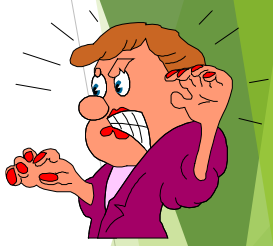


332

The effects of stress on the thyroid

Stress hormones causes inhibition of the thyroid gland. It can interfere with the conversion of T4 to T3, mimicking hypothyroidism.





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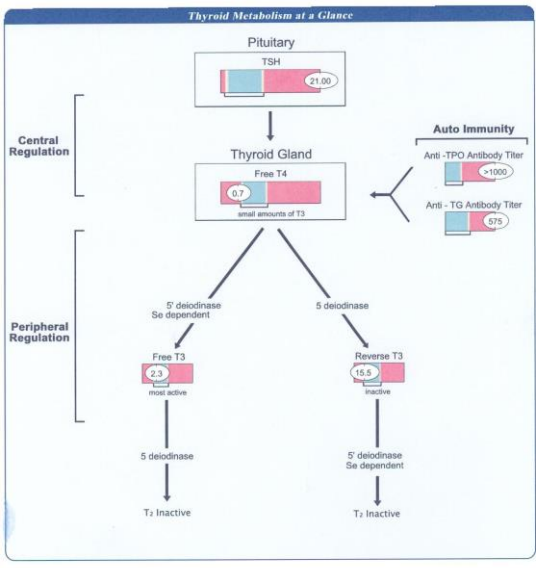
Patient: ID: Page 2



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Citation: Transl Psychiatry (2012) 2, e150; doi:10.1038/tp.2012.77

www.nature.com/tp

Dynamic changes in DNA methylation of stress-associated genes (*OXTR*, *BDNF*) after acute psychosocial stress

E Unternaehrer[1,6], P Luers[3,6], J Mill[3], E Dempster[3], AH Meyer[4], S Staehli[1,2], R Lieb[1], DH Hellhammer[2] and G Meinlschmidt[1,5]

Environmentally induced epigenetic alterations are related to mental health. We investigated quantitative DNA methylation status before and after an acute psychosocial stressor in two stress-related genes: oxytocin receptor (*OXTR*) and brain-derived

"This may enhance the understanding of how psychosocial events alter DNA methylation and cold provide new insights into the etiology of mental disorders."

Translational Psychiatry (2012) 2, e 150; 14 August 2012

Introduction

DNA methylation is an epigenetic mechanism related to mental and physical health and disease.[1–4] Aberrant DNA methylation has been implicated in the etiology of various mental disorders including, depression,[5–9] psychotic disorders,[10–15] post-traumatic stress disorder,[16,17] autism,[18,19] eating disorders[20,21] and substance dependence (for review see[22]), but also has an important role in the pathology of physical illnesses, such as cancer.[23] Thereby DNA methylation provides a biological basis for gene–environment interactions relevant to mental health[24]; animal and human studies have found that early life experiences can alter DNA methylation and affect gene expression and behavior.[25–32] Similarly, experiences later in life can modify the epigenome.[33,34] However, changes in DNA methylation immediately after adverse experiences, such as acute psychosocial stress, have not yet been investigated. Insight into how acute psychosocial stress affects DNA methylation may further elucidate our understanding of etiological mechanisms in mental health. Therefore, we investigated DNA methylation of two stress-related candidate genes—oxytocin receptor (*OXTR*)[35] and brain-derived neurotrophic factor (*BDNF*)[35,36]—before and after an acute psychosocial stressor.

We included the *OXTR* because the oxytocin system interacts with the hypothalamic-pituitary-adrenal axis[35,37–40] and cardiovascular stress reactivity.[41,42] To the best of our knowledge, there have been no studies investigating methylation of *OXTR* with reference to stress in humans or animals. A study on patients suffering from autism spectrum disorder revealed aberrant DNA methylation in an *OXTR* region in peripheral mononuclear blood cells; similar results were found for brain tissue.[43]

BDNF, the second candidate gene, encodes a neuronal growth factor involved in neuronal development, cell differentiation and synaptic plasticity.[44,45] In addition to its pivotal role in the central nervous system, *BDNF* is also expressed in the periphery where it shows neuro-protective action.[46] Peripheral BDNF concentration is decreased in various stress-related mental disorders[47] including depression[48] and post-traumatic stress disorder.[49] Previous work has also shown that early life- and chronic stress resulted in a higher methylation status of *Bdnf*,[32] and a decrease in *Bdnf* mRNA

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OPEN

Citation: Transl Psychiatry (2014) **4**, e448; doi:10.1038/tp.2014.94

www.nature.com/tp

ORIGINAL ARTICLE

Epigenomic profiling of men exposed to early-life stress reveals DNA methylation differences in association with current

… "Our results support the concept that DNA methylation differences may be important in the pathogenesis of psychiatric disease."

Transl Psychiatry (2014) 4, e448 September 2014

with roles in brain development and/or function in association with depressive symptoms. Pathway analysis revealed an enrichment of DNA methylation changes in pathways associated with development and morphogenesis, DNA and transcription factor binding and programmed cell death. Our results support the concept that DNA methylation differences may be important in the pathogenesis of psychiatric disease.

Translational Psychiatry (2014) **4**, e448; doi:10.1038/tp.2014.94; published online 23 September 2014

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BRIEF REPORT

Epigenetics 7:8, 853-857; August 2012; © 2012 Landes Bioscience

Methylation changes at *NR3C1* in newborns associate with maternal prenatal stress exposure and newborn birth weight

"…Increased methylation may constrain plasticity in subsequent gene expression and restrict the range of stress adaptation responses possible in affected individuals, thus increasing their risk for adult-onset diseases."

Epigenetics 7:8, 853-857, August 2012

According to the developmental origins of health and disease (DOHaD) hypothesis, events in early development are directly related to disease risk in later life.[1,2] The rationale is that fetal tissues are especially sensitive to the intrauterine environment, which results in selection of an optimal fetal phenotype. If the intrauterine environment is unusually limiting or unrepresentative of the environment in later life, the selected phenotype

maternal stress on newborn birth weight and investigate if any observed correlation is associated with epigenetic modifications at the newborn *NR3C1*. Specifically, we assay methylation status at 39 CpG sites in the upstream promoter of *NR3C1*, the same region that has been associated with changes in methylation and/or gene expression correlated with childhood abuse-related suicide,[11] prenatal exposure to intimate partner violence[12] and post-

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Yao et al. BMC Medicine 2014, 12:121
http://www.biomedcentral.com/1741-7015/12/121

BMC Medicine

RESEARCH ARTICLE Open Access

Ancestral exposure to stress epigenetically

Results: "Progressively up to the F2 generation stress gradually reduced gestational length……"

Yao et. All. BMC Medicine 2014, 12:121

endocrine, metabolic and behavioural manifestations of PTB possibly via microRNA (miRNA) regulation.

Methods: Pregnant dams of the parental generation were exposed to stress from gestational days 12 to 18. Their pregnant daughters (F1) and grand-daughters (F2) either were stressed or remained as non-stressed controls. Gestational length, maternal gestational weight gain, blood glucose and plasma corticosterone levels, litter size and offspring weight gain from postnatal days 1 to 30 were recorded in each generation, including F3. Maternal behaviours were analysed for the first hour after completed parturition, and offspring sensorimotor development was recorded on postnatal day (P) 7. F0 through F2 maternal brain frontal cortex, uterus and placenta miRNA and gene expression patterns were used to identify stress-induced epigenetic regulatory pathways of maternal behaviour and pregnancy maintenance.

Results: Progressively up to the F2 generation, stress gradually reduced gestational length, maternal weight gain and behavioural activity, and increased blood glucose levels. Reduced offspring growth and delayed behavioural development in the stress cohort was recognizable as early as P7, with the greatest effect in the F3 offspring of transgenerationally stressed mothers. Furthermore, stress altered miRNA expression patterns in the brain and uterus of F2 mothers, including the miR-200 family, which regulates pathways related to brain plasticity and parturition, respectively. Main miR-200 family target genes in the uterus, *Stat5b*, *Zeb1* and *Zeb2*, were downregulated by multigenerational stress in the F1 generation. *Zeb2* was also reduced in the stressed F2 generation, suggesting a causal mechanism for disturbed pregnancy maintenance. Additionally, stress increased placental miR-181a, a marker of human PTB.

Conclusions: The findings indicate that a family history of stress may program central and peripheral pathways regulating gestational length and maternal and newborn health outcomes in the maternal lineage. This new paradigm may model the origin of many human PTB causes.

Keywords: Preterm birth, maternal stress, prenatal stress, transgenerational inheritance, microRNA, epigenetic regulation, gestation, maternal health, behavioural development, perinatal programming, pregnancy

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Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2010 | 112 | 1115–1130

REVIEW

Stress and $GABA_A$ receptors

Kelly J. Skilbeck,*† Graham A. R. Johnston*† and Tina Hinton*†

*Department of Pharmacology, University of Sydney, Sydney, New South Wales, Australia
†Schizophrenia Research Institute, New South Wales, Australia

"… Interestingly, some studies have also provided evidence for long-lasting changes in GABA receptors as a result of exposure to stressors in early-life."

Journal of Neurology, 2010; 112: 1115 – 1130

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Biological Psychiatry

A Journal of Psychiatric Neuroscience and Therapeutics

Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders

…"central aspects of stress response, including activation of the hypothalamic-pituitary-adrenal (HPA) axis and dopamine neurotransmission, are modulated, and in some cases mediated, by glutamate neurotransmission…

Biol Psychiatry. 2002 May 15;51(10):775-87

cortical glutamate neurotransmission. Thus, understanding the contribution of glutamate-mediated processes to stress response through the use of experimental models that involve disrupted PFC function can provide insights to the fundamental pathophysiology of stress-sensitive psychiatric disorders and lead to novel strategies for treatment and prevention.

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Identifying Adrenal Dysfunction
With Urinary Laboratory Findings
Koenisburg's Test

Discussion:

This is a simple, yet highly accurate test at demonstrating adrenal function. The test demonstrates the effects of stress as well other factors associated with the stresses of daily living. Adrenal health is the cause of a number of health problems in America today.

The Koenisburg's test is actually measuring the amount of chloride being spilled into the urine. Whether the levels are elevated or decreased it provides a useful measure of adrenal stress. Adrenal stress is a major cause of illness in this country. As the body's level of aldosterone and cortisol change we can measure this by measuring chloride in the urine.

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Results

1 to 6 of silver nitrate needed

Low urinary chloride

Adrenal hyperfunctioning causing an increase in aldosterone secretion from the cortex of the adrenal which leads to an increase in renal resorption of sodium and chloride ions which leads to a decrease in urine chloride ions.

7 to 8 drops of silver nitrate needed

Normal urinary chloride

9 or greater of silver nitrate needed

High urinary chloride

Adrenal hypofuctioning causing a decrease in aldosterone secretion from the cortex of the adrenal which leads to a decrease in renal resorption of sodium and chloride ion which leads to an increase in urine chloride ions

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Koenisburg's Test

Summary of results:

↓ Koenisburg's(1-6)	**↑ (Hyperadrenia)**	**Compensation stage**	**↓ chloride**	**↑ aldosterone**
Normal Koenisburg's (7)				
↑ Koenisburg's (>8)	**↓ (Hypoadrenia)**	**Exhaustion stage**	**↑ chloride**	**↓ aldosterone**

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Identifying Adrenal Dysfunction With Blood Chemistry Laboratory Findings

Hypoadrenia

- ✓ ↑ potassium (K)
- ✓ ↓ sodium (Na)
- ✓ ↓ fasting glucose <80
- ✓ ↓ Aldosterone
- ✓ ↓ or N chloride (Cl)
- ✓ ↓ DHEA (generally)
- ✓ ↓ cortisol (salivary, serum)

Hyperadrenia

- ✓ ↓ potassium (K)
- ✓ ↑ sodium (Na)
- ✓ ↑ fasting glucose >100
- ✓ ↑ triglycerides
- ✓ ↑ Aldosterone
- ✓ ↑ or N chloride (Cl)
- ✓ ↑ DHEA (generally)
- ✓ ↑ cortisol (salivary, serum)

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Changes, Purposes, and Long-term Consequences of During Stress

Physiological Change	Purpose	Consequence
Higher BP, HR, and respiration rate	Provide more oxygenated blood to muscles	Hypertension, heart disease, stroke, kidney disease
Peripheral blood vessels constrict	Prevent bleeding if injured	Cold, clammy feeling, possible skin problems
Pupils dilate	See better in dark	?
Blood supply to digestive system & other organs reduced	Conserve blood for use elsewhere	Digestive upset, Diarrhea, constipation
Kidney function reduced	Conserve fluid to maintain blood volume if injured	Kidney damage, hypertension
Endorphins produced in brain	Block pain if injured	High risk behaviors may be addictive in some people
Immune response suppressed	Immune response after an injury interferes with ability to continue to resist	Lowered resistance to colds, cancer.

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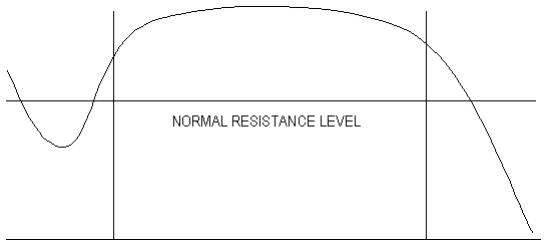
Hans Selye's General Adaptation Syndrome



The Alarm Reaction

The Stage of Resistance or Compensation Stage

The Stage of Exhaustion

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ADRENAL PROTOCOLS HYPERFUNCTION

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- ✓Bio-Ashwagandha™
- ✓Bio-GGG-B ™
- ✓De-Stress ™
- ✓Phophatidylserine ™

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- ✓Bio-3B-G ™
- ✓DHEA ™

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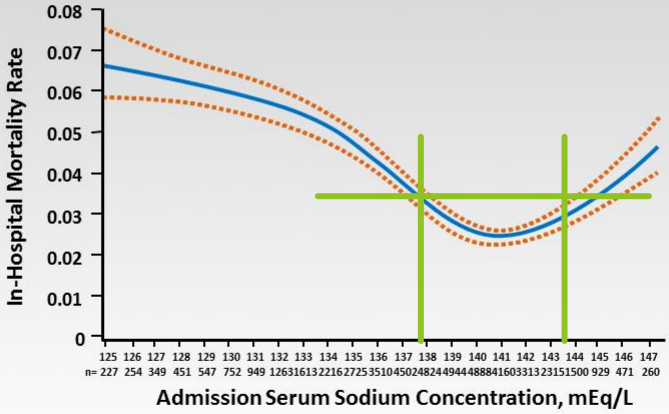
Relationship Between Serum Sodium Levels and In-Hospital Mortality (OPTIMIZE-HF Registry)



From Gheorghiade M, et al. Relationship between admission serum sodium concentration and clinical outcomes in patients hospitalized for heart failure: an analysis from the OPTIMIZE-HF registry. *Eur Heart J.* 2007;28:980-988. Republished with permission.

Heart failure

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Clinical Review & Education

JAMA Internal Medicine | Review

Reduced Salt Intake for Heart Failure
A Systematic Review

Kamal R. Mahtani, PhD; Carl Heneghan, DPhil; Igho Onakpoya, DPhil; Stephanie Tierney, MA, PhD; Jeffrey K. Aronson, DPhil; Nia Roberts, MSc; F. D. Richard Hobbs, FMedSci; David Nunan, MSc, PhD

IMPORTANCE Recent estimates suggest that more than 26 million people worldwide have heart failure. The syndrome is associated with major symptoms, significantly increased mortality, and extensive use of health care. Evidence-based treatments influence all these outcomes in a proportion of patients with heart failure. Current management also often includes advice to reduce dietary salt intake, although the benefits are uncertain.

OBJECTIVE To systematically review randomized clinical trials of reduced dietary salt in adult inpatients or outpatients with heart failure.

EVIDENCE REVIEW Several bibliographic databases were systematically searched, including the Cochrane Central Register of Controlled Trials, MEDLINE, Embase, and CINAHL. The methodologic quality of the studies was evaluated, and data associated with primary outcomes of interest (cardiovascular-associated mortality, all-cause mortality, and adverse events, such as stroke and myocardial infarction) and secondary outcomes (hospitalization, length of inpatient stay, change in New York Heart Association [NYHA] functional class, adherence to dietary low-salt intake, and changes in blood pressure) were extracted.

FINDINGS Of 2655 retrieved references, 9 studies involving 479 unique participants were included in the analysis. None of the studies included more than 100 participants. The risks of bias in the 9 studies were variable. None of the included studies provided sufficient data on the primary outcomes of interest. For the secondary outcomes of interest, 2 outpatient-based studies reported that NYHA functional class was not improved by restriction of salt intake, whereas 2 studies reported significant improvements in NYHA functional class.

CONCLUSIONS AND RELEVANCE Limited evidence of clinical improvement was available among outpatients who reduced dietary salt intake, and evidence was inconclusive for inpatients. Overall, a paucity of robust high-quality evidence to support or refute current guidance was available. This review suggests that well-designed, adequately powered studies are needed to reduce uncertainty about the use of this intervention.

PROTOCOL REGISTRATION PROSPERO Identifier: CRD42015019504

Invited Commentary
Supplemental content

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Invited Commentary

Sodium Restriction in Heart Failure: Too Much Uncertainty—Do the Trials

Clyde W. Yancy, MD, MSc

We have long treated the dictum to restrict sodium intake in heart failure as a pillar of best practices and a sacrosanct edict that populates the core database for all physicians treating cardiovascular disease. Guidelines have mandated empirical thresholds that are to be respected, and consensus statements from leading organizations further make the case for sodium restriction as a basic tenet of good cardiovascular care.[1] However, like many other dogmatic statements that were fully embedded in cardiovascular medicine—for example, suppression of premature ventricular contractions, avoidance of β-blockers in left ventricular dysfunction, and use of hormone replacement therapy in women at risk for cardiovascular disease—the time has now come for sodium restriction in heart failure to be critically reevaluated. There is simply too much uncertainty for a conviction we hold as truth. At a minimum, rigorous testing in well-performed randomized clinical trials is needed. There should be only 1 goal: valid evidence leading to a much more informed position, actionable guidelines, and personalized implementation.

Related article

At the outset, there is no question that sodium intake is associated with volume retention. Certainly, as a risk factor for hypertension (sodium intake) or as a nonpharmacologic intervention (sodium restriction) to treat hypertension through lifestyle interventions, the debates are less intense. The recent PURE (Prospective Urban Rural Epidemiology) data[2] argue that in those communities with high basal sodium intake, further increases in dietary sodium are associated with demonstrable increases in cardiovascular morbidity and mortality. In these populations, sodium restriction should be efficacious. In populations with less basal sodium intake, the risk of greater sodium intake was not evident.[2] Thus, not all patients respond similarly to sodium ingestion and by inference, sodium restriction. These findings emanate from unselected populations without known cardiovascular disease. How these concepts affect heart failure is a uniquely different question, for which meager evidence and empirical answers can no longer be deemed sufficient.

As experienced physicians, we hold fast to bedside observations that associate decompensated heart failure with dietary indiscretion. The evidence, based on experiential learning, would appear to be inviolate: indiscriminate intake of sodium leads to exacerbations of heart failure. Such deeply held beliefs are hard to change.

In this issue of *JAMA Internal Medicine*, Mahtani et al[3] provide a study worth contemplating. In a rigorous Cochrane systematic review of more than 2500 studies assessing the associations of sodium restriction in heart failure, only 9 were deemed suitable for inclusion in a systematic review, and none included outcomes of clinical interest.[3] Stated differently, only 0.3% of studies ever performed assessing sodium restriction in heart failure were deemed of sufficient quality to populate a systematic review, and none were found to be of high quality or free of bias. Numbers have been consistently small and methods have lacked rigor; yet guidelines, until recently, endorsed sodium restriction as de rigueur. In those studies included in the analysis by Mahtani et al,[3] no evidence of an important clinical benefit was found among patients with hospitalized heart failure who underwent any iteration of sodium restriction. That is a pause moment. For chronic ambulatory heart failure, a trend toward symptom improvement was noted that was modest and not consistent. Prior concerns of harm have been nullified because those data required retraction; nevertheless, even in the absence of overt harm, is it appropriate to persist with such strident advocacy at considerable cost to patients without more evidence of a demonstrable benefit? It is incredulous that in our evidence-based era, we have accepted such a low bar for this particular bedrock recommendation of cardiovascular care.

Multiple problems are at play, but the greatest challenges include completing high-quality randomized clinical trials in nutritional science and the ubiquity of sodium in westernized diets, especially considering the higher sodium consumption in higher-risk populations of lower socioeconomic status. Trials in nutrition science involving the addition of nutrients or supplements or the restriction or elimination of substances require rigorous dietary adherence, accurate diet recalls and/or diaries, and fairly sophisticated dietary monitoring. Accurately documenting the "dose" of sodium and controlling dietary foodstuffs are nearly insurmountable goals outside of carefully configured inpatient clinical research units. Given the presence of dietary sodium in the food manufacturing cycle with up to 65% of all sodium intake embedded imperceptibly in all foodstuffs, it becomes challenging if not frankly impossible to accurately gauge outpatient dietary sodium intake. Furthermore, an intricate balance exists between sodium delivered to the nephron and the clearance of free water. This balance is under the exquisite control of a plethora of neurohormones, including angiotensin II, aldosterone, vasopressin, and natriuretic peptides. These physiological loops have a certain plasticity that leads to substantial interindividual and intraindividual variability. Simply put, sodium balance is hard to measure and challenging to manage.

An even more provocative question is pertinent and could be the key question: is sodium really the villain or is it a surrogate for a more significant nutritional concern? Sodium plays important regulatory functions in renal and vascular homeostasis and is a necessary mineral. Emerging data may be expanding our nutritional concerns to include potassium-deficient diets, intake of inorganic phosphates, and lack of dietary fiber, all of

"However, like many other dogmatic statements that were fully embedded in cardiovascular medicine—for example, suppression of premature ventricular contractions, avoidance of ß-blockers in left ventricular dysfunction, and use of hormone replacement therapy in women at risk for cardiovascular disease—the time has now come for sodium restriction in heart failure to be critically reevaluated. There is simply too much uncertainty for a conviction we hold as truth.

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