The Vibrant Longevity Summit

The Gut Microbiome Connection

Advancing Systemic Health Protocols

Session 1

Dr. Tom O'Bryan, DC, CCN, DABCN, CIFM

Session 2

Dr. Dan Kalish, DC

Session 3

Dr. Kyle Gillett, MD

Session 4

Dr. Sue Mitchell, MD

The Gut Microbiome Connection

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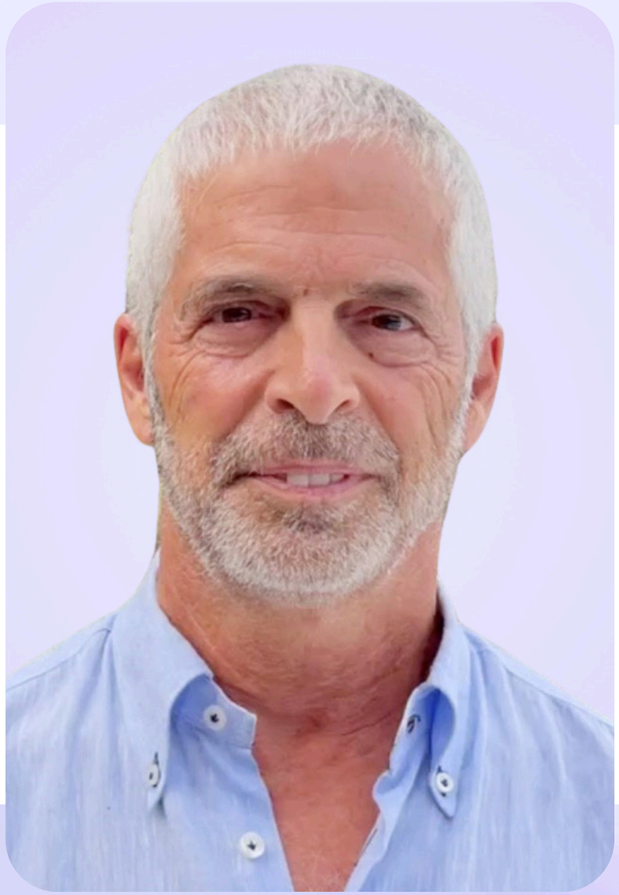

Session 1

Dr. Tom O'Bryan, DC, CCN, DABCN, CIFM

The Vibrant Longevity Summit

Beyond Digestion

Gut evaluation and stool antibodies in the development of chronic inflammatory diseases

Dr. Tom O'Bryan, DC, CCN, DACBN, CIFM

Towards Healing the Planet, One Person at a Time
theDr.com

Regulatory Statement

Limitations

Please note the information provided by Vibrant Wellness is intended solely for research and informational purposes to help inform lifestyle choices aimed at potential risk mitigation. The information is not intended to be used by the patient for any diagnostic purpose and is not a substitute for medical advice by a healthcare practitioner. Please consult a licensed healthcare practitioner for any questions regarding diagnosis, prevention, or treatment of any disease or health assessment.

Regulatory Disclaimer

Please note the information provided by Vibrant Wellness is intended solely for research and informational purposes to help inform lifestyle choices aimed at potential risk mitigation. The tests were developed, and their performance characteristics were determined by Vibrant America and Vibrant Genomics. They have not been cleared or approved by US Food and Drug Administration. The laboratory is certified under the Clinical Laboratory Improvements Amendments (CLIA) as qualified to perform high complexity testing. The laboratory is also in compliance with College of American Pathologists (CAP) regulations and New York State Department of Health (NYSDOH) regulations.

Waves of Gratitude

Vibrant Wellness

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Big Boss

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A Gift For You

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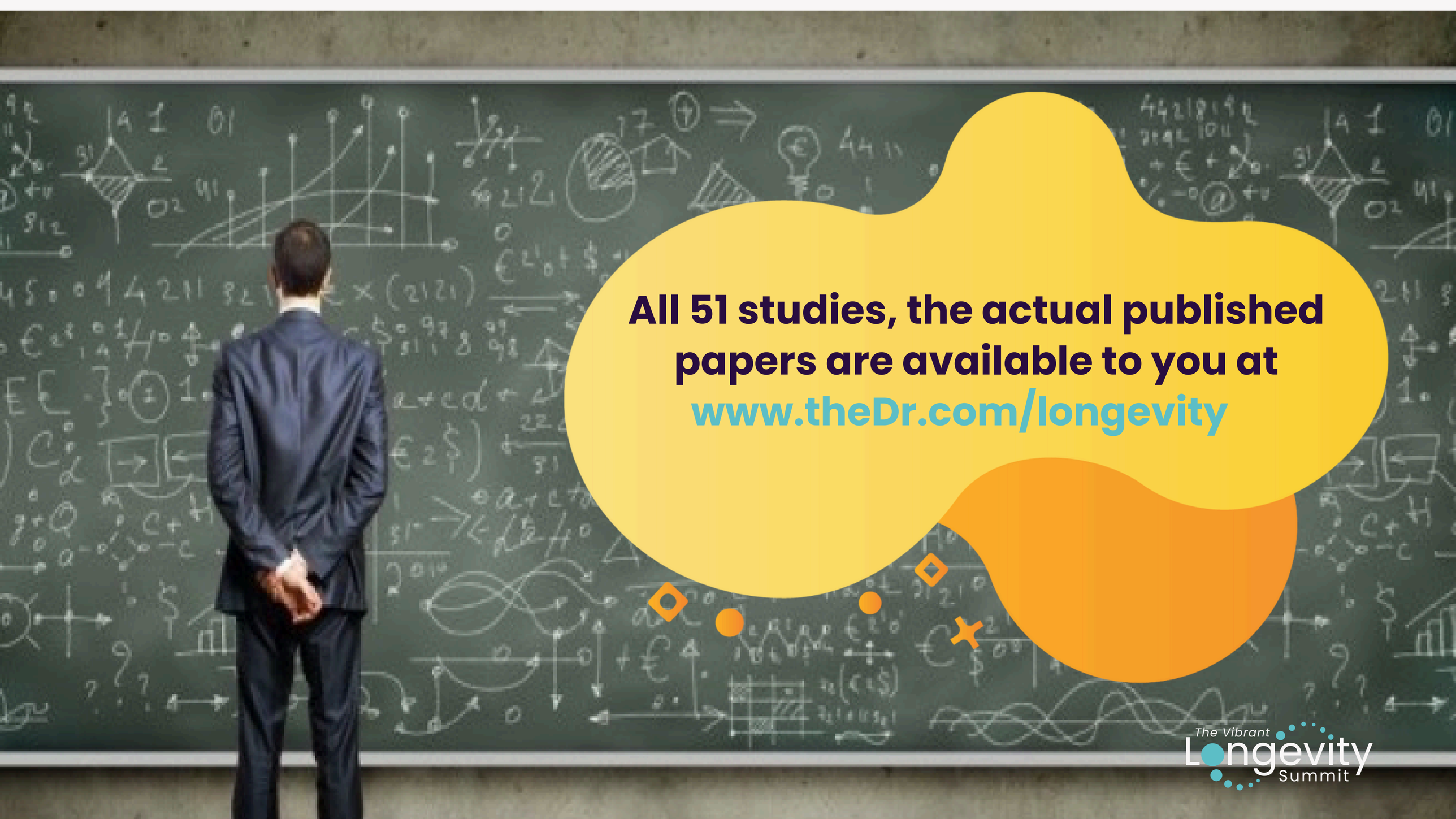
All 51 studies, the actual published papers are available to you at
www.theDr.com/longevity
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Premise #1

Where Do We Begin Educating Our Patients?

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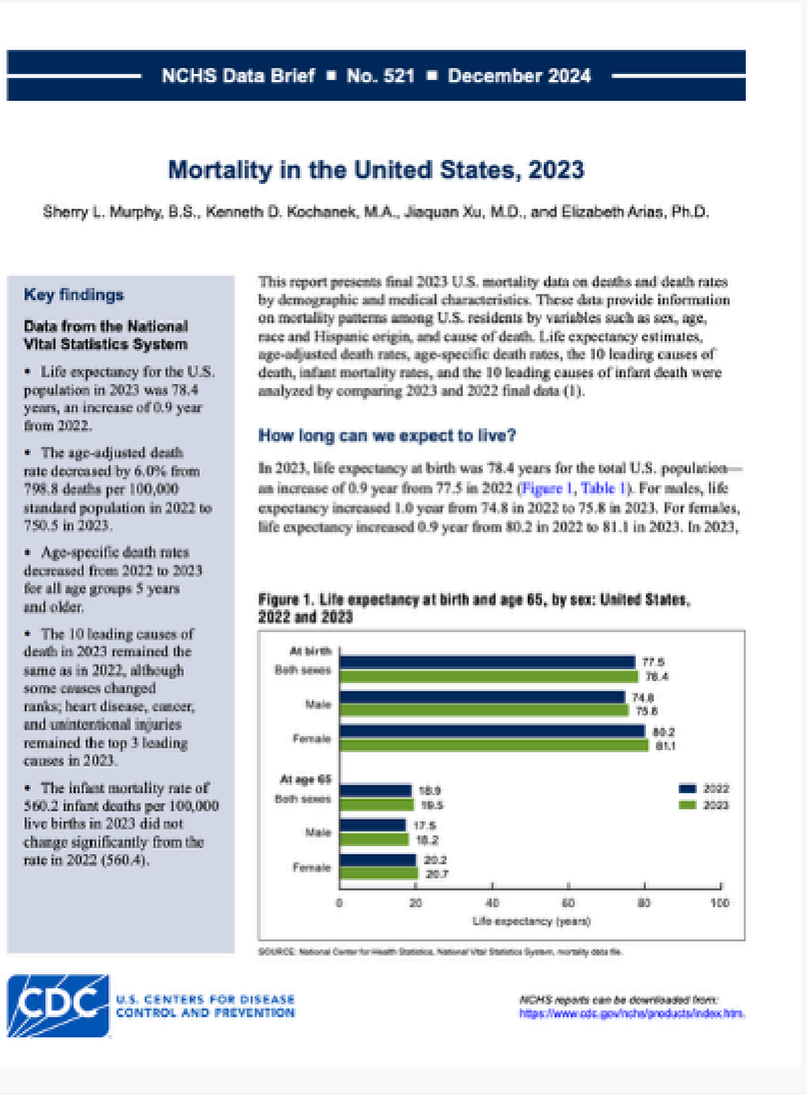
The 10 Leading Causes of Death in 2023 were:

1.	Diseases of Heart	21.4%
2.	Malignant Neoplasms (Cancer)	18.5%
3.	Accidents (unintentional injuries)	6.9%
4.	Cerebrovascular diseases (strokes)	5.0%
5.	Chronic lower respiratory diseases	4.5%
6.	Alzheimer's disease	3.7%
7.	Diabetes mellitus	3.1%
8.	Kidney disease	1.8%
9.	Chronic liver disease and cirrhosis	1.7%
10.	Covid-19	5.7%



NCHS Data Brief ■ No. 521 ■ December 2024

Mortality in the United States, 2023

Sherry L. Murphy, B.S., Kenneth D. Kochanek, M.A., Jiaquan Xu, M.D., and Elizabeth Arias, Ph.D.

Key findings

Data from the National Vital Statistics System

- Life expectancy for the U.S. population in 2023 was 78.4 years, an increase of 0.9 year from 2022.
- The age-adjusted death rate decreased by 6.0% from 798.8 deaths per 100,000 standard population in 2022 to 750.5 in 2023.
- Age-specific death rates decreased from 2022 to 2023 for all age groups 5 years and older.
- The 10 leading causes of death in 2023 remained the same as in 2022, although some causes changed ranks; heart disease, cancer, and unintentional injuries remained the top 3 leading causes in 2023.
- The infant mortality rate of 560.2 infant deaths per 100,000 live births in 2023 did not change significantly from the rate in 2022 (560.4).

This report presents final 2023 U.S. mortality data on deaths and death rates by demographic and medical characteristics. These data provide information on mortality patterns among U.S. residents by variables such as sex, age, race and Hispanic origin, and cause of death. Life expectancy estimates, age-adjusted death rates, age-specific death rates, the 10 leading causes of death, infant mortality rates, and the 10 leading causes of infant death were analyzed by comparing 2023 and 2022 final data (1).

How long can we expect to live?

In 2023, life expectancy at birth was 78.4 years for the total U.S. population—an increase of 0.9 year from 77.5 in 2022 (Figure 1, Table 1). For males, life expectancy increased 1.0 year from 74.8 in 2022 to 75.8 in 2023. For females, life expectancy increased 0.9 year from 80.2 in 2022 to 81.1 in 2023. In 2023,

Figure 1. Life expectancy at birth and age 65, by sex: United States, 2022 and 2023

SOURCE: National Center for Health Statistics, National Vital Statistics System, mortality data file.

CDC U.S. CENTERS FOR DISEASE CONTROL AND PREVENTION

NCHS reports can be downloaded from: https://www.cdc.gov/nchs/products/index.htm.

https://www.cdc.gov/nchs/data/databriefs/db521.pdf

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HHS Public Access

Author manuscript

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Published in final edited form as:

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Chronic inflammation in the etiology of disease across the life span

David Furman[1,2,3,4,*], **Judith Campisi**[1,5], **Eric Verdin**[1], **Pedro Carrera-Bastos**[6], **Sasha Targ**[4,7], **Claudio Franceschi**[8,9], **Luigi Ferrucci**[10], **Derek W. Gilroy**[11], **Alessio Fasano**[12], **Gary W. Miller**[13], **Andrew H. Miller**[14], **Alberto Mantovani**[15,16,17], **Cornelia M. Weyand**[18], **Nir Barzilai**[19], **Jorg J. Goronzy**[20], **Thomas A. Rando**[20,21,22], **Rita B. Effros**[23], **Alejandro Lucia**[24,25], **Nicole Kleinstreuer**[26,27], **George M. Slavich**[28]

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Indeed, chronic inflammatory diseases have been recognized as the most significant cause of death in the world today,

Nat Med. 2019 December ; 25(12): 1822–1832

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The overexpression of inflammation genes
and immune-response genes
J Clin Immunol 29:397405, 2009

Inflammaging describes the low-grade, chronic, systemic inflammation in aging, in the absence of overt infection (“sterile” inflammation).

Journal of Alzheimer's Disease xx (2020) x–xx
DOI 10.3233/JAD-200006
IOS Press

Short-Chain Fatty Acids and Lipopolysaccharide as Mediators Between Gut Dysbiosis and Amyloid Pathology in Alzheimer's Disease

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[c]*Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy*
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Handling Associate Editor: Vincenzo Solfrizzi

Accepted 28 August 2020

Abstract.
Background: Metagenomic data support an association between certain bacterial strains and Alzheimer's disease (AD), but their functional dynamics remain elusive.
Objective: To investigate the association between amyloid pathology, bacterial products such as lipopolysaccharide (LPS) and short chain fatty acids (SCFAs: acetate, valerate, butyrate), inflammatory mediators, and markers of endothelial dysfunction in AD.
Methods: Eighty-nine older persons with cognitive performance from normal to dementia underwent florbetapir amyloid PET and blood collection. Brain amyloidosis was measured with standardized uptake value ratio versus cerebellum. Blood levels of LPS were measured by ELISA, SCFAs by mass spectrometry, cytokines by using real-time PCR, and biomarkers of endothelial dysfunction by flow cytometry. We investigated the association between the variables listed above with Spearman's rank test.
Results: Amyloid SUVR uptake was positively associated with blood LPS (rho ≥ 0.32, $p \leq 0.006$), acetate and valerate (rho ≥ 0.45, $p < 0.001$), pro-inflammatory cytokines (rho ≥ 0.25, $p \leq 0.012$), and biomarkers of endothelial dysfunction (rho ≥ 0.25, $p \leq 0.042$). In contrast, it was negatively correlated with butyrate (rho ≤ -0.42, $p \leq 0.020$) and the anti-inflammatory cytokine IL10 (rho ≤ -0.26, $p \leq 0.009$). Endothelial dysfunction was positively associated with pro-inflammatory cytokines, acetate and valerate (rho ≥ 0.25, $p \leq 0.045$) and negatively with butyrate and IL10 levels (rho ≤ -0.25, $p \leq 0.038$).

[1]These authors contributed equally to this work.
*Correspondence to: Moira Marizzoni, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. Tel.: +39 030 35 01 362; Fax: +39 030 35 01 592; E-mail: mmarizzoni@gmail.com.

J Alzheimers Dis. 2020;78(2):683-697

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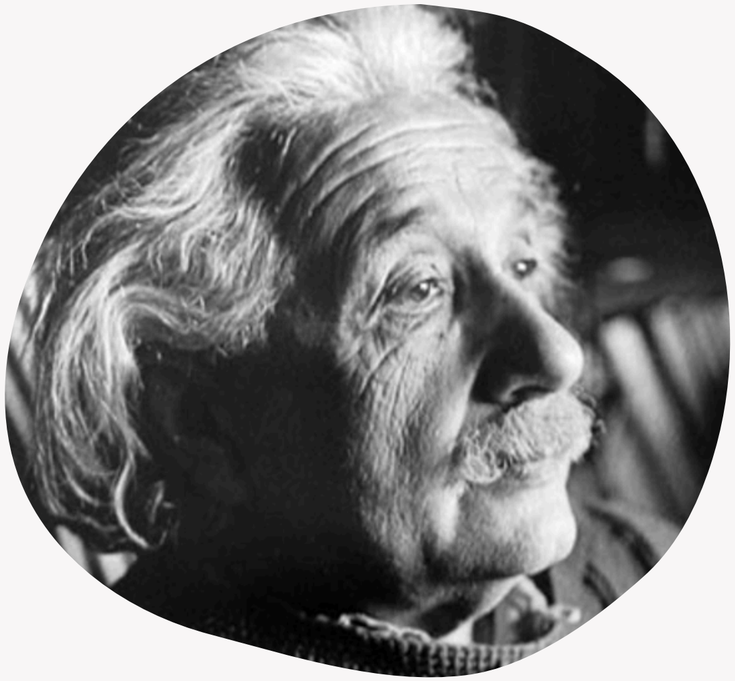
“Everything should be made as simple as possible, but not simpler”



First and Primary, is an Understanding that Reducing Inflammation is the Target to Address

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nutrients

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Review

Undigested Food and Gut Microbiota May Cooperate in the Pathogenesis of Neuroinflammatory Diseases: A Matter of Barriers and a Proposal on the Origin of Organ Specificity

Paolo Riccio and Rocco Rossano *

Department of Sciences, University of Basilicata, 85100 Potenza, Italy; paoloriccio@gmail.com
* Correspondence: rocco.rossano@unibas.it

Received: 1 October 2019; Accepted: 8 November 2019; Published: 9 November 2019

check for updates

Abstract: As food is an active subject and may have anti-inflammatory or pro-inflammatory effects, dietary habits may modulate the low-grade neuroinflammation associated with chronic neurodegenerative diseases. Food is living matter different from us, but made of our own nature. Therefore, it is at the same time foreign to us (*non-self*), if not yet digested, and like us (*self*), after its complete digestion. To avoid the efflux of undigested food from the lumen, the intestinal barrier must remain intact. What and how much we eat shape the composition of gut microbiota. Gut dysbiosis, as a consequence of Western diets, leads to intestinal inflammation and a leaky intestinal barrier. The efflux of undigested food, microbes, endotoxins, as well as immune-competent cells and molecules, causes chronic systemic inflammation. Opening of the blood-brain barrier may trigger microglia and astrocytes and set up neuroinflammation. We suggest that what determines the organ specificity of the autoimmune-inflammatory process may depend on food antigens resembling proteins of the organ being attacked. This applies to the brain and neuroinflammatory diseases, as to other organs and other diseases, including cancer. Understanding the cooperation between microbiota and undigested food in inflammatory diseases may clarify organ specificity, allow the setting up of adequate experimental models of disease and develop targeted dietary interventions.

Keywords: diet; gut microbiota; inflammation; intestinal barrier; blood-brain barrier; Alzheimer's disease; Parkinson's disease; multiple sclerosis; autism spectrum disorders; amyotrophic lateral sclerosis

1. Chronic Neurodegenerative Diseases are Associated with Low-Grade Chronic Inflammation

Despite having different etiology and different pathogenic mechanisms, chronic neurodegenerative diseases, such as multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and autism spectrum disorder (ASD), all have an inflammatory nature in common [1] (Figure 1).

Fighting the inflammatory processes that underlie these diseases may reduce their progression and their severity. Inflammation is an innate, non-specific defense process [2,3]. It occurs in response to the presence of foreign material (*non-self*), or as a consequence of tissue damage caused by physical, chemical or biological agents, or by abnormalities such as the failure to eliminate waste or digest nutrients. If the cause of inflammation persists, the inflammation also persists, usually with low intensity, and is called low-grade chronic inflammation. As for the chronic neuroinflammatory diseases, in most cases, the neuroinflammatory state does not originate in the central nervous system (CNS), but is thought to come from a chronic systemic inflammation (CSI) [4–6]. Recent evidence suggests that CSI may in turn result from a persistent intestinal inflammation spreading through the intestinal

Recent evidence suggests that CSI results from a persistent intestinal inflammation spreading through the intestinal barrier, so as to cause a systemic inflammatory and immune response.

Nutrients. 2019 Nov 9;11(11):2714

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Propagation of the inflammatory state from the intestine to the CNS involves the breakdown of two biological barriers: the intestinal barrier and the blood-brain barrier (BBB).

Nutrients. 2019 Nov 9;11(11):2714

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Review

Undigested Food and Gut Microbiota May Cooperate in the Pathogenesis of Neuroinflammatory Diseases: A Matter of Barriers and a Proposal on the Origin of Organ Specificity

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check for updates

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To avoid neurological diseases of intestinal origin, the two barriers must remain intact.

Nutrients. 2019 Nov 9;11(11):2714

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Review

Leaky Gut, Leaky Brain?

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check for updates

Abstract: 'Leaky gut' syndrome, long-associated with celiac disease, has attracted much attention in recent years and for decades, was widely known in complementary/alternative medicine circles. It is often described as an increase in the permeability of the intestinal mucosa, which could allow bacteria, toxic digestive metabolites, bacterial toxins, and small molecules to 'leak' into the bloodstream. Nervous system involvement with celiac disease is know to occur even at subclinical levels. Gluten and gluten sensitivity are considered to trigger this syndrome in individuals genetically predisposed to celiac disease. However, the incidence of celiac disease in the general population is quite low. Nevertheless, increased public interest in gluten sensitivity has contributed to expanded food labels stating 'gluten-free' and the proliferation of gluten-free products, which further drives gluten-free lifestyle changes by individuals without frank celiac disease. Moreover, systemic inflammation is associated with celiac disease, depression, and psychiatric comorbidities. This mini-review focuses on the possible neurophysiological basis of leaky gut; leaky brain disease; and the microbiota's contribution to inflammation, gastrointestinal, and blood-brain barrier integrity, in order to build a case for possible mechanisms that could foster further 'leaky' syndromes. We ask whether a gluten-free diet is important for anyone or only those with celiac disease.

Keywords: leaky gut; leaky brain; microbiota; microbiome; celiac disease; gluten; gluten-free; microbiota-gut-brain axis; metabolic interactome; inflammation; blood barriers

1. Introduction

The mutually beneficial relationship between the host and its resident gut microbiota has been described by many [1–3]. Bacterial products and metabolites from gut commensal micro-organisms are largely useful for the host and our overall health. Arguably, the most important of these is co-metabolism, which occurs between the microbiota and host systems, and the same microbes can control integral segments of our neurobiology and even affect several mammalian systems like the brain and digestive system [2]. The microbiota-gut is an integral component of the gut–brain neuroendocrine metabolic axis [2] and any disruption that can occur, such as antibiotic use and during disease, could upset homeostasis and share an inflammatory component [2]. Celiac disease, ulcerative colitis, or Crohn's disease—the latter two are collectively referred to as inflammatory bowel disease—are chronic conditions that affect the gastrointestinal tract and have an inflammatory component. The microbiota gastrointestinal barrier, together with transport proteins, act at the interface of blood permeability barriers to help regulate trafficking of macromolecules between the digestive

Microorganisms 2018, 6, 107; doi:10.3390

Leaky gut may be one of the underlying causes in illnesses involving concomitant downstream disruptions in the blood brain barrier and numerous studies indicate that inflammation increases BBB paracellular permeability

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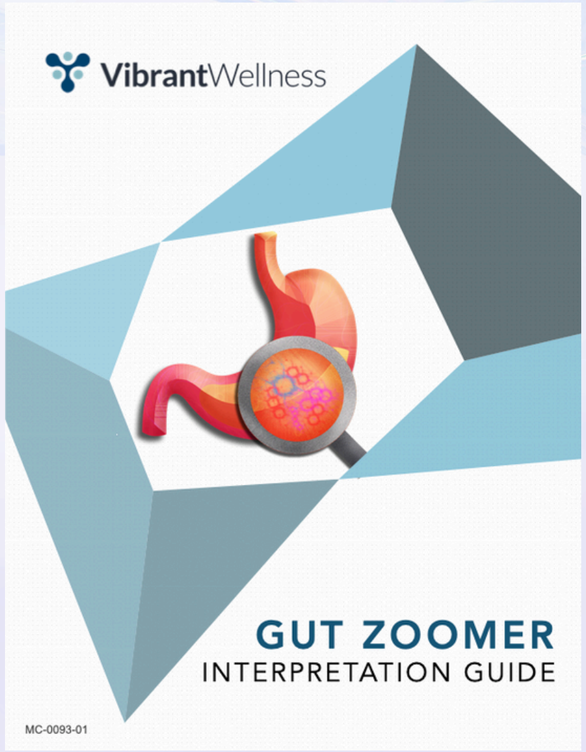
Premise #2

Where Does the Importance of the Gut Zoomer Sit in the Priorities of Patient Evaluation?



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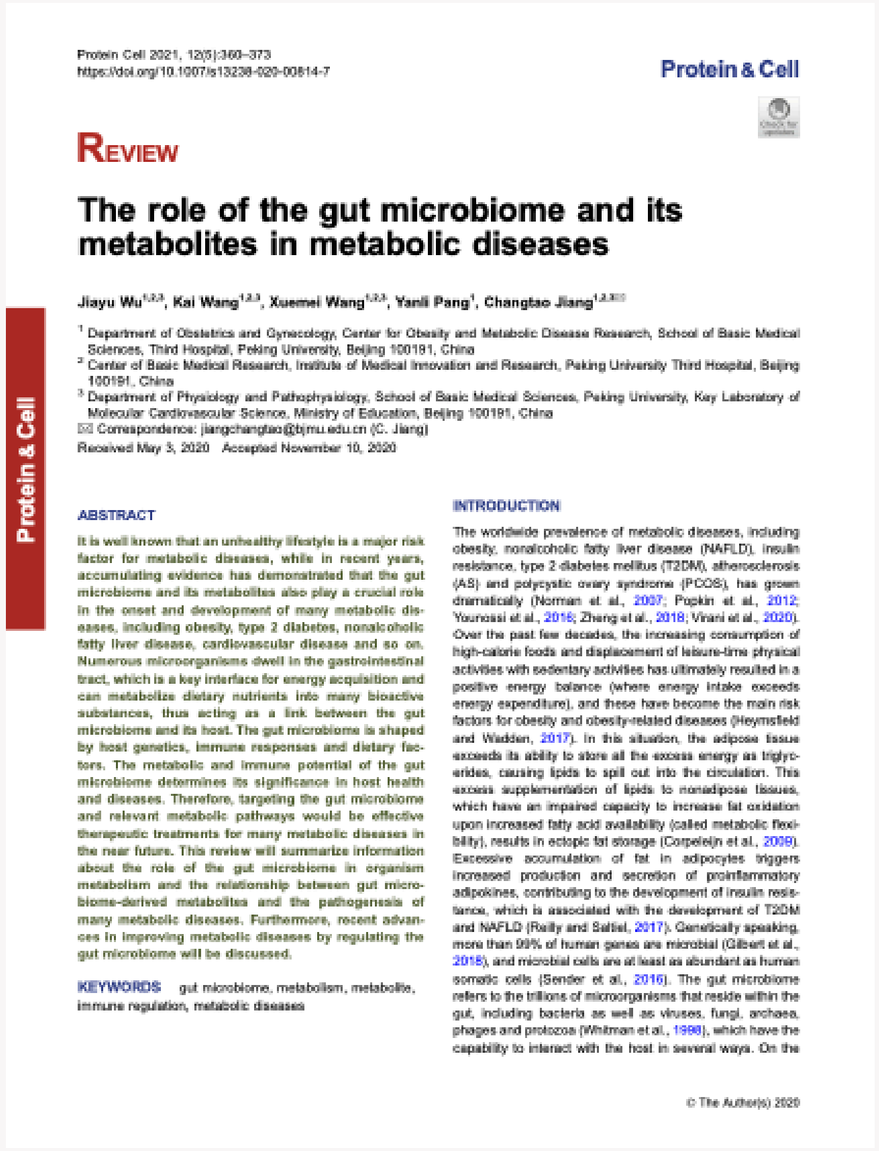

Protein Cell 2021, 12(5):360–373
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Protein & Cell

REVIEW

The role of the gut microbiome and its metabolites in metabolic diseases

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ABSTRACT

It is well known that an unhealthy lifestyle is a major risk factor for metabolic diseases, while in recent years, accumulating evidence has demonstrated that the gut microbiome and its metabolites also play a crucial role in the onset and development of many metabolic diseases, including obesity, type 2 diabetes, nonalcoholic fatty liver disease, cardiovascular disease and so on. Numerous microorganisms dwell in the gastrointestinal tract, which is a key interface for energy acquisition and can metabolize dietary nutrients into many bioactive substances, thus acting as a link between the gut microbiome and its host. The gut microbiome is shaped by host genetics, immune responses and dietary factors. The metabolic and immune potential of the gut microbiome determines its significance in host health and diseases. Therefore, targeting the gut microbiome and relevant metabolic pathways would be effective therapeutic treatments for many metabolic diseases in the near future. This review will summarize information about the role of the gut microbiome in organism metabolism and the relationship between gut microbiome-derived metabolites and the pathogenesis of many metabolic diseases. Furthermore, recent advances in improving metabolic diseases by regulating the gut microbiome will be discussed.

KEYWORDS gut microbiome, metabolism, metabolite, immune regulation, metabolic diseases

INTRODUCTION

The worldwide prevalence of metabolic diseases, including obesity, nonalcoholic fatty liver disease (NAFLD), insulin resistance, type 2 diabetes mellitus (T2DM), atherosclerosis (AS) and polycystic ovary syndrome (PCOS), has grown dramatically (Norman et al., 2007; Popkin et al., 2012; Younossi et al., 2016; Zheng et al., 2018; Virani et al., 2020). Over the past few decades, the increasing consumption of high-calorie foods and displacement of leisure-time physical activities with sedentary activities has ultimately resulted in a positive energy balance (where energy intake exceeds energy expenditure), and these have become the main risk factors for obesity and obesity-related diseases (Heymsfield and Wadden, 2017). In this situation, the adipose tissue exceeds its ability to store all the excess energy as triglycerides, causing lipids to spill out into the circulation. This excess supplementation of lipids to nonadipose tissues, which have an impaired capacity to increase fat oxidation upon increased fatty acid availability (called metabolic flexibility), results in ectopic fat storage (Corpeleijn et al., 2009). Excessive accumulation of fat in adipocytes triggers increased production and secretion of proinflammatory adipokines, contributing to the development of insulin resistance, which is associated with the development of T2DM and NAFLD (Reilly and Saltiel, 2017). Genetically speaking, more than 99% of human genes are microbial (Gilbert et al., 2018), and microbial cells are at least as abundant as human somatic cells (Sender et al., 2016). The gut microbiome refers to the trillions of microorganisms that reside within the gut, including bacteria as well as viruses, fungi, archaea, phages and protozoa (Whitman et al., 1998), which have the capability to interact with the host in several ways. On the

Protein Cell 2021, 12(5):360–373

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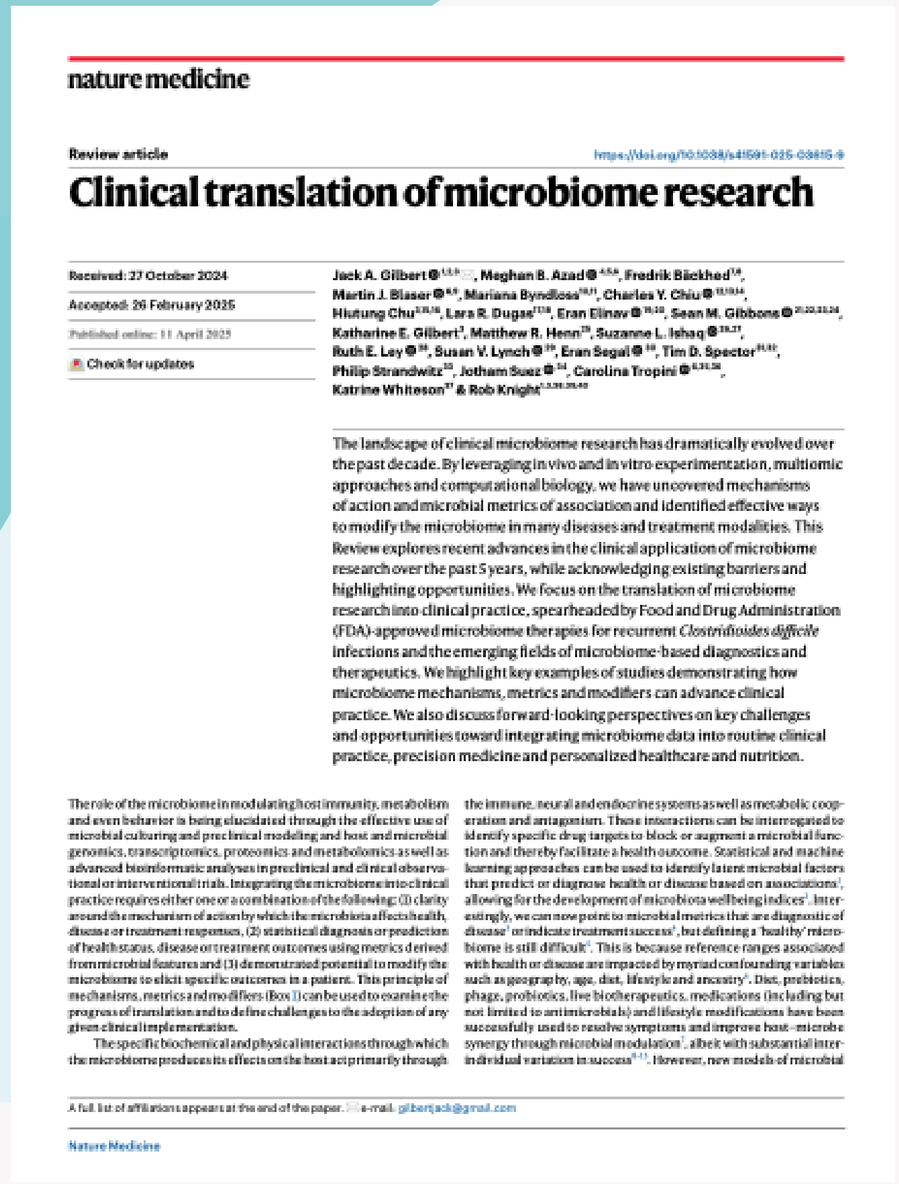

nature medicine

Review article

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Clinical translation of microbiome research

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Broadly speaking, the adult gut microbiome (defined as after weaning) helps regulate the immune system through metabolites such as short-chain fatty acids (SCFAs), reduces infectious disease burden through bile acid metabolism and ecological competition, aids digestion by fermenting fibers and other nutrients and produces a cornucopia of metabolites that shape myriad functions throughout the body

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Gut microbes and food reward: From the gut to the brain

Alice de Wouters d'Oplinter, Sabrina J. P. Huwart, Patrice D. Cani and Amandine Everard*

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Inappropriate food intake behavior is one of the main drivers for fat mass development leading to obesity. Importantly the gut microbiota-mediated signals have emerged as key actors regulating food intake acting mainly on the hypothalamus, and thereby controlling hunger or satiety/satiation feelings. However, food intake is also controlled by the hedonic and reward systems leading to food intake based on pleasure (i.e., non-homeostatic control of food intake). This review focus on both the homeostatic and the non-homeostatic controls of food intake and the implication of the gut microbiota on the control of these systems. The gut-brain axis is involved in the communications between the gut microbes and the brain to modulate host food intake behaviors through systemic and nervous pathways. Therefore, here we describe several mediators of the gut-brain axis including gastrointestinal hormones, neurotransmitters, bioactive lipids as well as bacterial metabolites and compounds. The modulation of gut-brain axis by gut microbes is deeply addressed in the context of host food intake with a specific focus on hedonic feeding. Finally, we also discuss possible gut microbiota-based therapeutic approaches that could lead to potential clinical applications to restore food reward alterations. Therapeutic applications to tackle these dysregulations is of utmost importance since most of the available solutions to treat obesity present low success rate.

KEYWORDS
food reward, food intake, gut microbes, gut microbiome, gut-brain axis, obesity

Gut-brain axis related to food intake

The gut-brain axis is a complex bi-directional communication system connecting the gastrointestinal (GI) tract and the central nervous system (CNS). This connection allows the brain to be informed among other components of the energy status in the periphery. The CNS sends then feedbacks to maintain energy homeostasis (Cryan et al., 2019). Two pathways are involved in this communication: the nervous and the systemic pathways.

Thirty hormones have been identified in the GI tract and therefore the intestine represents an incredible reservoir of peptides acting at distance from the gut and on different organs.

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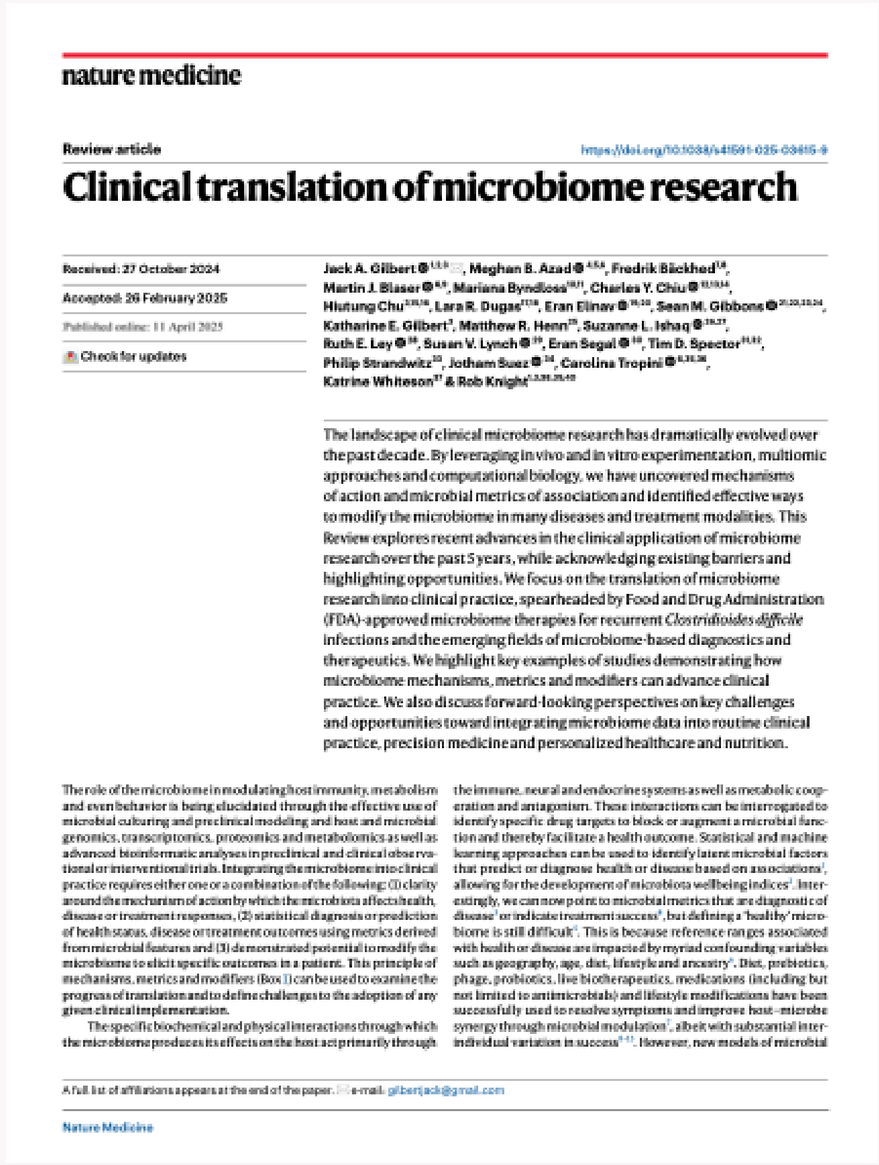

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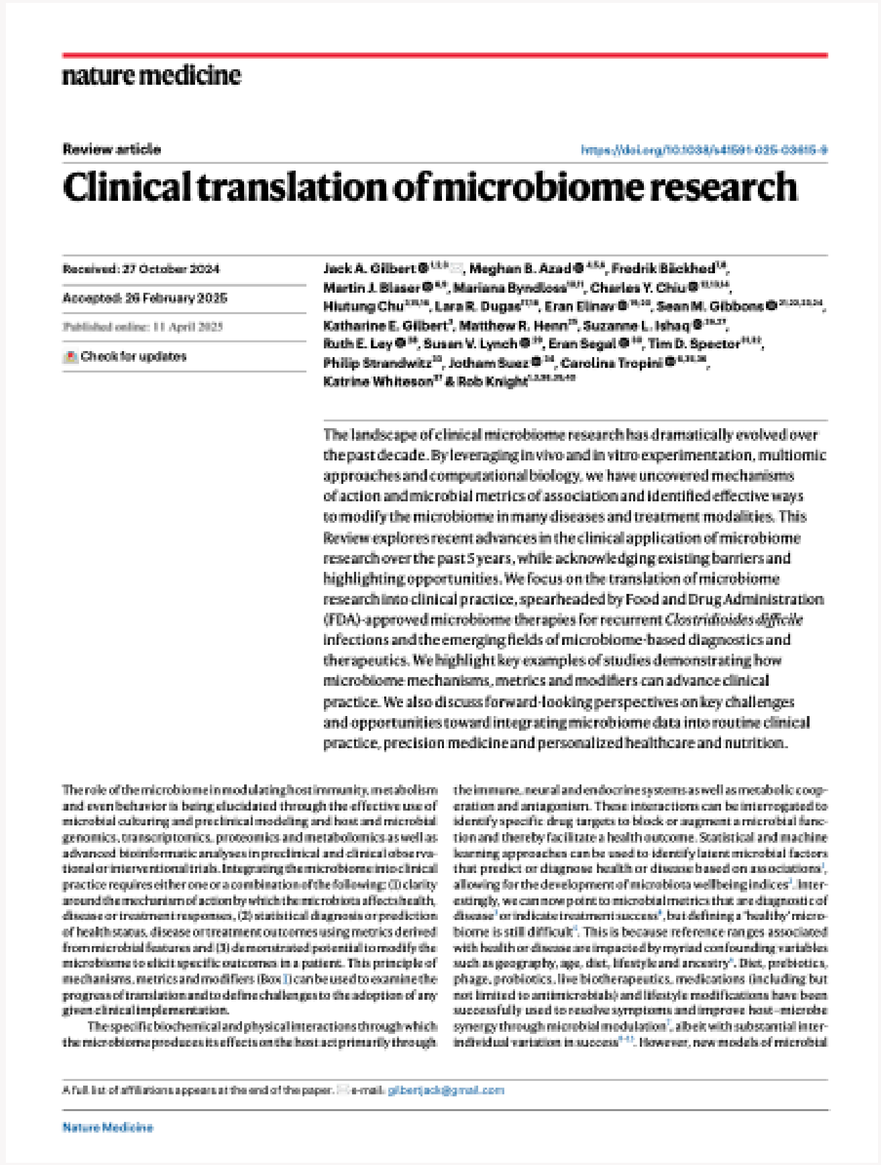

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The most well-studied ways to modify the microbiome include microbiome transplants (vaginal, oral, gut and so on), dietary changes and the use of prebiotics, probiotics and synbiotics. Imprecise strategies such as antibiotic therapy are now being augmented or replaced with more specific interventions such as phage therapy.

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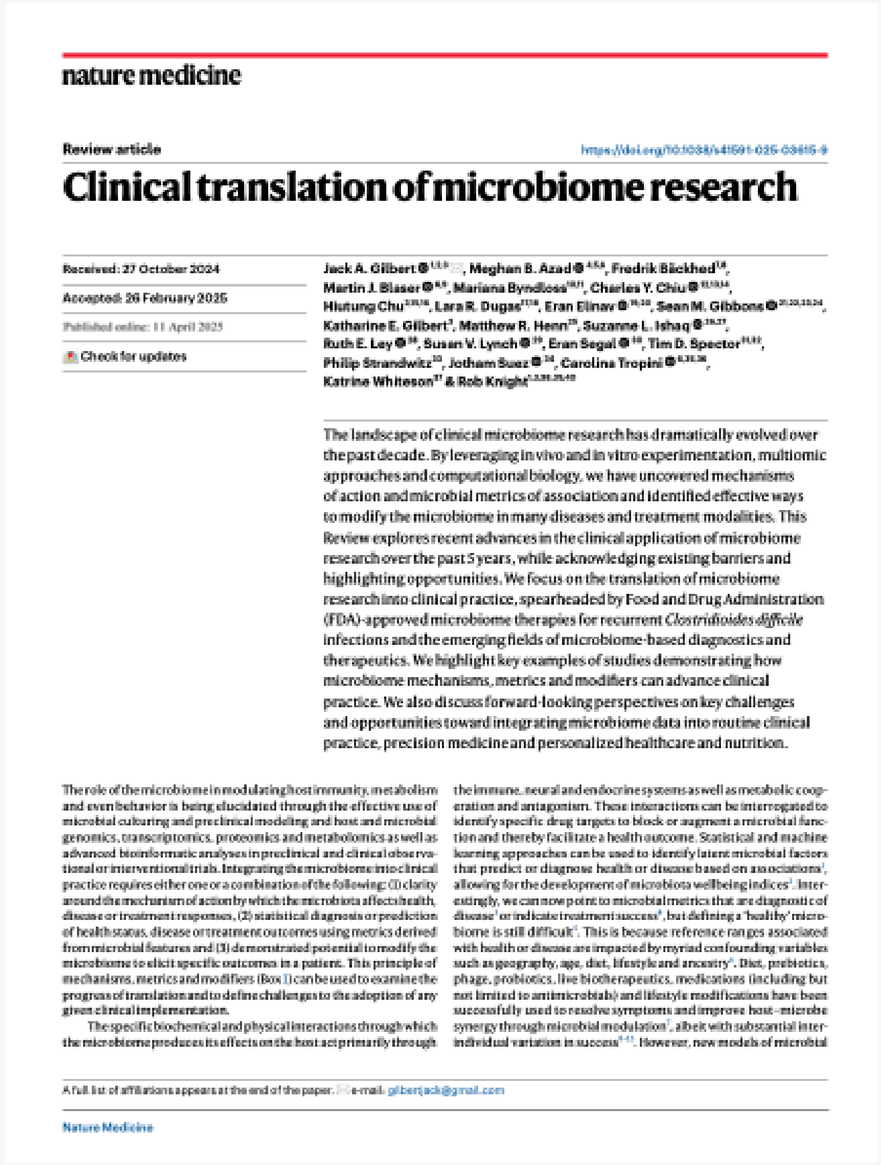

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Once we understand that the microbiome is exhibiting features that are associated with detrimental outcomes, such as disease or nonresponse to treatment, it becomes imperative to identify how to remove, reverse or alter these features

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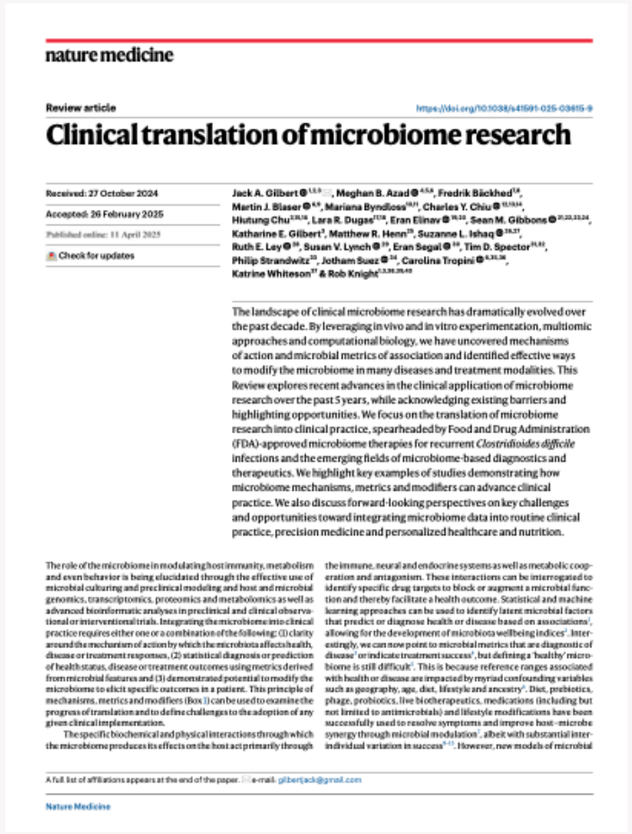

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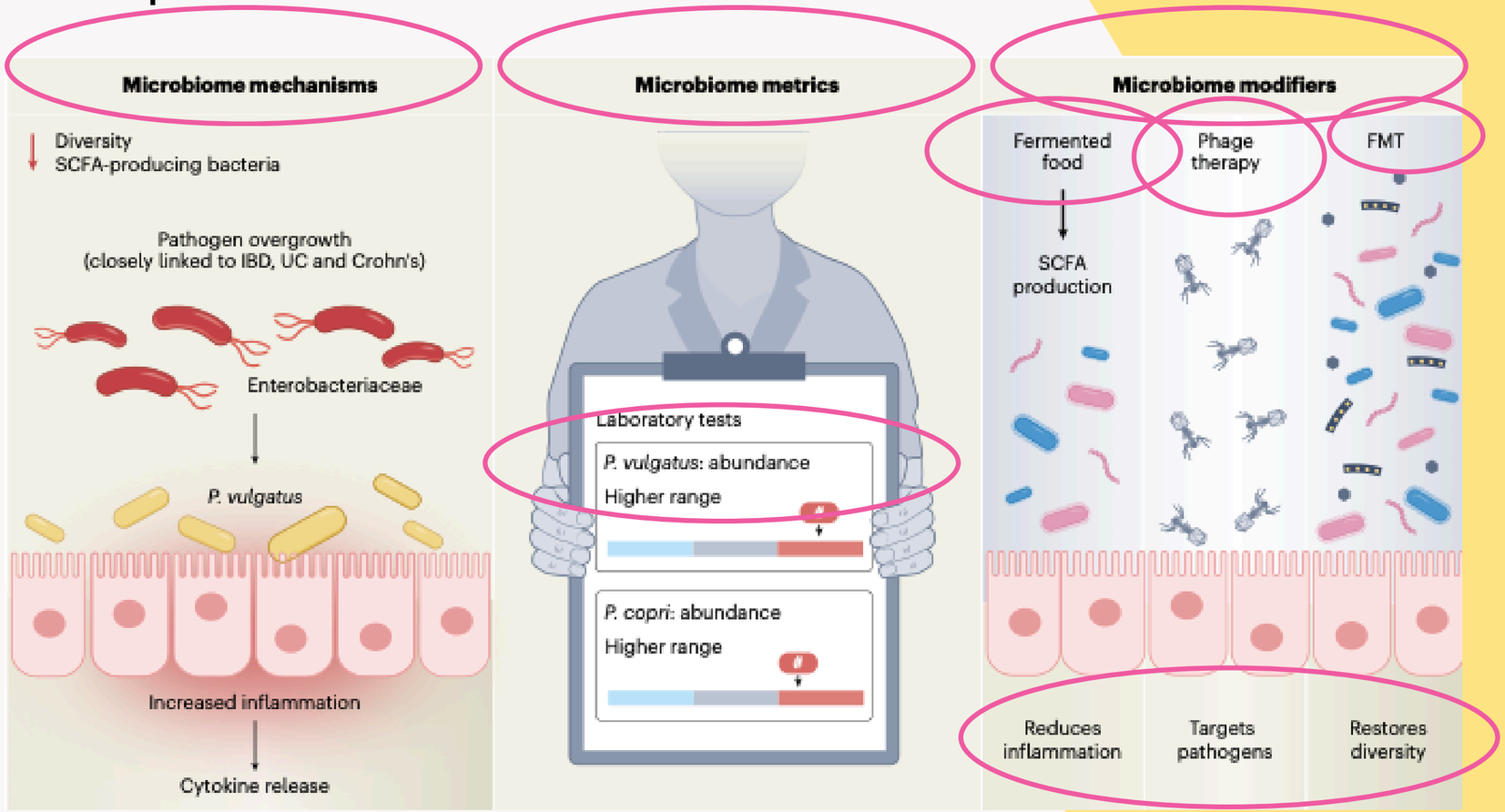

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Is the Gut Zoomer Comprehensive?

This test reveals:

- the diversity and keystone health of your microbiome
- highlights levels associated with conditions such as metabolic dysfunction, intestinal permeability, digestive health challenges
- detects biomarkers associated with infections, inflammation, autoimmunity, immune health status, factors impacting estrogen metabolism, and gut-brain axis activity.

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Is the Gut Zoomer Comprehensive?

It is so comprehensive, it would be very easy to overwhelm the patient and lose their connection with you (while they continue to smile and “uh huh” you every once in awhile).

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Is the Gut Zoomer Comprehensive?

Pick what you think is most important, and let them know: “we’re going to talk about the most relevant aspects of this test today. But this test is so comprehensive, we could spend 2+ hours reviewing all of these markers.

Today our focus is on the most important features to move your body towards a higher functioning, balanced gut. But anytime you have questions, we can review in the future”

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Gut Commensals

- Akkermansia muciniphila
- Enterobacteriaceae
- Butyrivibrio
- Alloprevotella
- Faecalibacterium prausnitzii
- Roseburia intestinalis
- Eubacterium rectale
- Bacteroides vulgatus
- Coprococcus
- Prevotella
- Lachnospiraceae
- Lactobacillus
- Roseburia
- Clostridium
- Faecalibacterium
- Ruminococcaceae
- Bacteroidetes
- Ruminococcus spp
- Enterobacteriaceae
- Escherichia coli
- Bifidobacterium adolescentis
- Bacteroides
- Dialister invisus
- Enterococcus
- Ruminococcus gnavus
- Veillonella
- Ruminococcus
- Haemophilus
- Bacteroidales
- Bacteroides caccae
- Bifidobacterium animalis
- Blautia hydrogenotrophica
- Christensenella minuta
- Clostridium hathewayi
- Clostridium ramosum
- Clostridium symbiosum
- Eggerthella lenta
- Oscillospira
- Blautia obeum
- Collinsella
- Phascolarctobacterium
- Hafnia
- Parabacteroides
- Ruminococcus bromii
- Bacteroides
- Eubacterium
- Ruminococcus gnavus
- Marvinbryantia
- Bifidobacterium catenulatum
- Dorea
- Enterobacteriaceae
- Methanobrevibacter smithii
- Ruminococcus
- Bifidobacterium adolescentis
- Enterococcus
- Desulfovibrio piger
- Streptococcus
- Eubacterium rectale
- Atopobium parvulum
- Catenibacterium
- Klebsiella aerogenes
- Escherichia coli
- Prevotella copri
- Solobacterium moorei
- Streptococcus species
- Tyzzerella
- Tyzzerella 4
- Atopobium
- Lactobacillus ruminis
- Lactobacillus sakei
- Bradyrhizobiaceae
- Clostridiales incertae sedis iv
- Lactobacillaceae
- Blautia
- Butyricimonas
- Coprococcus
- Desulfovibrio
- Veillonellaceae
- Lachnospiraceae
- Alistipes
- Holdemania
- Bacillus subtilis
- β-galactosidase producing bacteria
- β-glucuronidase producing bacteria
- Acinetobacter
- Enterococcus species
- Methanobrevibacter smithii
- Staphylococcus species
- Fusobacterium
- Methanobrevibacter smithii
- Streptococcus thermophilus
- Clostridium
- Porphyromonas gingivalis
- Proteus mirabilis
- Pseudobutyrivibrio
- Bifidobacterium
- Lactobacillus
- Staphylococcaceae
- Clostridiales incertae sedis iv
- Staphylococcus epidermidis
- Staphylococcus pasteuri
- Clostridia clusters IV
- Clostridia clusters XIVa
- Clostridia clusters xviii
- Enterococcus gallinarum
- Propionibacterium freudenreichi
- Bifidobacterium animalis subspecies lactis
- Lactobacillus animalis
- Streptococcus spp.
- Lactobacillus spp.
- Clostridium species
- Peptostreptococcus species
- Enterococcus spp.
- Staphylococcus
- Lactobacillus bulgaricus
- Lactobacillus plantarum
- Clostridium spp
- Eubacterium spp
- Clostridiales Family XIV Incertae Sedis
- Enterobacteria
- Faecalibacterium prausnitzii
- Streptococci
- Lactobacillus
- Lactococcus
- Leuconostoc
- Pediococcus
- Bacillus coagulans
- Bifidobacterium bifidum
- Bifidobacterium breve
- Bifidobacterium infantis
- Bifidobacterium lactis
- Bifidobacterium longum
- Escherichia coli nissle
- Lactobacillus acidophilus
- Lactobacillus brevis
- Lactobacillus casei
- Lactobacillus fermentum
- Lactobacillus paracasei
- Lactobacillus reuteri
- Lactobacillus rhamnosus
- Lactobacillus rhamnosus GG
- Lactobacillus salivarius
- Saccharomyces boulardii
- Streptococcus thermophilus
- Bifidobacterium dentium
- Mycoplana
- Pseudomonas

Gut Diversity Indices

- Shannon's Diversity Index
- Simpson's Diversity Index
- Firmicutes/Bacteroidetes
- Prevotella/Bacteroidetes (P/B)

Gut Phyla

- Intestinal permeability
- Intestinal Gas
- SIBO
- Irritable Bowel Syndrome
- Inflammatory bowel disease
- Autoimmune Health
- Metabolic Health
- Liver Health
- Hormones
- Nutrition
- Cardiovascular Health
- Neurological Health
- Probiotic Health
- Keystone Health

Antibiotic Resistance Genes

- Helicobacter - Clarithromycin
- Helicobacter - Fluoroquinolones
- Fluoroquinolones
- Vancomycin
- b-lactamase
- Macrolides
- Tetracycline
- Aminoglycoside
- Bactrim
- Carbapenem
- Rifampin
- Polymyxins

Gut Pathogens

- Clostridium difficile
- Adenovirus F40/41
- Aeromonas spp.
- Ancylostoma duodenale
- Ascaris lumbricoides
- Astrovirus
- Bacillus cereus
- Balantidium coli
- Blastocystis hominis
- Campylobacter coli
- Campylobacter jejuni
- Campylobacter spp.
- Campylobacter upsaliensis
- Candida albicans
- Candida glabrata
- Candida spp.
- Chilomastix mesnili
- Clostridium difficile
- Clostridium difficile Toxin A Gene
- Clostridium difficile Toxin B Gene
- Clostridium perfringens
- Cryptosporidium
- Cyclospora cayetanensis
- Cyclospora spp.
- Cytomegalovirus
- Dientamoeba fragilis
- Diphyllobothrium latum
- Dipylidium caninum
- E.coli O157
- Edwardsiella tarda
- Endolimax nana
- Entamoeba coli
- Entamoeba histolytica
- Enteroaggregative E.coli (EAEC)
- Enterobius vermicularis
- Enteropathogenic E.coli (EPEC)
- Enterotoxigenic E.coli (Etec) Lt/St
- Enterovirus
- Epstein-Barr virus
- Fasciola/Fasciolopsis
- Geotrichum spp.
- Giardia lamblia
- Helicobacter pylori
- Human bocavirus
- Hymenolepis
- Isospora belli
- Klebsiella pneumoniae
- Larval nematode
- Listeria spp.
- Mansonella
- Microsporidia spp.
- Necator americanus
- Non-pylori Helicobacter spp.
- Norovirus GI
- Norovirus GII
- Pentatrichomonas hominis
- Plesiomonas shigelloides
- Rhodotorula spp.
- Rotavirus A
- Salmonella spp.
- Sapovirus I
- Sapovirus II
- Sapovirus IV
- Sapovirus V
- Schistosoma
- Shiga-Like Toxin Producing E.coli (STEC)
- Stx1/Stx2
- Shigella/EIEC
- Staphylococcus aureus
- Strongyloides stercoralis
- Taenia solium
- Taenia spp.
- Trichomonas hominis
- Trichuris trichiura
- Vibrio cholerae
- Vibrio parahaemolyticus
- Vibrio vulnificus
- Yersinia enterocolitica

Reload this page

Gut Commensals - Risk Category

- Intestinal permeability
- Intestinal Gas
- SIBO
- Irritable Bowel Syndrome
- Inflammatory bowel disease
- Autoimmune Health
- Metabolic Health
- Liver Health
- Hormones
- Nutrition
- Cardiovascular Health
- Neurological Health
- Probiotic Health
- Keystone Health

Gut Inflammatory Markers

- Beta defensin 2 (ng/mL)
- Lysozyme (ng/mL)
- MMP 9 (ng/mL)
- S100A12 (mcg/ml)
- Calprotectin (mcg/g)
- Fecal lactoferrin (mcg/ml)
- Fecal Eosinophil Protein X (mcg/g)

Digestion and Immune Balance

- Pancreatic elastase 1 (mcg/g)
- Fecal Immunochemical Test (FIT)
- Fecal Zonulin (ng/mL)
- pHsIgA (mcg/g)

Gut Antibodies

- Lipopolysaccharide antibody
- Anti-Saccharomyces cerevisiae antibody
- Tissue transglutaminase
- Deamidated gliadin peptide
- Fecal Anti Gliadin (U/L)
- Actin antibody

Malabsorption

- Meat fiber
- Vegetable fiber
- Total Fecal Fat (mg/g)
- Total Fecal Triglycerides (mg/g)
- Long chain fatty acids (mg/g)
- Total Cholesterol (mg/g)
- Total Phospholipids (mg/g)

Gut Metabolites

- Acetate (%)
- Butyrate (%)
- Chenodeoxycholic acid (CDCA) (%)
- Cholic acid (CA) (%)
- Deoxycholic acid (DCA) (%)
- LCA/DCA ratio
- Lithocholic acid (LCA) (%)
- Propionate (%)
- ß-glucuronidase (U/mL)
- Total Short chain fatty acids (micromol/g)
- Valerate (%)

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nature communications

Article

https://doi.org/10.1038/s41467-023-43431-8

Health-related quality of life is linked to the gut microbiome in kidney transplant recipients

Received: 12 February 2023

Accepted: 8 November 2023

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Check for updates

J. Casper Swarte[1,2,10], Tim J. Knobbe[2,10], Johannes R. Björk[1,3], Ranko Gacesa[1,3], Lianne M. Nieuwenhuis[1], Shuyan Zhang[1], Arnau Vich Vila[1,3], Daan Kremer[2], Rianne M. Douwes[1,2], Adrian Post[2], Evelien E. Quint[4], Robert A. Pol[4], Bernadien H. Jansen[5], TransplantLines investigators*, Martin H. de Borst[2], Vincent E. de Meijer[5], Hans Blokzijl[1], Stefan P. Berger[2], Eleonora A. M. Festen[1,3], Alexandra Zhernakova[1], Jingyuan Fu[1,6], Hermie J. M. Harmsen[7], Stephan J. L. Bakker[2,11] & Rinse K. Weersma[1,11]

Kidney transplant recipients (KTR) have impaired health-related quality of life (HRQoL) and suffer from intestinal dysbiosis. Increasing evidence shows that gut health and HRQoL are tightly related in the general population. Here, we investigate the association between the gut microbiome and HRQoL in KTR, using metagenomic sequencing data from fecal samples collected from 507 KTR. Multiple bacterial species are associated with lower HRQoL, many of which have previously been associated with adverse health conditions. Gut microbiome distance to the general population is highest among KTR with an impaired physical HRQoL ($R = -0.20$, $P = 2.3 \times 10^{-13}$) and mental HRQoL ($R = -0.14$, $P = 1.3 \times 10^{-5}$). Physical and mental HRQoL explain a significant part of variance in the gut microbiome ($R^2 = 0.58\%$, FDR = 5.43×10^{-4} and $R^2 = 0.37\%$, FDR = 1.38×10^{-3}, respectively). Additionally, multiple metabolic and neuroactive pathways (gut brain modules) are associated with lower HRQoL. While the observational design of our study does not allow us to analyze causality, we provide a comprehensive overview of the associations between the gut microbiome and HRQoL while controlling for confounders.

Kidney transplantation is the preferred treatment of patients with end-stage kidney disease and improves survival after transplantation compared with patients who are treated with dialysis[1,2]. However, health-related quality of life (HRQoL) of kidney transplant recipients (KTR) still remains lower after transplantation compared with the general population, especially regarding physical HRQoL[3]. Improving HRQoL in the long term after transplantation would greatly improve the outcomes of kidney transplantation.

The gut-brain axis refers to the bidirectional communication between the gut and the brain, which plays a role in regulating mood, behavior, and overall well-being. The gut and the central nervous system are known to communicate via neural, immunological and chemical pathways[4]. Therefore, it is not surprising that gut health and HRQoL are tightly connected[5]. The gut microbiome can influence the central nervous system via the gut-brain axis[6] with, for example, bacterial cell wall components[7] or short chain fatty acids[8]. Translation of these mostly animal-based studies to human subjects remains difficult, although it has previously been shown that modifying dietary fiber intake is associated with improved mental HRQoL[9], which could be mediated by the gut microbiome[10].

A full list of affiliations appears at the end of the paper. *A list of authors and their affiliations appears at the end of the paper. e-mail: r.k.weersma@umcg.nl

Nature Communications (2023)14:7968

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How Tightly Connected?

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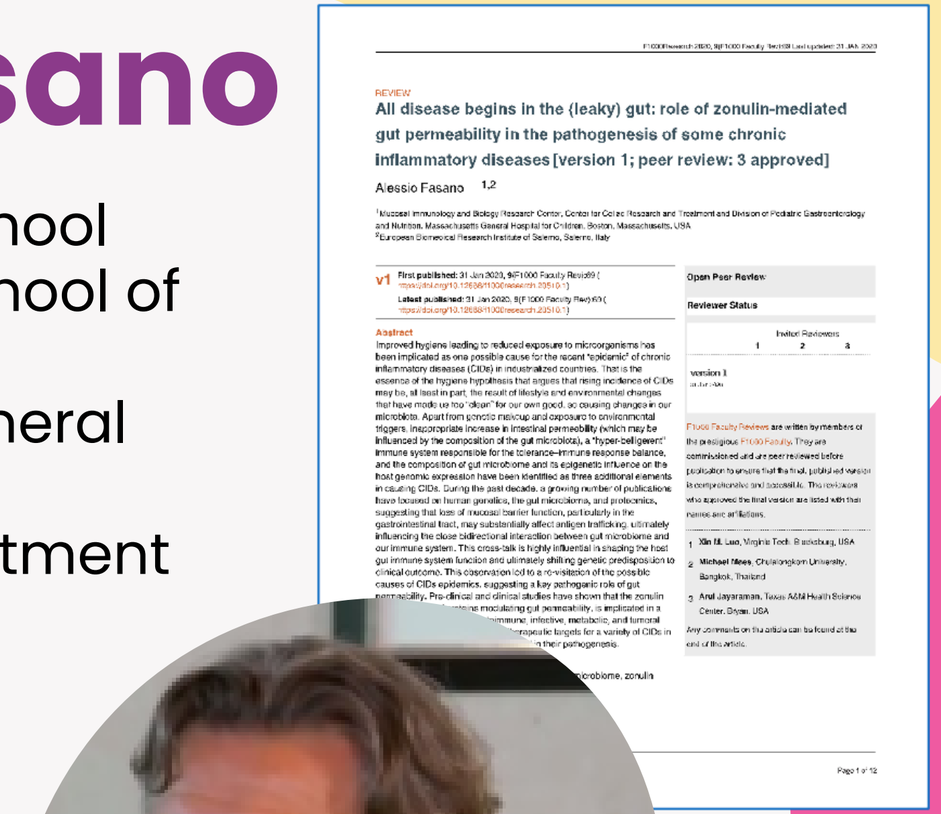
Professor Alessio Fasano

- Professor of Pediatrics at Harvard Medical School
- Professor of Nutrition at Harvard T.H. Chan School of Public Health
- Chief of Pediatric Gastroenterology, Mass General Hospital
- Director, Center for Celiac Research and Treatment
- Director, Mucosal Immunology and Biology Research Center;
- Associate Chief for Basic, Clinical and Translational Research
- 339 publications on pubmed.gov
- Identified zonulin as the protein activated in intestinal permeability



REVIEW

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Alessio Fasano [1,2]

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[illegible] microbiome, zonulin

Open Peer Review

Reviewer Status

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Page 1 of 12

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Keywords

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Page 1 of 12

The idea that a chronic inflammatory disease iscaused by a gene is much too simplistic to explain the hundreds of thousands of interactions occurring in the body literally every second that eventually produces a chronic inflammatory disease

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Page 1 of 12

The premise of “one gene, one protein, one disease” can not explain the complexity of the balance between health and disease and, most definitively, the CIDs epidemics.

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Twenty-three thousand genes are insufficient to explain all the permutations of human pathophysiology, including if, and when, and why we develop diseases.

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Rather, it is the interplay between us as individuals and the environment in which we live that dictates our clinical destiny.

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This interplay is physically and mechanistically regulated by biological interfaces that divide our body from the surrounding environment.
(thus the study of mucosal immunology)

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There is growing evidence that the elements of gut permeability, immune system response, and gut microbiome—together with genetic predisposition and exposure to environmental triggers— make the “*perfect storm*” for Chronic Inflammatory Disease development.

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Any comments on the article can be found at the end of the article.

https://f1000research.com/articles/9-69/v1

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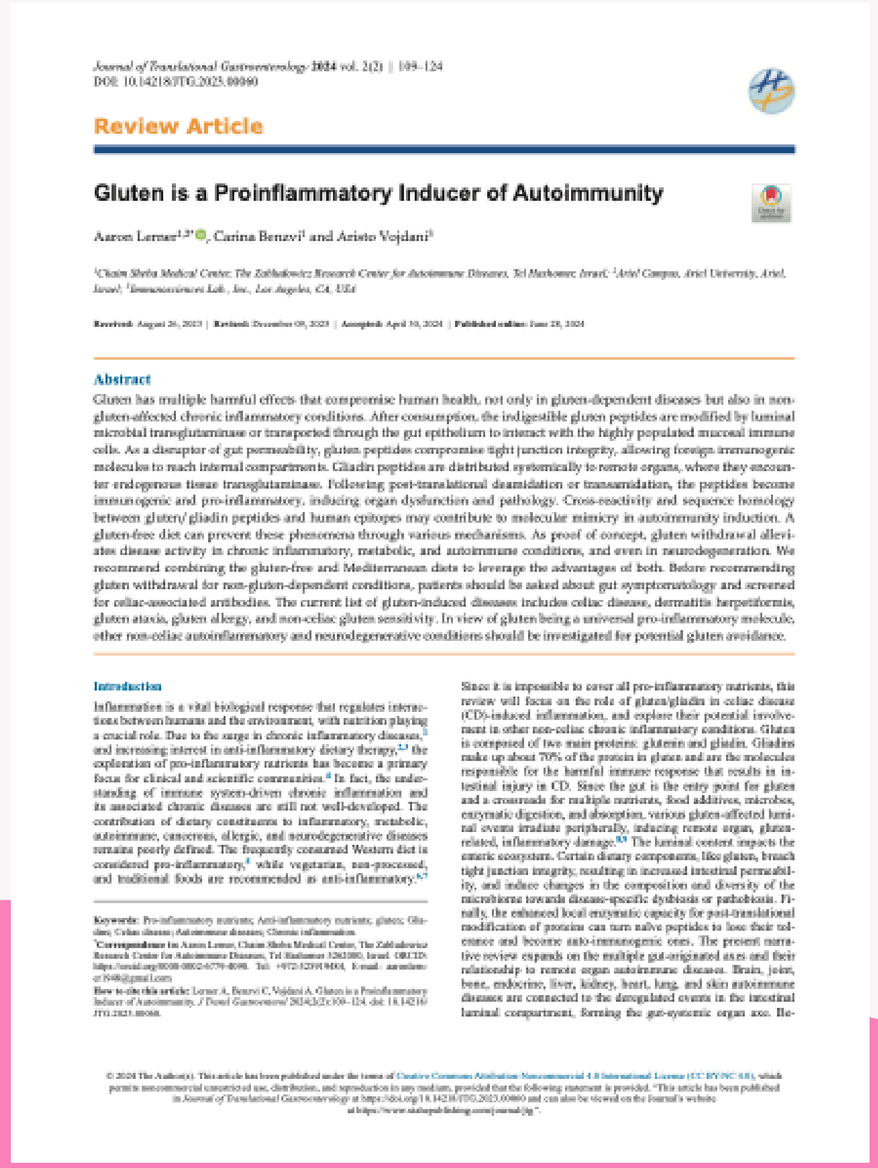

Journal of Translational Gastroenterology 2024 vol. 2(2) | 109–124

Intestinal tight junction functional integrity is one of the most conserved protective mechanisms for human survival and is crucial for maintaining intestinal homeostasis.

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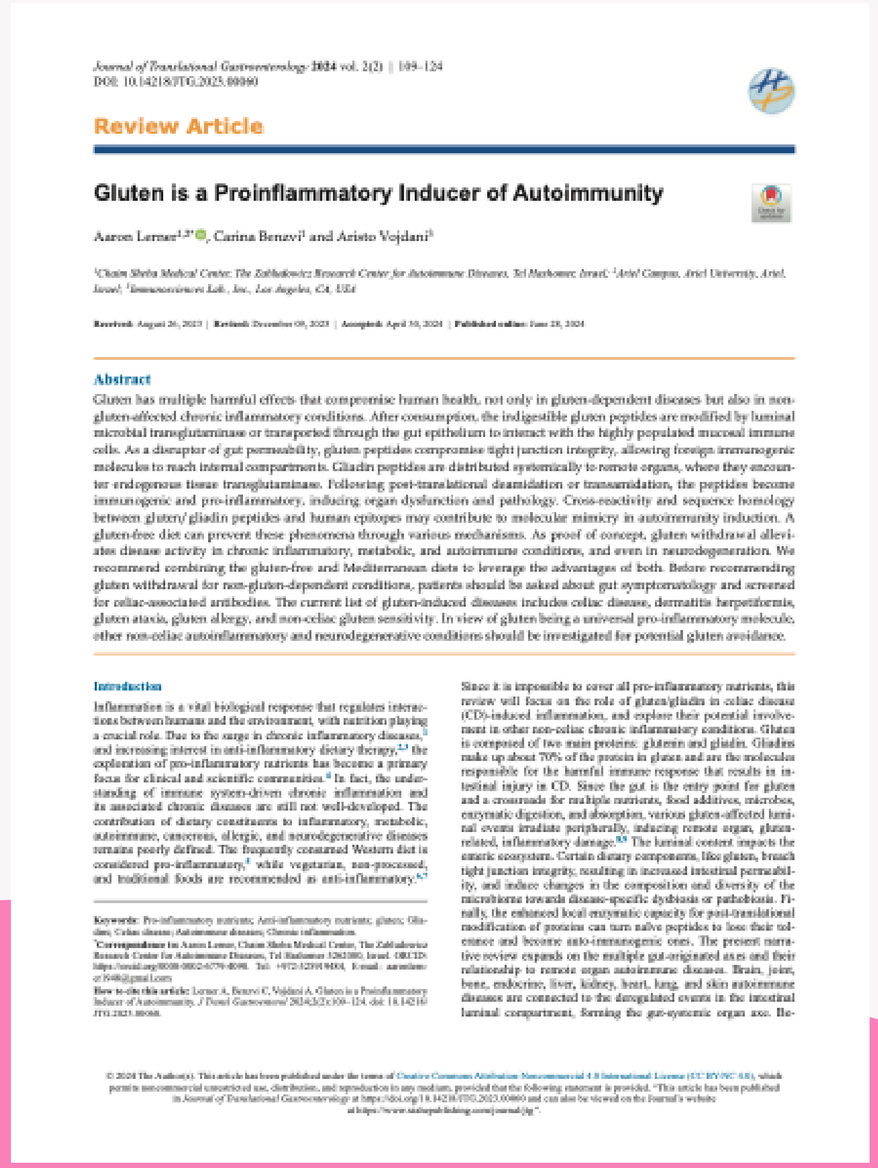

Journal of Translational Gastroenterology 2024 vol. 2(2) | 109–124

When disrupted, foreign molecules enter the epithelial barrier, come into contact with the subepithelial dense immune systems, and initiate chronic inflammation and autoimmunity.

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What System of the Body Specifically Screens and Responds to ‘the environment in which we live’ ?

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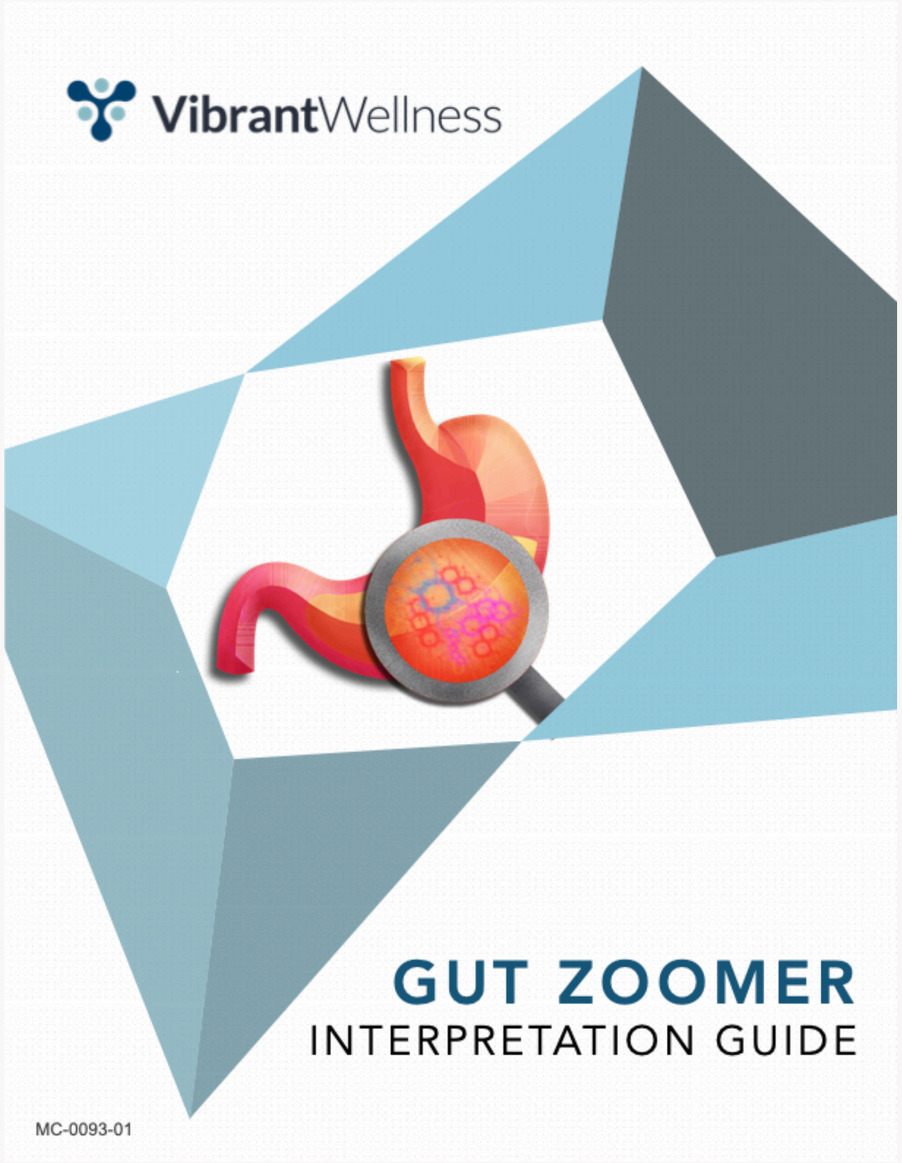

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"That's not quite the stool sample we had in mind, Mr. O'Donnell."

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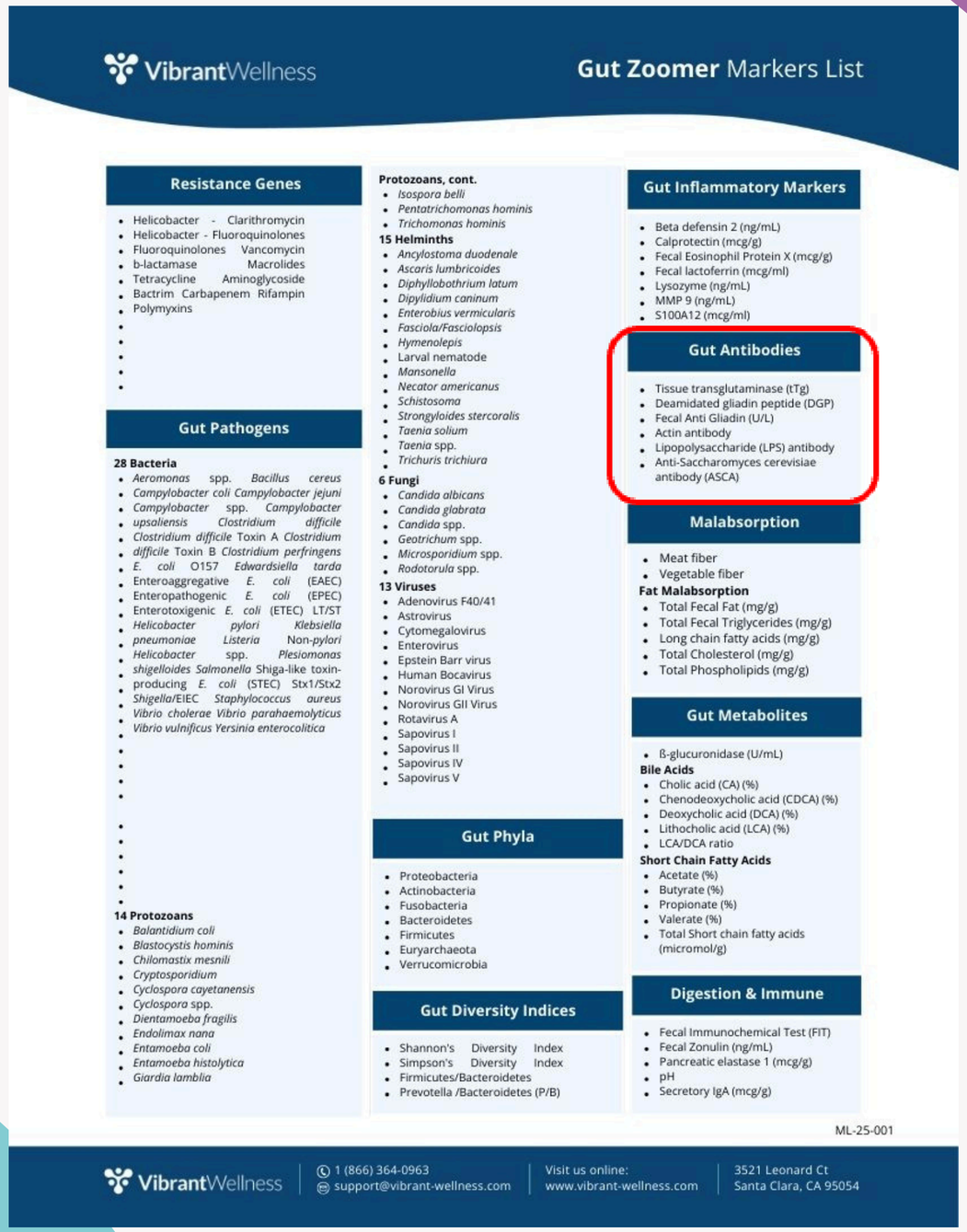

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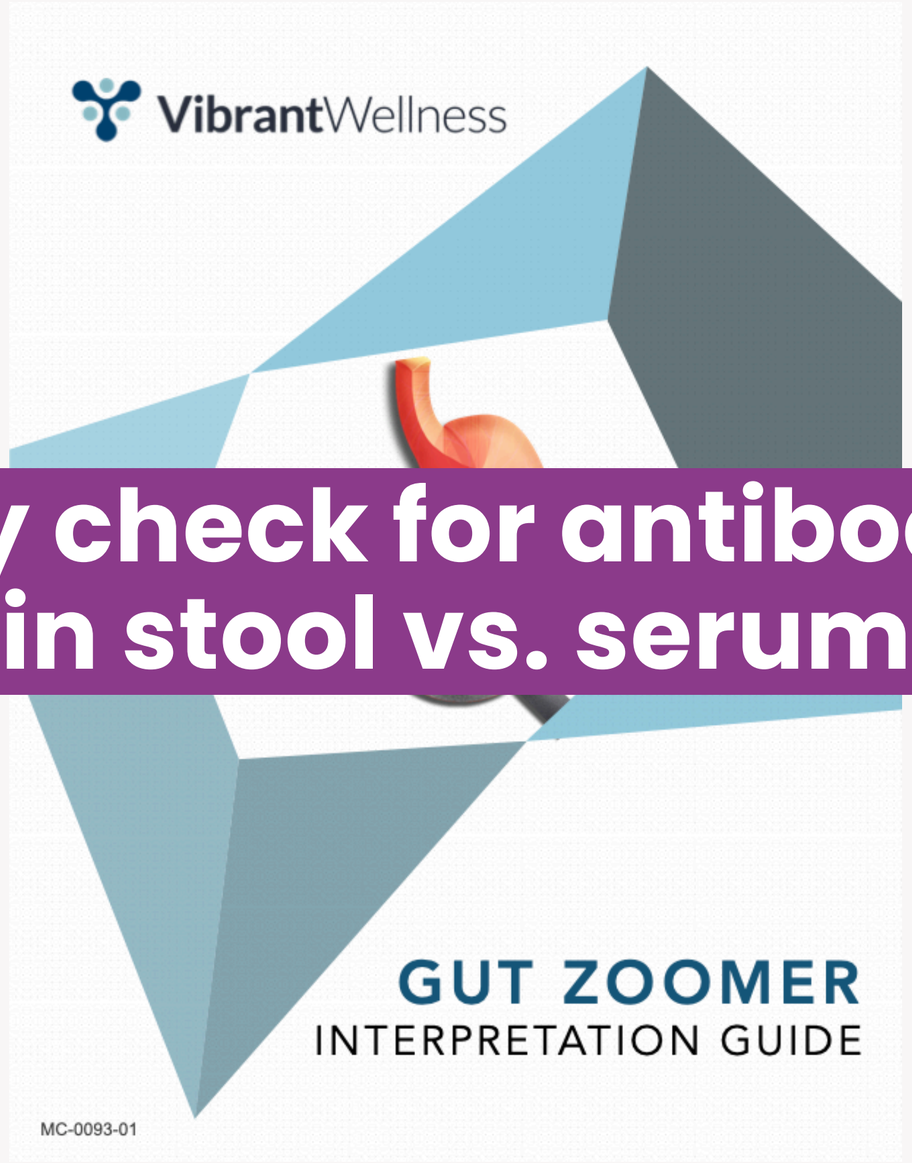

Why check for antibodies in stool vs. serum

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Local immune surveillance: Reflects activity in the gut-associated lymphoid tissue (GALT).

Early detection: Antibodies in stool may appear before serum antibodies, since mucosal surfaces are the first site of contact with food antigens, microbes, and toxins.

Barrier integrity: Elevated stool antibodies can indicate loss of oral tolerance and ongoing mucosal immune activation ("leaky gut physiology").

Non-systemic responders: Some patients may never mount a strong systemic response, so serum testing alone can miss local gluten or pathogen reactivity.

Functional insight: Stool antibodies reflect what's happening at the epithelial interface, where symptoms like bloating, diarrhea, and abdominal pain originate.

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Lipopolysaccharide Antibodies

The Vibrant Longevity Summit

REVIEW

All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases [version 1; peer review: 3 approved]

Alessio Fasano [1,2]

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[2]European Biomedical Research Institute of Salerno, Salerno, Italy

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Abstract

Improved hygiene leading to reduced exposure to microorganisms has been implicated as one possible cause for the recent "epidemic" of chronic inflammatory diseases (CIDs) in industrialized countries. That is the essence of the hygiene hypothesis that argues that rising incidence of CIDs may be, at least in part, the result of lifestyle and environmental changes that have made us too "clean" for our own good, so causing changes in our microbiota. Apart from genetic makeup and exposure to environmental triggers, inappropriate increase in intestinal permeability (which may be influenced by the composition of the gut microbiota), a "hyper-belligerent" immune system responsible for the tolerance–immune response balance, and the composition of gut microbiome and its epigenetic influence on the host genomic expression have been identified as three additional elements in causing CIDs. During the past decade, a growing number of publications have focused on human genetics, the gut microbiome, and proteomics, suggesting that loss of mucosal barrier function, particularly in the gastrointestinal tract, may substantially affect antigen trafficking, ultimately influencing the close bidirectional interaction between gut microbiome and our immune system. This cross-talk is highly influential in shaping the host gut immune system function and ultimately shifting genetic predisposition to clinical outcome. This observation led to a re-visitation of the possible causes of CIDs epidemics, suggesting a key pathogenic role of gut permeability. Pre-clinical and clinical studies have shown that the zonulin family, a group of proteins modulating gut permeability, is implicated in a variety of CIDs, including autoimmune, infective, metabolic, and tumoral diseases. These data offer novel therapeutic targets for a variety of CIDs in which the zonulin pathway is implicated in their pathogenesis.

Keywords

Chronic inflammatory diseases, Gut permeability, microbiome, zonulin

Among the several potential intestinal luminal stimuli that can stimulate zonulin release (thus Intestinal Permeability), small exposure to large amounts of bacteria (with its 'vest' - LPS) and gluten, have been identified as the two most powerful triggers

https://f1000research.com/articles/9-69/v1

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This is exactly why EVERY new patient will be tested comprehensively for an immune reaction to the many peptides of wheat – AND to LPS, as they are the two '*most powerful triggers of inflammation*' in the gut fueling excessive intestinal permeability.

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What is LPS? And why does Prof. Fasano say it is one of the two ’most powerful triggers’ of excessive intestinal permeability?

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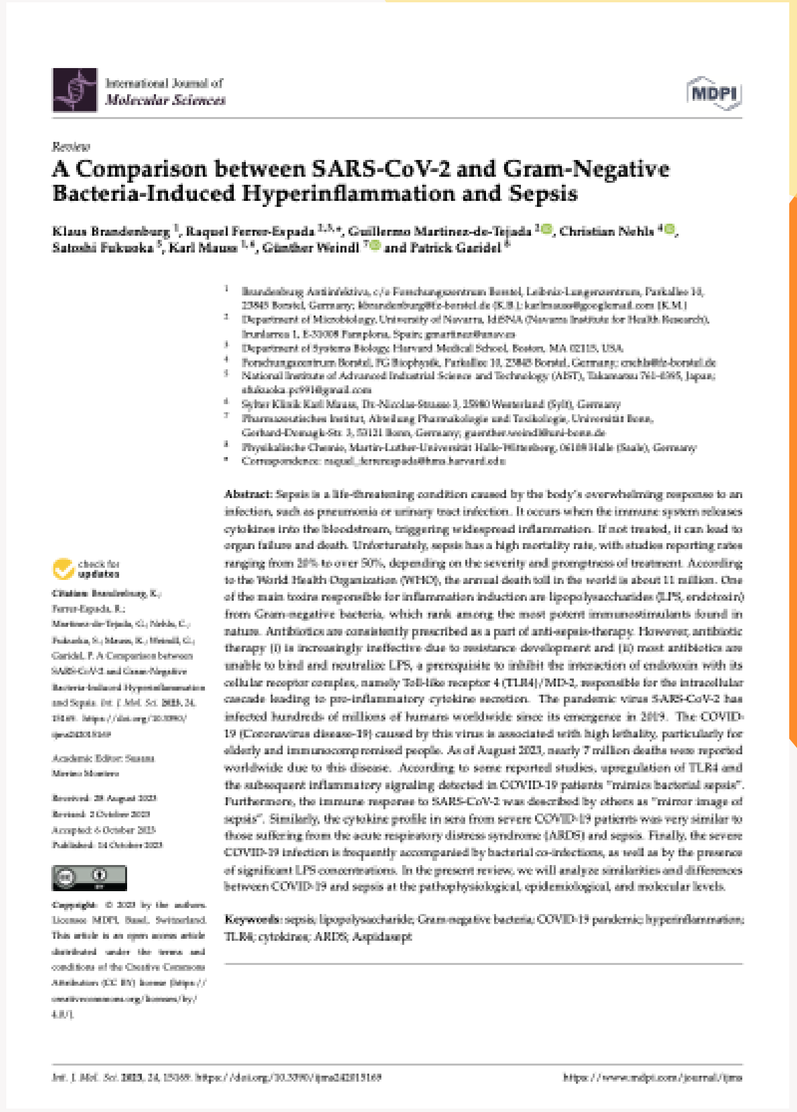
One of the main toxins responsible for inflammation induction are lipopolysaccharides (LPS, endotoxin) from Gram-negative bacteria, which rank amongthe most potent immunostimulants found in nature.



International Journal of Molecular Sciences

MDPI

Review

A Comparison between SARS-CoV-2 and Gram-Negative Bacteria-Induced Hyperinflammation and Sepsis

Klaus Brandenburg [1], Raquel Ferrer-Espada [1,2,*], Guillermo Martínez-de-Tejada [3], Christian Nehls [4], Satoshi Fukuoka [5], Karl Mauss [1,6], Günther Weindl [7] and Patrick Garidel [8]

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Abstract: Sepsis is a life-threatening condition caused by the body's overwhelming response to an infection, such as pneumonia or urinary tract infection. It occurs when the immune system releases cytokines into the bloodstream, triggering widespread inflammation. If not treated, it can lead to organ failure and death. Unfortunately, sepsis has a high mortality rate, with studies reporting rates ranging from 28% to over 50%, depending on the severity and promptness of treatment. According to the World Health Organization (WHO), the annual death toll in the world is about 11 million. One of the main toxins responsible for inflammation induction are lipopolysaccharides (LPS, endotoxin) from Gram-negative bacteria, which rank among the most potent immunostimulants found in nature. Antibiotics are consistently prescribed as a part of anti-sepsis therapy. However, antibiotic therapy (i) is increasingly ineffective due to resistance development and (ii) most antibiotics are unable to bind and neutralize LPS, a prerequisite to inhibit the interaction of endotoxin with its cellular receptor complex, namely Toll-like receptor 4 (TLR4)/MD-2, responsible for the intracellular cascade leading to pro-inflammatory cytokine secretion. The pandemic virus SARS-CoV-2 has infected hundreds of millions of humans worldwide since its emergence in 2019. The COVID-19 (Coronavirus disease-19) caused by this virus is associated with high lethality, particularly for elderly and immunocompromised people. As of August 2023, nearly 7 million deaths were reported worldwide due to this disease. According to some reported studies, upregulation of TLR4 and the subsequent inflammatory signaling detected in COVID-19 patients "mimics bacterial sepsis". Furthermore, the immune response to SARS-CoV-2 was described by others as "mirror image of sepsis". Similarly, the cytokine profile in sera from severe COVID-19 patients was very similar to those suffering from the acute respiratory distress syndrome (ARDS) and sepsis. Finally, the severe COVID-19 infection is frequently accompanied by bacterial co-infections, as well as by the presence of significant LPS concentrations. In the present review, we will analyze similarities and differences between COVID-19 and sepsis at the pathophysiological, epidemiological, and molecular levels.

Keywords: sepsis; lipopolysaccharide; Gram-negative bacteria; COVID-19 pandemic; hyperinflammation; TLR4; cytokines; ARDS; Aspidasept

check for updates

Citation: Brandenburg, K.; Ferrer-Espada, R.; Martínez-de-Tejada, G.; Nehls, C.; Fukuoka, S.; Mauss, K.; Weindl, G.; Garidel, P. A Comparison between SARS-CoV-2 and Gram-Negative Bacteria-Induced Hyperinflammation and Sepsis. *Int. J. Mol. Sci.* **2023**, *24*, 15169. https://doi.org/10.3390/ijms242015169

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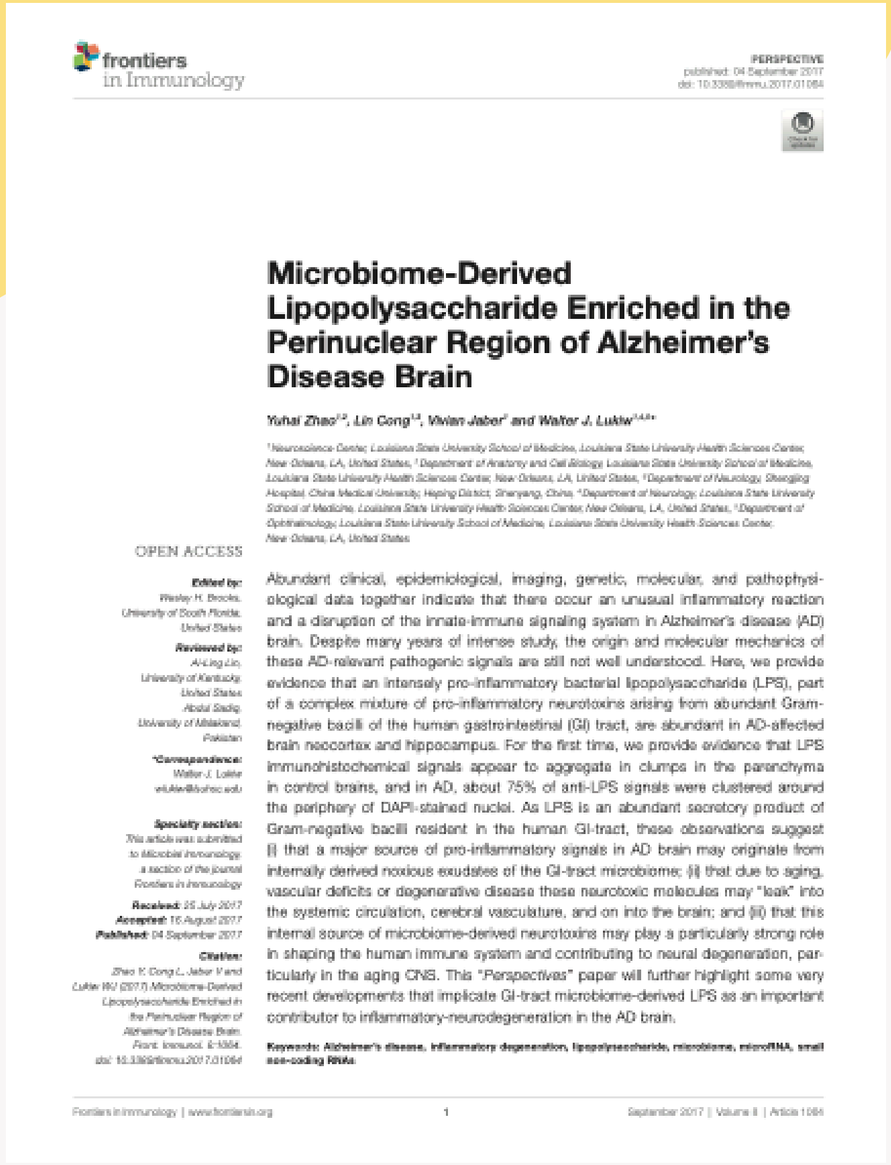

frontiers in Immunology

PERSPECTIVE
published: 04 September 2017
doi: 10.3389/fimmu.2017.01064

Microbiome-Derived Lipopolysaccharide Enriched in the Perinuclear Region of Alzheimer's Disease Brain

Yuhai Zhao[1,2], Lin Gong[1,3], Vivian Jaber[1] and Walter J. Lukiw[1,4,5]

[1] Neuroscience Center, Louisiana State University School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, United States, [2] Department of Anatomy and Cell Biology, Louisiana State University School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, United States, [3] Department of Neurology, Shengjing Hospital, China Medical University, Heping District, Shenyang, China, [4] Department of Neurology, Louisiana State University School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, United States, [5] Department of Ophthalmology, Louisiana State University School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, United States

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Abundant clinical, epidemiological, imaging, genetic, molecular, and pathophysiological data together indicate that there occur an unusual inflammatory reaction and a disruption of the innate-immune signaling system in Alzheimer's disease (AD) brain. Despite many years of intense study, the origin and molecular mechanics of these AD-relevant pathogenic signals are still not well understood. Here, we provide evidence that an intensely pro-inflammatory bacterial lipopolysaccharide (LPS), part of a complex mixture of pro-inflammatory neurotoxins arising from abundant Gram-negative bacilli of the human gastrointestinal (GI) tract, are abundant in AD-affected brain neocortex and hippocampus. For the first time, we provide evidence that LPS immunohistochemical signals appear to aggregate in clumps in the parenchyma in control brains, and in AD, about 75% of anti-LPS signals were clustered around the periphery of DAPI-stained nuclei. As LPS is an abundant secretory product of Gram-negative bacilli resident in the human GI-tract, these observations suggest (i) that a major source of pro-inflammatory signals in AD brain may originate from internally derived noxious exudates of the GI-tract microbiome; (ii) that due to aging, vascular deficits or degenerative disease these neurotoxic molecules may "leak" into the systemic circulation, cerebral vasculature, and on into the brain; and (iii) that this internal source of microbiome-derived neurotoxins may play a particularly strong role in shaping the human immune system and contributing to neural degeneration, particularly in the aging CNS. This "Perspectives" paper will further highlight some very recent developments that implicate GI-tract microbiome-derived LPS as an important contributor to inflammatory-neurodegeneration in the AD brain.

Keywords: Alzheimer's disease, inflammatory degeneration, lipopolysaccharide, microbiome, microRNA, small non-coding RNAs

Front. Immunol. 8:1064. doi: 10.3389/fimmu.2017

LPS, the major molecular component of the outer membrane of Gram-negative bacteria, normally serves as a physical barrier providing the (Gram negative) bacteria protection from its surroundings.

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nutrients

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Review

Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids

Marcelo Macedo Rogero [1,2,*] and Philip C. Calder [3,4]

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2 Food Research Center (FoRC), CEPID-FAPESP, Research Innovation and Dissemination Centers São Paulo Research Foundation, São Paulo 05468-140, Brazil
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4 National Institute for Health Research Southampton Biomedical Research Centre, University Hospital Southampton National Health Service Foundation Trust and University of Southampton, Southampton SO16 6YD, UK
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Abstract: Obesity leads to an inflammatory condition that is directly involved in the etiology of cardiovascular diseases, type 2 diabetes mellitus, and certain types of cancer. The classic inflammatory response is an acute reaction to infections or to tissue injuries, and it tends to move towards resolution and homeostasis. However, the inflammatory process that was observed in individuals affected by obesity and metabolic syndrome differs from the classical inflammatory response in certain respects. This inflammatory process manifests itself systemically and it is characterized by a chronic low-intensity reaction. The toll-like receptor 4 (TLR4) signaling pathway is acknowledged as one of the main triggers of the obesity-induced inflammatory response. The aim of the present review is to describe the role that is played by the TLR4 signaling pathway in the inflammatory response and its modulation by saturated and omega-3 polyunsaturated fatty acids. Studies indicate that saturated fatty acids can induce inflammation by activating the TLR4 signaling pathway. Conversely, omega-3 polyunsaturated fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, exert anti-inflammatory actions through the attenuation of the activation of the TLR4 signaling pathway by either lipopolysaccharides or saturated fatty acids.

Keywords: inflammation; toll-like receptor 4; obesity; fatty acids

1. Obesity

Obesity is a multifactorial and polygenic condition that has become a very concerning public health issue that is affecting both developed and developing countries [1–3]. Overweight individuals (defined as body mass index (BMI) $\geq$ 25 kg/m^2) account for approximately 30% of the global population, i.e., 2.1 billion people, of whom more than 600,000 are classified as obese (defined as BMI $\geq$ 30 kg/m^2) [4]. The analysis conducted by the Global Burden of Disease Study 2013 showed that the overweight prevalence increased to 27.5% of adults and 47.1% of children in the past three decades [5]. The prevalence of obesity is currently higher in developed countries; nevertheless, approximately two-thirds of the obese population lives in developing countries [6]. Based on the current scenario, it is estimated that up to 50% of the global population will be classified as overweight or obese by 2030 [7]. Approximately 35% of adult individuals and 17% of children and adolescents (2 to 19 years old) are considered to be obese (defined by values above the 95th percentile of the BMI

Nutrients 2018, 10, 432;

LPS is one of the most powerful microbial inflammation indicators.

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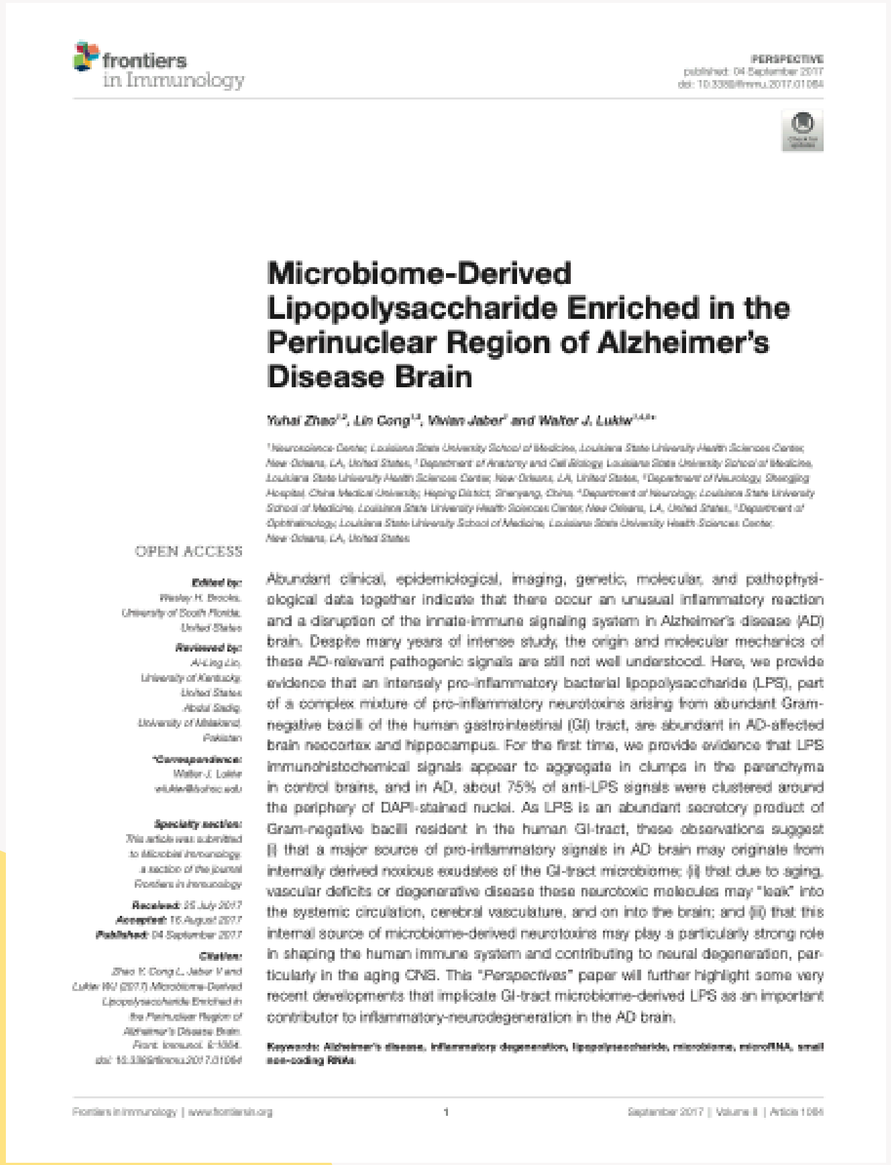

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Keywords: Alzheimer's disease, inflammatory degeneration, lipopolysaccharide, microbiome, microRNA, small non-coding RNAs

LPS is responsible for the development of inflammatory response.

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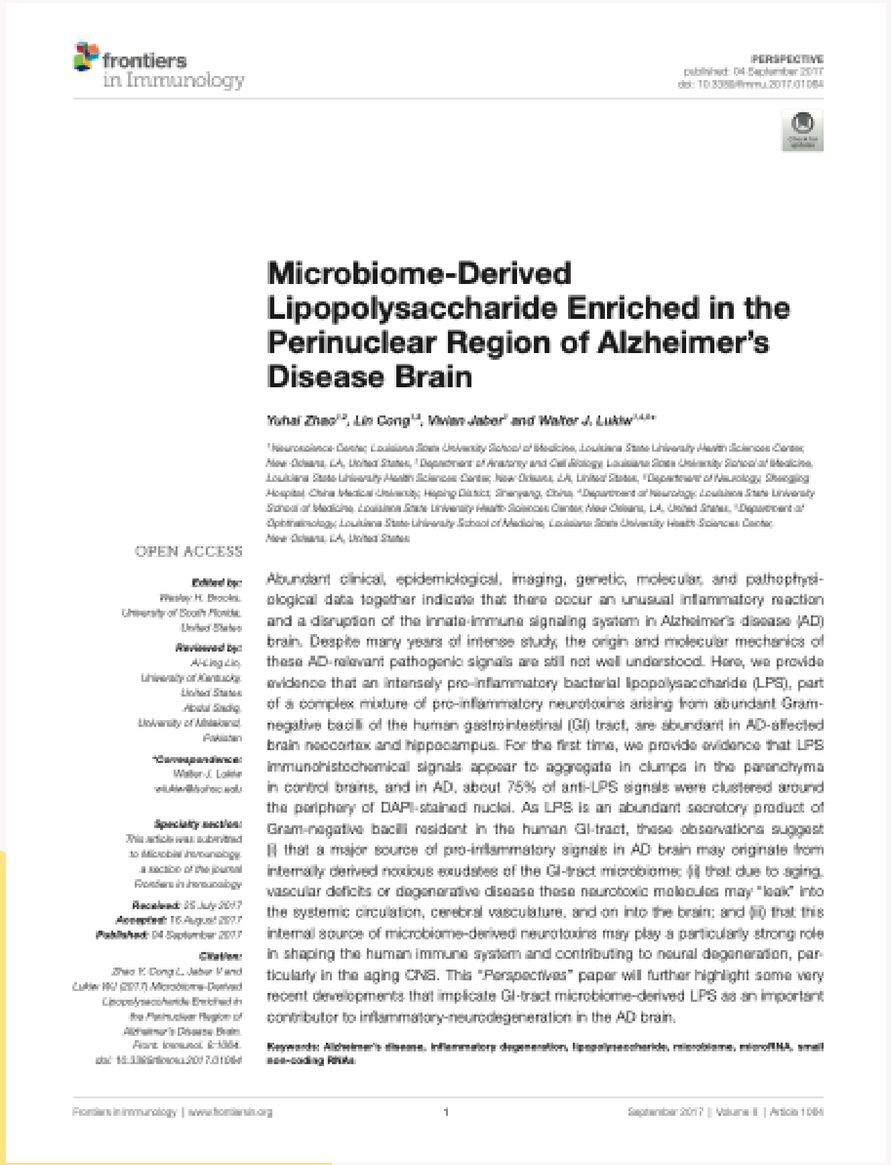

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Keywords: Alzheimer's disease, inflammatory degeneration, lipopolysaccharide, microbiome, microRNA, small non-coding RNAs

It is perhaps the most potent stimulator and trigger of inflammation known

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Cells 2022, 11,4038

LPS-induced systemic inflammatory toxicity is termed 'endotoxemia'

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For example, in the brain...

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molecules

MDPI

Review

Lipopolysaccharide-Induced Model of Neuroinflammation: Mechanisms of Action, Research Application and Future Directions for Its Use

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Abstract: Despite advances in antimicrobial and anti-inflammatory therapies, inflammation and its consequences still remain a significant problem in medicine. Acute inflammatory responses are responsible for directly life-threating conditions such as septic shock; on the other hand, chronic inflammation can cause degeneration of body tissues leading to severe impairment of their function. Neuroinflammation is defined as an inflammatory response in the central nervous system involving microglia, astrocytes, and cytokines including chemokines. It is considered an important cause of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Lipopolysaccharide (LPS) is a strong immunogenic particle present in the outer membrane of Gram-negative bacteria. It is a major triggering factor for the inflammatory cascade in response to a Gram-negative bacteria infection. The use of LPS as a strong pro-inflammatory agent is a well-known model of inflammation applied in both in vivo and in vitro studies. This review offers a summary of the pathogenesis associated with LPS exposure, especially in the field of neuroinflammation. Moreover, we analyzed different in vivo LPS models utilized in the area of neuroscience. This paper presents recent knowledge and is focused on new insights in the LPS experimental model.

Keywords: lipopolysaccharide; neuroinflammation; neurodegenerative diseases; Alzheimer's disease; Toll-like receptor 4

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1. Introduction

Neuroinflammation is defined as an inflammatory response in the central nervous system (CNS), mediated by production of cytokines together with chemokines, and inflammatory enzymes. CNS, as a structure separated by the blood–brain barrier (BBB), is equipped with resident immunocompetent cells, namely microglia and astrocytes. Microglia are a type of glial cells related to macrophages that constitute the main pool of immune cells within the brain and spinal cord. Microglia plays a crucial role in maintaining homeostasis in the nervous tissue of the CNS by sensing and eliminating unnecessary metabolic products, foreign materials and cellular debris. For this reason it is sometimes known as "housekeeping cells" [?]. However, it has been proven that role of microglia exceeds beyond housekeeping, as it participates in the brain development, neuromodulation, synaptic plasticity, and it contributes to learning and memory processing [? ?]. Another group of CNS cells that possess immunological properties are astrocytes. Similarl to microglia, astrocytes have many different functions. They are essential for both the developing and adult brain, and their most prominent role is to maintain BBB [?]. However, as mentioned above, both microglia and astrocytes are immunocompetent cells, and they play a pivotal role in the neuroinflammation [?]. They express membrane receptors and molecules, such as the receptor for advanced glycation end-products (RAGE), clusters of

LPS causes inflammatory response in the brain... which results in the degeneration of neurons, synaptic loss and finally neuronal cell death.

Molecules 2022, 27, 5481.

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Molecules 2022, 27, 5481.

Amyloidogenesis caused by LPS (exposure in the brain) is the most prominent phenomenon in the cortical and hippocampal areas

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LPS causes inflammatory response in the brain
One of the 2 most powerful triggers stimulating zonulin release
LPS is one of the most powerful microbial inflammation indicators.
LPS exhibits the strongest induction of pro-inflammatory signaling in human neuronal–glial cells of any single inducer
perhaps the most potent stimulator and trigger of inflammation known
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What percentage of your patients of child-bearing age have had complications (infertility, unexplained miscarriage, IUGR…) without outright symptoms of infection, or any trigger?

I would wager that the vast majority, if checked, perhaps close to every one of them, would test positive for low-grade chronic inflammation and a systemic immune reaction to LPS .

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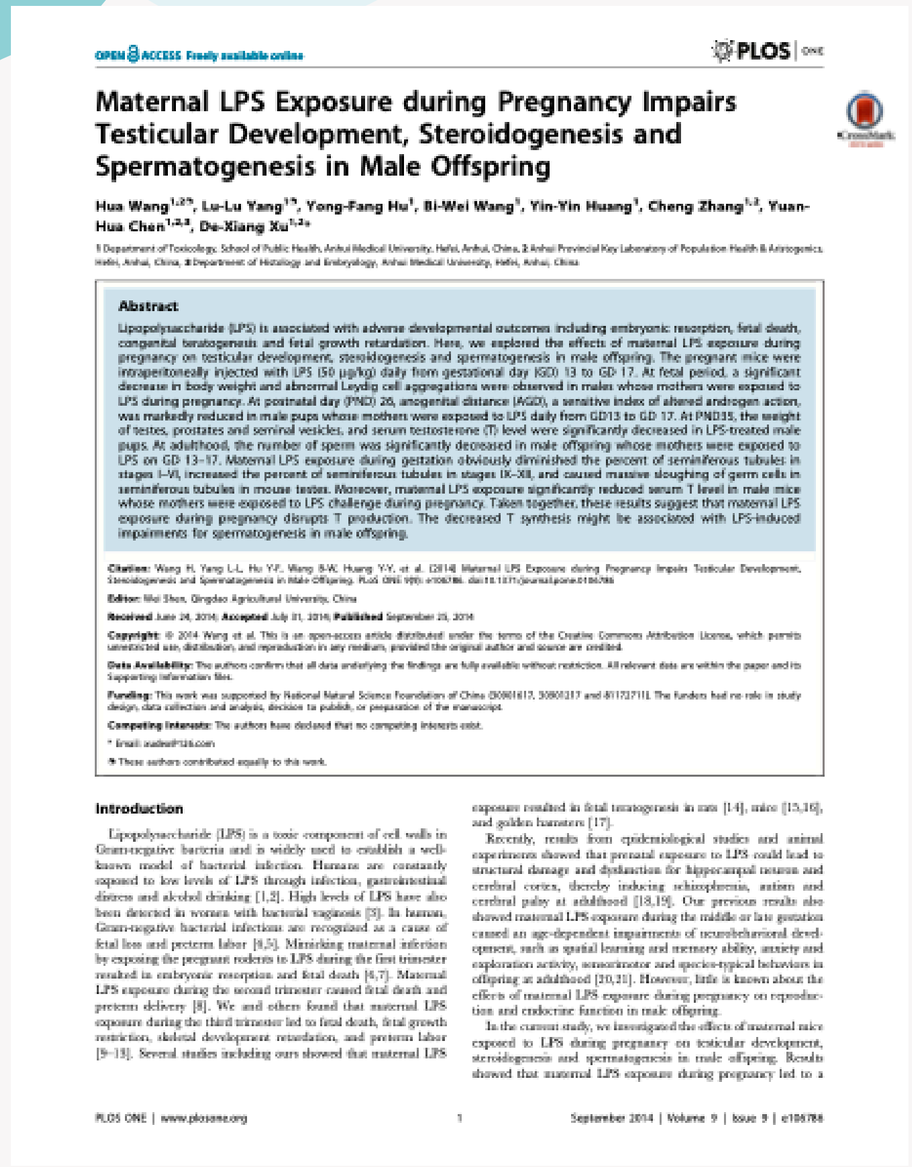

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PLOS ONE

Maternal LPS Exposure during Pregnancy Impairs Testicular Development, Steroidogenesis and Spermatogenesis in Male Offspring

Hua Wang[1,2¶], Lu-Lu Yang[1¶], Yong-Fang Hu[1], Bi-Wei Wang[1], Yin-Yin Huang[1], Cheng Zhang[1,2], Yuan-Hua Chen[1,2,3], De-Xiang Xu[1,2]*

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Abstract

Lipopolysaccharide (LPS) is associated with adverse developmental outcomes including embryonic resorption, fetal death, congenital teratogenesis and fetal growth retardation. Here, we explored the effects of maternal LPS exposure during pregnancy on testicular development, steroidogenesis and spermatogenesis in male offspring. The pregnant mice were intraperitoneally injected with LPS (50 μg/kg) daily from gestational day (GD) 13 to GD 17. At fetal period, a significant decrease in body weight and abnormal Leydig cell aggregations were observed in males whose mothers were exposed to LPS during pregnancy. At postnatal day (PND) 28, anogenital distance (AGD), a sensitive index of altered androgen action, was markedly reduced in male pups whose mothers were exposed to LPS daily from GD13 to GD 17. At PND35, the weight of testes, prostates and seminal vesicles, and serum testosterone (T) level were significantly decreased in LPS-treated male pups. At adulthood, the number of sperm was significantly decreased in male offspring whose mothers were exposed to LPS on GD 13–17. Maternal LPS exposure during gestation obviously diminished the percent of seminiferous tubules in stages I–VI, increased the percent of seminiferous tubules in stages IX–XII, and caused massive sloughing of germ cells in seminiferous tubules in mouse testes. Moreover, maternal LPS exposure significantly reduced serum T level in male mice whose mothers were exposed to LPS challenge during pregnancy. Taken together, these results suggest that maternal LPS exposure during pregnancy disrupts T production. The decreased T synthesis might be associated with LPS-induced impairments for spermatogenesis in male offspring.

Citation: Wang H, Yang L-L, Hu Y-F, Wang B-W, Huang Y-Y, et al. (2014) Maternal LPS Exposure during Pregnancy Impairs Testicular Development, Steroidogenesis and Spermatogenesis in Male Offspring. PLoS ONE 9(9): e106786. doi:10.1371/journal.pone.0106786

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Lipopolysaccharide (LPS) is a toxic component of cell walls in Gram-negative bacteria and is widely used to establish a well-known model of bacterial infection. Humans are constantly exposed to low levels of LPS through infection, gastrointestinal distress and alcohol drinking [1,2]. High levels of LPS have also been detected in women with bacterial vaginosis [3]. In human, Gram-negative bacterial infections are recognized as a cause of fetal loss and preterm labor [4,5]. Mimicking maternal infection by exposing the pregnant rodents to LPS during the first trimester resulted in embryonic resorption and fetal death [6,7]. Maternal LPS exposure during the second trimester caused fetal death and preterm delivery [8]. We and others found that maternal LPS exposure during the third trimester led to fetal death, fetal growth restriction, skeletal development retardation, and preterm labor [9–13]. Several studies including ours showed that maternal LPS exposure resulted in fetal teratogenesis in rats [14], mice [15,16], and golden hamsters [17].

Recently, results from epidemiological studies and animal experiments showed that prenatal exposure to LPS could lead to structural damage and dysfunction for hippocampal neuron and cerebral cortex, thereby inducing schizophrenia, autism and cerebral palsy at adulthood [18,19]. Our previous results also showed maternal LPS exposure during the middle or late gestation caused an age-dependent impairments of neurobehavioral development, such as spatial learning and memory ability, anxiety and exploration activity, sensorimotor and species-typical behaviors in offspring at adulthood [20,21]. However, little is known about the effects of maternal LPS exposure during pregnancy on reproduction and endocrine function in male offspring.

In the current study, we investigated the effects of maternal mice exposed to LPS during pregnancy on testicular development, steroidogenesis and spermatogenesis in male offspring. Results showed that maternal LPS exposure during pregnancy led to a

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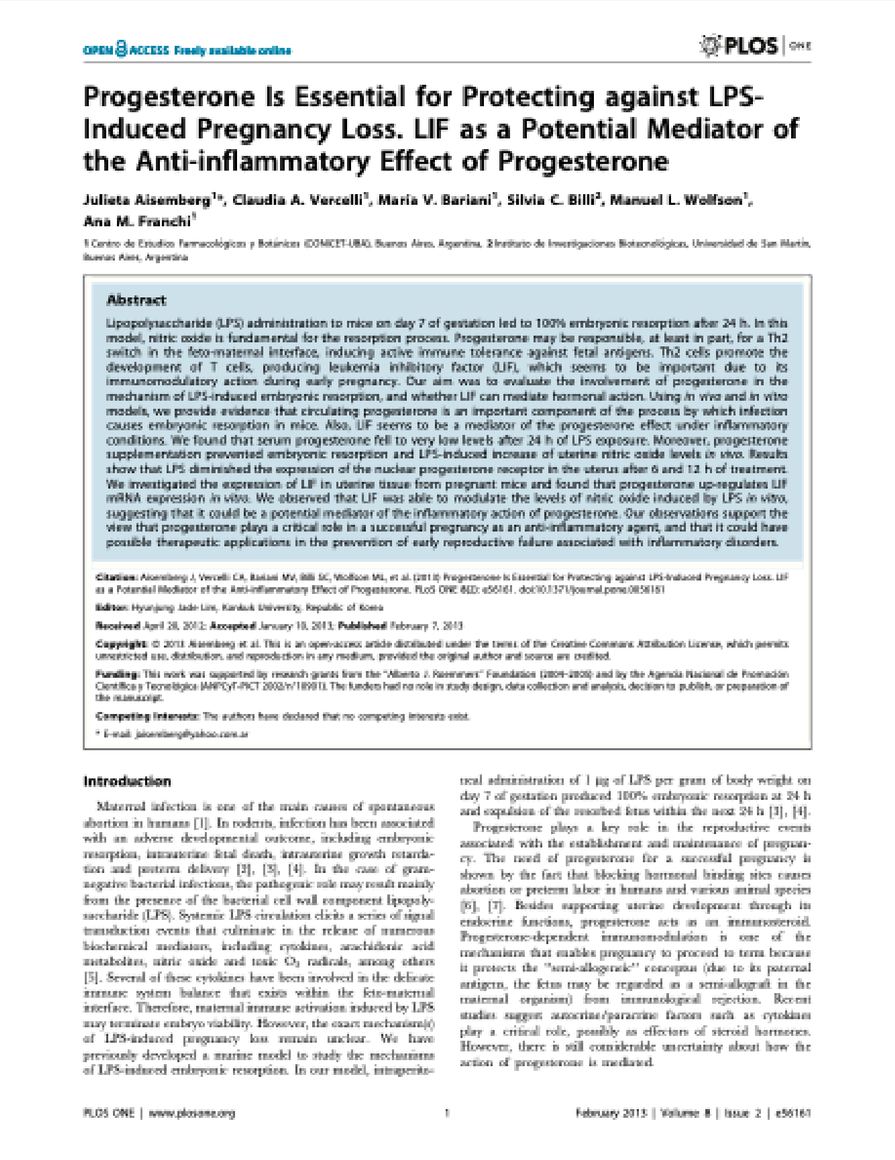

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PLOS ONE

Progesterone Is Essential for Protecting against LPS-Induced Pregnancy Loss. LIF as a Potential Mediator of the Anti-inflammatory Effect of Progesterone

Julieta Aisemberg[1]*, Claudia A. Vercelli[1], María V. Bariani[1], Silvia C. Billi[2], Manuel L. Wolfson[1], Ana M. Franchi[1]

1 Centro de Estudios Farmacológicos y Botánicos (CONICET-UBA), Buenos Aires, Argentina, 2 Instituto de Investigaciones Biotecnológicas, Universidad de San Martín, Buenos Aires, Argentina

Abstract

Lipopolysaccharide (LPS) administration to mice on day 7 of gestation led to 100% embryonic resorption after 24 h. In this model, nitric oxide is fundamental for the resorption process. Progesterone may be responsible, at least in part, for a Th2 switch in the feto-maternal interface, inducing active immune tolerance against fetal antigens. Th2 cells promote the development of T cells, producing leukemia inhibitory factor (LIF), which seems to be important due to its immunomodulatory action during early pregnancy. Our aim was to evaluate the involvement of progesterone in the mechanism of LPS-induced embryonic resorption, and whether LIF can mediate hormonal action. Using *in vivo* and *in vitro* models, we provide evidence that circulating progesterone is an important component of the process by which infection causes embryonic resorption in mice. Also, LIF seems to be a mediator of the progesterone effect under inflammatory conditions. We found that serum progesterone fell to very low levels after 24 h of LPS exposure. Moreover, progesterone supplementation prevented embryonic resorption and LPS-induced increase of uterine nitric oxide levels *in vivo*. Results show that LPS diminished the expression of the nuclear progesterone receptor in the uterus after 6 and 12 h of treatment. We investigated the expression of LIF in uterine tissue from pregnant mice and found that progesterone up-regulates LIF mRNA expression *in vivo*. We observed that LIF was able to modulate the levels of nitric oxide induced by LPS *in vitro*, suggesting that it could be a potential mediator of the inflammatory action of progesterone. Our observations support the view that progesterone plays a critical role in a successful pregnancy as an anti-inflammatory agent, and that it could have possible therapeutic applications in the prevention of early reproductive failure associated with inflammatory disorders.

Citation: Aisemberg J, Vercelli CA, Bariani MV, Billi SC, Wolfson ML, et al. (2013) Progesterone Is Essential for Protecting against LPS-Induced Pregnancy Loss. LIF as a Potential Mediator of the Anti-inflammatory Effect of Progesterone. PLoS ONE 8(2): e56161. doi:10.1371/journal.pone.0056161

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Maternal infection is one of the main causes of spontaneous abortion in humans [1]. In rodents, infection has been associated with an adverse developmental outcome, including embryonic resorption, intrauterine fetal death, intrauterine growth retardation and preterm delivery [2], [3], [4]. In the case of gram-negative bacterial infections, the pathogenic role may result mainly from the presence of the bacterial cell wall component lipopolysaccharide (LPS). Systemic LPS circulation elicits a series of signal transduction events that culminate in the release of numerous biochemical mediators, including cytokines, arachidonic acid metabolites, nitric oxide and toxic O_2 radicals, among others [5]. Several of these cytokines have been involved in the delicate immune system balance that exists within the feto-maternal interface. Therefore, maternal immune activation induced by LPS may terminate embryo viability. However, the exact mechanism(s) of LPS-induced pregnancy loss remain unclear. We have previously developed a murine model to study the mechanisms of LPS-induced embryonic resorption. In our model, intraperitoneal administration of 1 μg of LPS per gram of body weight on day 7 of gestation produced 100% embryonic resorption at 24 h and expulsion of the resorbed fetus within the next 24 h [3], [4].

Progesterone plays a key role in the reproductive events associated with the establishment and maintenance of pregnancy. The need of progesterone for a successful pregnancy is shown by the fact that blocking hormonal binding sites causes abortion or preterm labor in humans and various animal species [6], [7]. Besides supporting uterine development through its endocrine functions, progesterone acts as an immunosteroid. Progesterone-dependent immunomodulation is one of the mechanisms that enables pregnancy to proceed to term because it protects the "semi-allogeneic" conceptus (due to its paternal antigens, the fetus may be regarded as a semi-allograft in the maternal organism) from immunological rejection. Recent studies suggest autocrine/paracrine factors such as cytokines play a critical role, possibly as effectors of steroid hormones. However, there is still considerable uncertainty about how the action of progesterone is mediated.

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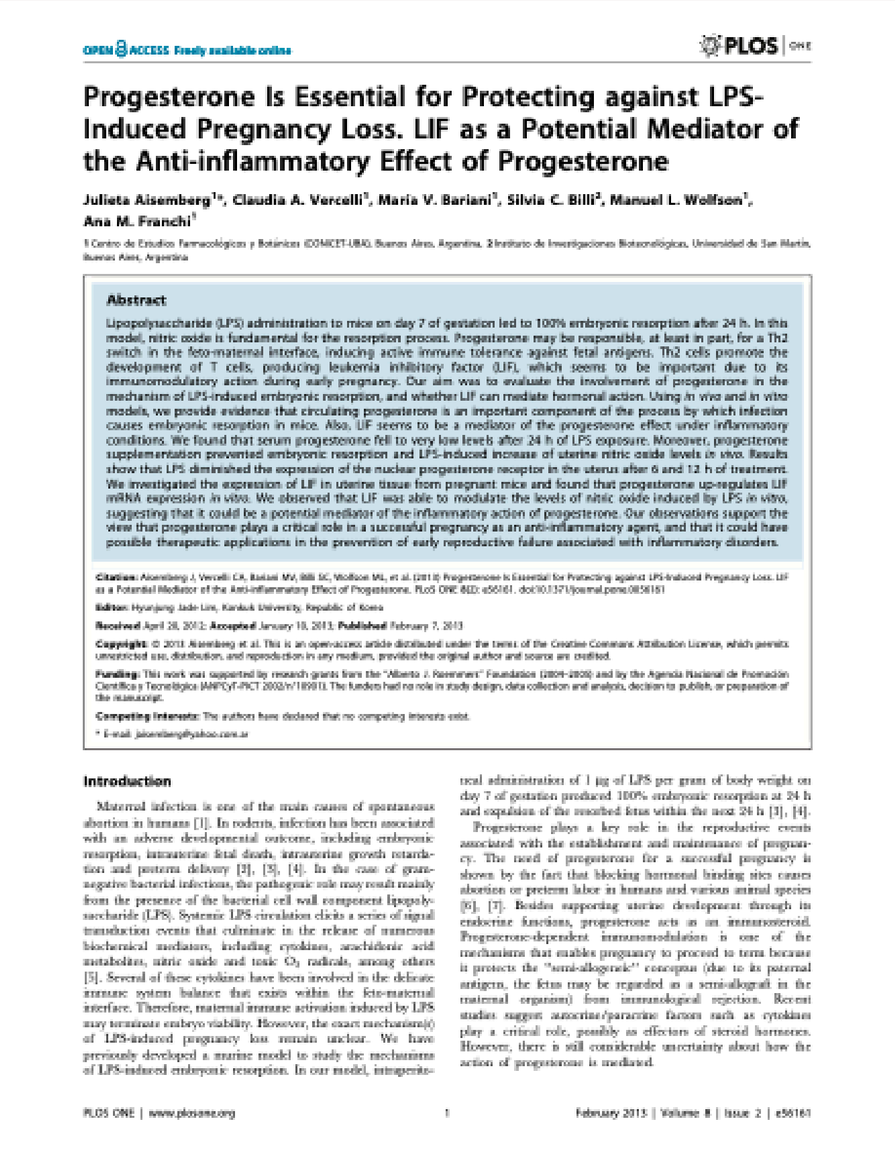

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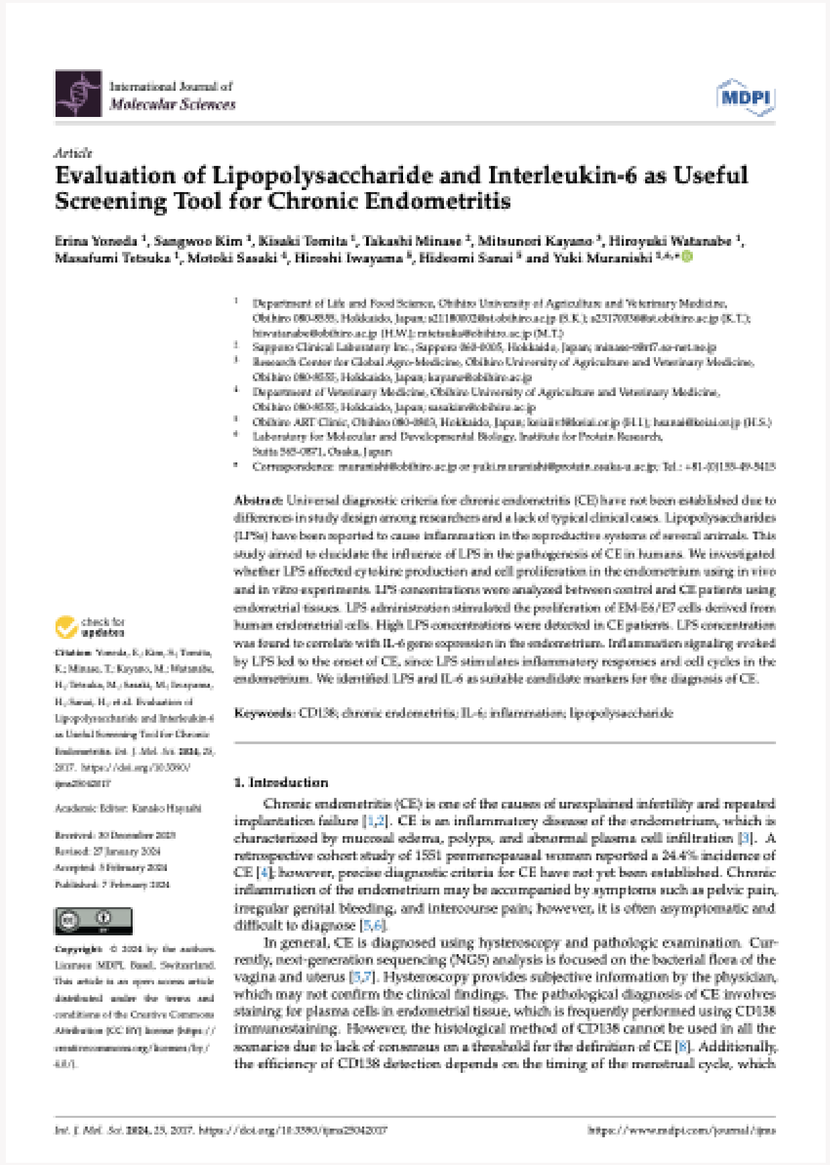

International Journal of *Molecular Sciences*

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Article

Evaluation of Lipopolysaccharide and Interleukin-6 as Useful Screening Tool for Chronic Endometritis

Erina Yoneda [1], Sangwoo Kim [2], Kisaki Tomita [1], Takashi Minase [2], Mitsunori Kayano [3], Hiroyuki Watanabe [1], Masafumi Tetsuka [1], Motoki Sasaki [4], Hiroshi Iwayama [5], Hideomi Sanai [5] and Yuki Muranishi [3,6,*]

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Abstract: Universal diagnostic criteria for chronic endometritis (CE) have not been established due to differences in study design among researchers and a lack of typical clinical cases. Lipopolysaccharides (LPSs) have been reported to cause inflammation in the reproductive systems of several animals. This study aimed to elucidate the influence of LPS in the pathogenesis of CE in humans. We investigated whether LPS affected cytokine production and cell proliferation in the endometrium using in vivo and in vitro experiments. LPS concentrations were analyzed between control and CE patients using endometrial tissues. LPS administration stimulated the proliferation of EM-E6/E7 cells derived from human endometrial cells. High LPS concentrations were detected in CE patients. LPS concentration was found to correlate with IL-6 gene expression in the endometrium. Inflammation signaling evoked by LPS led to the onset of CE, since LPS stimulates inflammatory responses and cell cycles in the endometrium. We identified LPS and IL-6 as suitable candidate markers for the diagnosis of CE.

Keywords: CD138; chronic endometritis; IL-6; inflammation; lipopolysaccharide

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Citation: Yoneda, E.; Kim, S.; Tomita, K.; Minase, T.; Kayano, M.; Watanabe, H.; Tetsuka, M.; Sasaki, M.; Iwayama, H.; Sanai, H.; et al. Evaluation of Lipopolysaccharide and Interleukin-6 as Useful Screening Tool for Chronic Endometritis. *Int. J. Mol. Sci.* **2024**, *25*, 2017. https://doi.org/10.3390/ijms25042017

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1. Introduction

Chronic endometritis (CE) is one of the causes of unexplained infertility and repeated implantation failure [1,2]. CE is an inflammatory disease of the endometrium, which is characterized by mucosal edema, polyps, and abnormal plasma cell infiltration [3]. A retrospective cohort study of 1551 premenopausal women reported a 24.4% incidence of CE [4]; however, precise diagnostic criteria for CE have not yet been established. Chronic inflammation of the endometrium may be accompanied by symptoms such as pelvic pain, irregular genital bleeding, and intercourse pain; however, it is often asymptomatic and difficult to diagnose [5,6].

In general, CE is diagnosed using hysteroscopy and pathologic examination. Currently, next-generation sequencing (NGS) analysis is focused on the bacterial flora of the vagina and uterus [3,7]. Hysteroscopy provides subjective information by the physician, which may not confirm the clinical findings. The pathological diagnosis of CE involves staining for plasma cells in endometrial tissue, which is frequently performed using CD138 immunostaining. However, the histological method of CD138 cannot be used in all the scenarios due to lack of consensus on a threshold for the definition of CE [8]. Additionally, the efficiency of CD138 detection depends on the timing of the menstrual cycle, which

In humans, LPS affects the trophoblastic spheroids and endometrial epithelial cells and decreases uterine receptivity to implantation.

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Binding and Neutralization of Lipopolysaccharides by Plant Proanthocyanidins

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Proanthocyanidins (PACs), polyphenolic metabolites that are widely distributed in higher plants, have been associated with potential positive health benefits including antibacterial, chemotherapeutic, and antiatherosclerotic activities. In this paper, we analyze the binding of PACs from cranberries, tea, and grapes to lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria and the cause of several human illnesses. We demonstrate that in the case of cranberries, the most potent LPS-binding activity is contained within a PAC fraction composed of polymers with an average degree of polymerization of 21. The PAC fraction modestly inhibits the binding of LPS to the surface of HEK 293 cells expressing the full complement of LPS receptors (TLR4/MD2 and CD14), while it significantly abrogates the endocytosis of LPS. This PAC fraction also inhibits LPS-induced nuclear factor-κB activation in a manner that is not readily overcome by excess LPS. Such an effect is mediated through the inhibition of LPS interaction with TLR4/MD2 and the partial abrogation of LPS interaction with CD14. Importantly, PAC concentrations that mediate effective LPS neutralization elicit minimal *in vitro* cytotoxicity. Our results identify PACs as a new class of LPS-binding compound and suggest that they have potential utility in applications that necessitate either the purification and removal of LPS or the *in vivo* neutralization of LPS.

Proanthocyanidins (PACs) are plant-derived polyphenolic compounds composed of flavanoid subunits and have recently been associated with several potential positive health benefits. For example, PACs have been shown to possess cardioprotective properties through the inhibition of both LDL oxidation and platelet aggregation.[1] PACs have also been shown to have antioxidant properties, scavenging free radicals in biological systems.[2] Of particular note is the observation that PACs from cranberries are effective in the mitigation of urinary tract infections through the decreased adhesion of pathogenic bacteria to uroepithelial cells.[3−5] Detailed studies have attributed this activity to PACs with a degree of polymerization of 4 to 5 containing at least one unique interflavan subunit linkage consisting of one carbon−carbon and one carbon−oxygen bond (referred to as an A-type bond).[3] More recently, it has been shown that PACs induce conformational changes in bacterial P-fimbriae that reduce the adhesive forces between these proteins and epithelial cell surface receptors.[6] Recent work in our laboratory pointed to still further activities of high molecular weight polymers from cranberry juice that inhibited the nonspecific adhesion of bacteria to a protein-functionalized immunosensor surface.[5] On the basis of this observation, we were prompted to investigate the potential for previously undescribed interactions of cranberry juice components with other molecules comprising the bacterial cell surface.

Lipopolysaccharide (LPS), the major component of the outer cell membrane of Gram-negative bacteria, is the primary cause of sepsis, an inflammatory syndrome characterized by an overwhelming systemic response to bacterial infection. Sepsis has become the most common cause of death in intensive care units in the United States, with 120 000 deaths annually and associated health-care costs of $16.7 billion.[10] Commonly referred to as bacterial "endotoxin", LPS is composed primarily of three domains: (1) a bacterial membrane-proximal lipid A moiety, (2) a core oligosaccharide region, which connects to (3) the O-antigen, a branched polysaccharide that extends from the core oligosaccharide.[11] LPS present in blood binds to LPS-binding protein,[12] which transfers LPS to the membrane-anchored receptor, CD14, on mononuclear macrophages. CD14 then mediates the interaction of LPS with the bipartite receptor complex, Toll-like receptor 4/MD2 (TLR4/MD2), resulting in intracellular signaling and production of nuclear factor-κB (NF-κB)-activated inflammatory cytokines.[13]

Given the important role of LPS in the onset of sepsis, much effort has been focused on the isolation of robust LPS-binding compounds. The ultimate application of these compounds ranges from the purification and removal of LPS from solutions where its presence is not desirable (e.g., from pharmaceutical preparations) to the *in vivo* neutralization of LPS in septic patients. This pursuit has resulted in the identification of several classes of compounds possessing desirable LPS-binding characteristics such as the cyclic decapeptide polymyxin B[14] and the polyamine spermine and its structural analogues.[15] Polymyxin B, which has a moderately high affinity for LPS (~0.4 μM),[14] has been used for the successful removal of LPS from tissue culture media[16] and blood.[17] However, its *in vivo* applications remain limited due to its high toxicity. Polyamines such as spermine are often limited in their specificity, as the mode of recognition is largely electrostatic. Hence, the need for the identification of alternative LPS-binding substances remains.

In the present study, we report the first description of the LPS-binding properties of PACs from cranberries, tea, and grapes. Focusing more closely on PACs from cranberries, we demonstrate the binding of LPS from multiple bacterial species with an apparent affinity for LPS that is comparable to that reported for polymyxin B.[14] The recognition of LPS by PACs appears to be mediated largely through interaction with the conserved lipid A moiety. We also demonstrate the ability of PACs to inhibit the interaction of LPS with cells expressing the full complement of LPS receptors. PACs inhibit LPS interaction with mammalian cells largely through abrogation of LPS interaction with TLR4/MD2, an activity that also mediates the inhibition of LPS-induced NF-κB activation. This is the first report of the LPS-binding activity of PACs, and we discuss our findings in the context of the potential utility of PACs for endotoxin purification and removal or the *in vivo* treatment of sepsis.

Results and Discussion

PACs from Multiple Sources Bind to LPS. PACs are naturally occurring plant-derived polymers composed chiefly of the

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Lipopolysaccharide (LPS), is the primary cause of sepsis, an inflammatory syndrome characterized by an overwhelming systemic response to bacterial infection.

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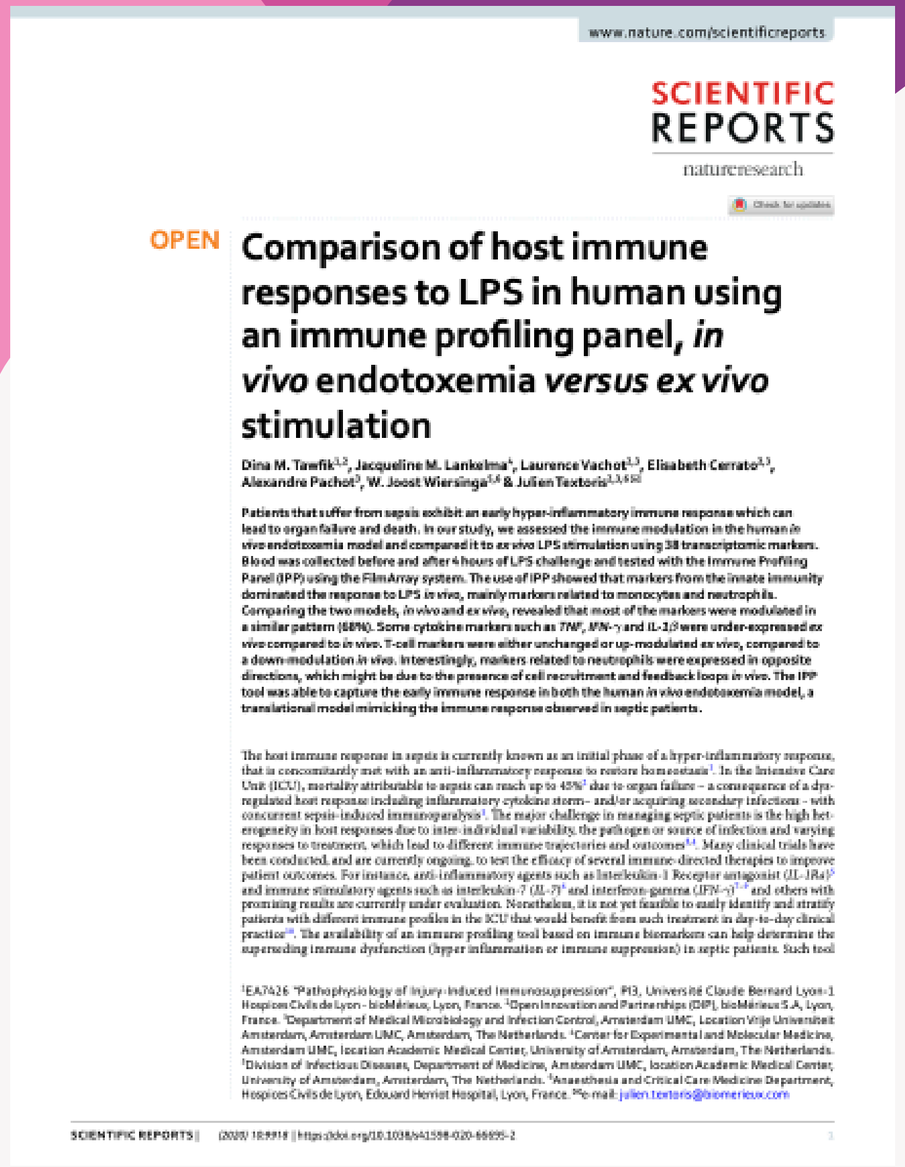

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Comparison of host immune responses to LPS in human using an immune profiling panel, *in vivo* endotoxemia *versus ex vivo* stimulation

Dina M. Tawfik[1,2], Jacqueline M. Lankelma[4], Laurence Vachot[1,3], Elisabeth Cerrato[3,5], Alexandre Pachot[3], W. Joost Wiersinga[4,6] & Julien Textoris[1,3,6]✉

Patients that suffer from sepsis exhibit an early hyper-inflammatory immune response which can lead to organ failure and death. In our study, we assessed the immune modulation in the human *in vivo* endotoxemia model and compared it to *ex vivo* LPS stimulation using 38 transcriptomic markers. Blood was collected before and after 4 hours of LPS challenge and tested with the Immune Profiling Panel (IPP) using the FilmArray system. The use of IPP showed that markers from the innate immunity dominated the response to LPS *in vivo*, mainly markers related to monocytes and neutrophils. Comparing the two models, *in vivo* and *ex vivo*, revealed that most of the markers were modulated in a similar pattern (88%). Some cytokine markers such as *TNF*, *IFN-γ* and *IL-2Rβ* were under-expressed *ex vivo* compared to *in vivo*. T-cell markers were either unchanged or up-modulated *ex vivo*, compared to a down-modulation *in vivo*. Interestingly, markers related to neutrophils were expressed in opposite directions, which might be due to the presence of cell recruitment and feedback loops *in vivo*. The IPP tool was able to capture the early immune response in both the human *in vivo* endotoxemia model, a translational model mimicking the immune response observed in septic patients.

The host immune response in sepsis is currently known as an initial phase of a hyper-inflammatory response, that is concomitantly met with an anti-inflammatory response to restore homeostasis[1]. In the Intensive Care Unit (ICU), mortality attributable to sepsis can reach up to 45%[2] due to organ failure – a consequence of a dysregulated host response including inflammatory cytokine storm– and/or acquiring secondary infections - with concurrent sepsis-induced immunoparalysis[3]. The major challenge in managing septic patients is the high heterogeneity in host responses due to inter-individual variability, the pathogen or source of infection and varying responses to treatment, which lead to different immune trajectories and outcomes[4,5]. Many clinical trials have been conducted, and are currently ongoing, to test the efficacy of several immune-directed therapies to improve patient outcomes. For instance, anti-inflammatory agents such as Interleukin-1 Receptor antagonist (*IL-1Ra*)[6] and immune stimulatory agents such as interleukin-7 (*IL-7*)[4] and interferon-gamma (*IFN-γ*)[7–9] and others with promising results are currently under evaluation. Nonetheless, it is not yet feasible to easily identify and stratify patients with different immune profiles in the ICU that would benefit from such treatment in day-to-day clinical practice[10]. The availability of an immune profiling tool based on immune biomarkers can help determine the superseding immune dysfunction (hyper-inflammation or immune suppression) in septic patients. Such tool

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More than 100 clinical trials were conducted on novel immunotherapies in sepsis, those trials mainly targeted the modulation of the systemic inflammatory response in patients with acute clinical manifestations. However, almost none of these trials have resulted in new treatments available in the market. Those clinical trials might have failed as all patients were treated in the same manner, and few patient stratification approaches were adopted

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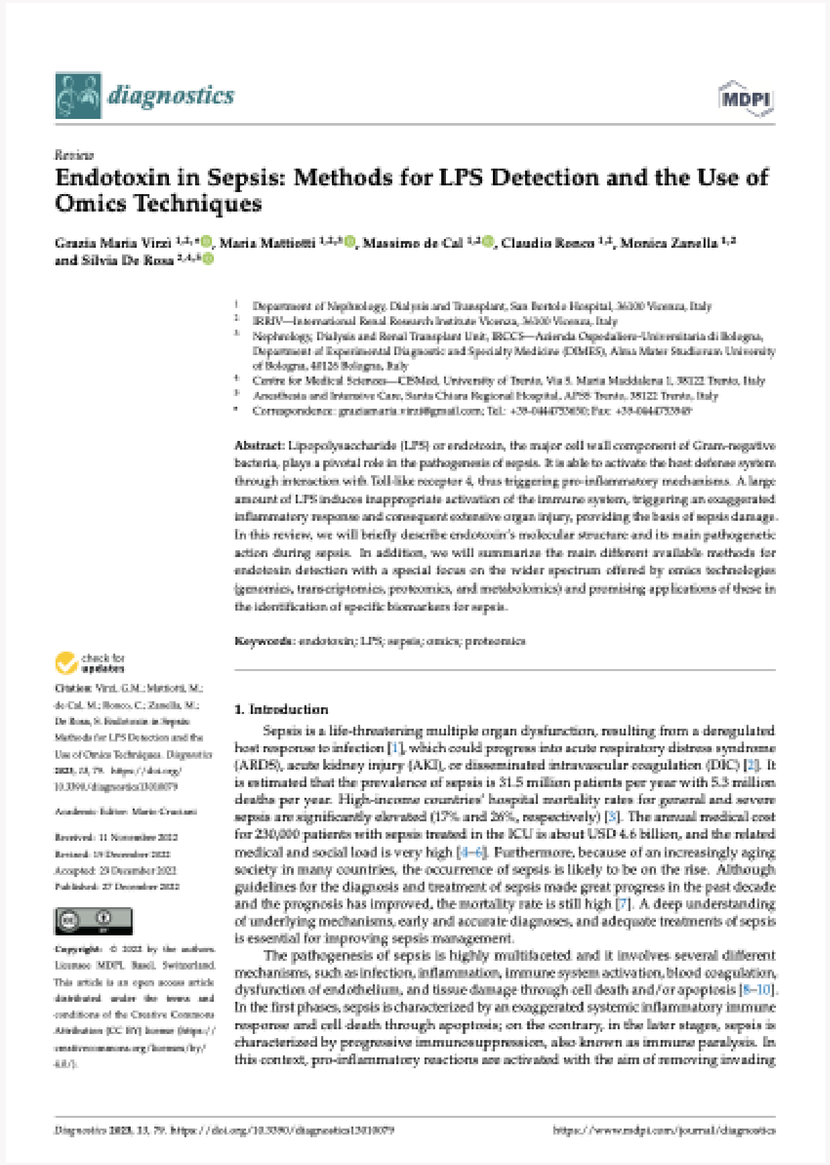

diagnostics

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Review

Endotoxin in Sepsis: Methods for LPS Detection and the Use of Omics Techniques

Grazia Maria Virzì [1,2,*], Maria Mattiotti [1,2,3], Massimo de Cal [1,2], Claudio Ronco [1,2], Monica Zanella [1,2] and Silvia De Rosa [2,4,5]

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Abstract: Lipopolysaccharide (LPS) or endotoxin, the major cell wall component of Gram-negative bacteria, plays a pivotal role in the pathogenesis of sepsis. It is able to activate the host defense system through interaction with Toll-like receptor 4, thus triggering pro-inflammatory mechanisms. A large amount of LPS induces inappropriate activation of the immune system, triggering an exaggerated inflammatory response and consequent extensive organ injury, providing the basis of sepsis damage. In this review, we will briefly describe endotoxin's molecular structure and its main pathogenetic action during sepsis. In addition, we will summarize the main different available methods for endotoxin detection with a special focus on the wider spectrum offered by omics technologies (genomics, transcriptomics, proteomics, and metabolomics) and promising applications of these in the identification of specific biomarkers for sepsis.

Keywords: endotoxin; LPS; sepsis; omics; proteomics

check for updates

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1. Introduction

Sepsis is a life-threatening multiple organ dysfunction, resulting from a deregulated host response to infection [1], which could progress into acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), or disseminated intravascular coagulation (DIC) [2]. It is estimated that the prevalence of sepsis is 31.5 million patients per year with 5.3 million deaths per year. High-income countries' hospital mortality rates for general and severe sepsis are significantly elevated (17% and 26%, respectively) [3]. The annual medical cost for 230,000 patients with sepsis treated in the ICU is about USD 4.6 billion, and the related medical and social load is very high [4–6]. Furthermore, because of an increasingly aging society in many countries, the occurrence of sepsis is likely to be on the rise. Although guidelines for the diagnosis and treatment of sepsis made great progress in the past decade and the prognosis has improved, the mortality rate is still high [7]. A deep understanding of underlying mechanisms, early and accurate diagnoses, and adequate treatments of sepsis is essential for improving sepsis management.

The pathogenesis of sepsis is highly multifaceted and it involves several different mechanisms, such as infection, inflammation, immune system activation, blood coagulation, dysfunction of endothelium, and tissue damage through cell death and/or apoptosis [8–10]. In the first phases, sepsis is characterized by an exaggerated systemic inflammatory immune response and cell death through apoptosis; on the contrary, in the later stages, sepsis is characterized by progressive immunosuppression, also known as immune paralysis. In this context, pro-inflammatory reactions are activated with the aim of removing invading

Diagnostics 2023, 13, 79.

Sepsis is a life-threatening multiple organ dysfunction, resulting from a deregulated host response to infection.

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Journal of *Clinical Medicine*

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Review

Lipid and Lipoprotein Dysregulation in Sepsis: Clinical and Mechanistic Insights into Chronic Critical Illness

Grant Barker [1], Christiaan Loeuwenburgh [2], Todd Brusko [3], Lyle Moldawer [4], Srinivasa T. Reddy [5] and Faheem W. Guirgis [1,*]

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Abstract: In addition to their well-characterized roles in metabolism, lipids and lipoproteins have pleiotropic effects on the innate immune system. These undergo clinically relevant alterations during sepsis and acute inflammatory responses. High-density lipoprotein (HDL) plays an important role in regulating the immune response by clearing bacterial toxins, supporting corticosteroid release, decreasing platelet aggregation, inhibiting endothelial cell apoptosis, reducing the monocyte inflammatory response, and inhibiting expression of endothelial cell adhesion molecules. It undergoes quantitative as well as qualitative changes which can be measured using the HDL inflammatory index (HII). Pro-inflammatory, or dysfunctional HDL (dysHDL) lacks the ability to perform these functions, and we have also found it to independently predict adverse outcomes and organ failure in sepsis. Another important class of lipids known as specialized pro-resolving mediators (SPMs) positively affect the escalation and resolution of inflammation in a temporal fashion. These undergo phenotypic changes in sepsis and differ significantly between survivors and non-survivors. Certain subsets of sepsis survivors go on to have perilous post-hospitalization courses where this inflammation continues in a low grade fashion. This is associated with immunosuppression in a syndrome of persistent inflammation, immunosuppression, and catabolism syndrome (PICS). The continuous release of tissue damage-related patterns and viral reactivation secondary to immunosuppression feed this chronic cycle of inflammation. Animal data indicate that dysregulation of endogenous lipids and SPMs play important roles in this process. Lipids and their associated pathways have been the target of many clinical trials in recent years which have not shown mortality benefit. These results are limited by patient heterogeneity and poor animal models. Considerations of sepsis phenotypes and novel biomarkers in future trials are important factors to be considered in future research. Further characterization of lipid dysregulation and chronic inflammation during sepsis will aid mortality risk stratification, detection of sepsis, and inform individualized pharmacologic therapies.

Keywords: sepsis; lipids; lipoproteins; chronic critical illness

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Citation: Barker, G.; Loeuwenburgh, C.; Brusko, T.; Moldawer, L.; Reddy, S.T.; Guirgis, F.W. Lipid and Lipoprotein Dysregulation in Sepsis: Clinical and Mechanistic Insights into Chronic Critical Illness. *J. Clin. Med.* **2021**, *10*, 1693. https://doi.org/10.3390/jcm10081693

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1. Introduction

1.1. Sepsis Overview

Deriving from the ancient Greek word 'sepo' meaning "I rot", the semantics of sepsis have proven nearly as complex as elucidating new treatments [1]. The current definition of sepsis (Sepsis-3) is a "life-threatening organ dysfunction caused by a dysregulated host

J. Clin. Med. 2021, 10, 1693.

The word sepsis is derived from the ancient Greek word ‘sepo’ meaning “I rot”

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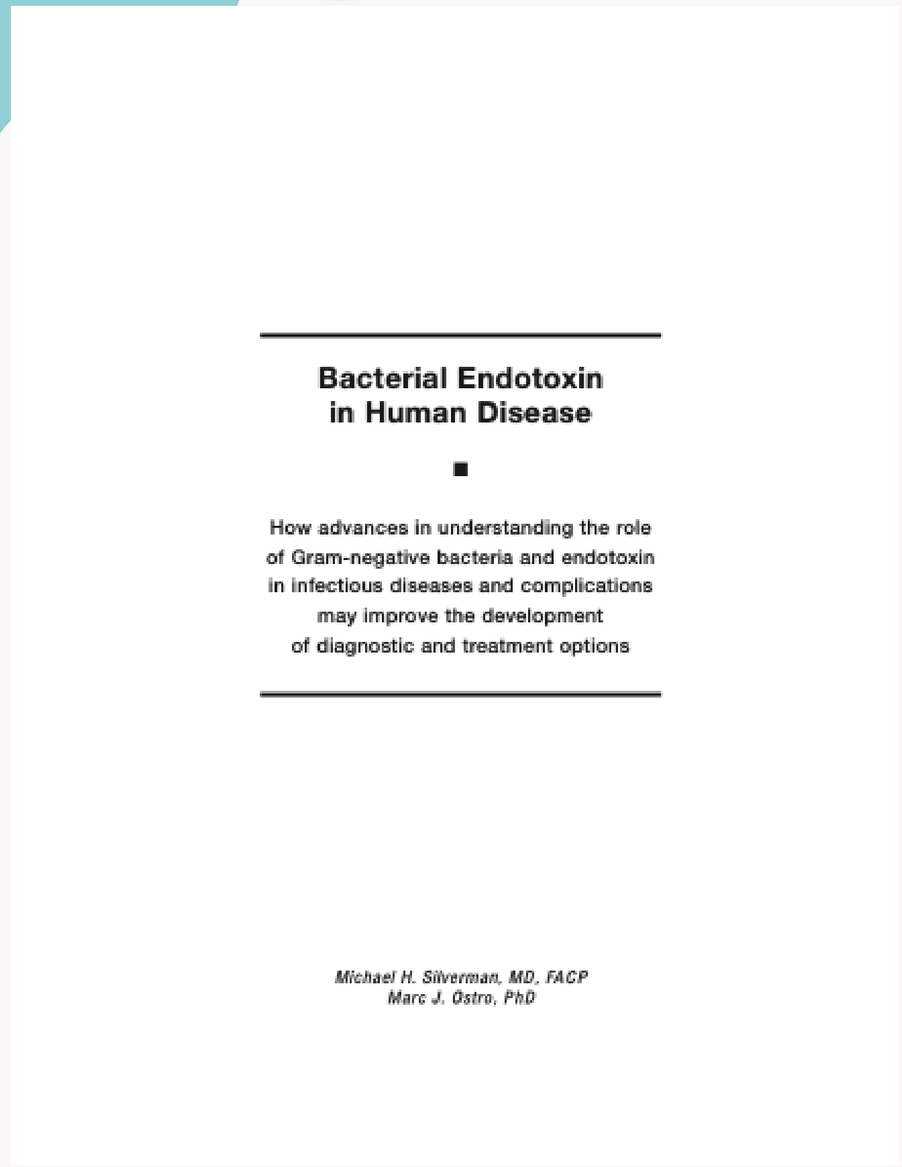

In the old model, sepsis was viewed as a unique clinical syndrome, difficult to treat, but the obvious target for therapy.

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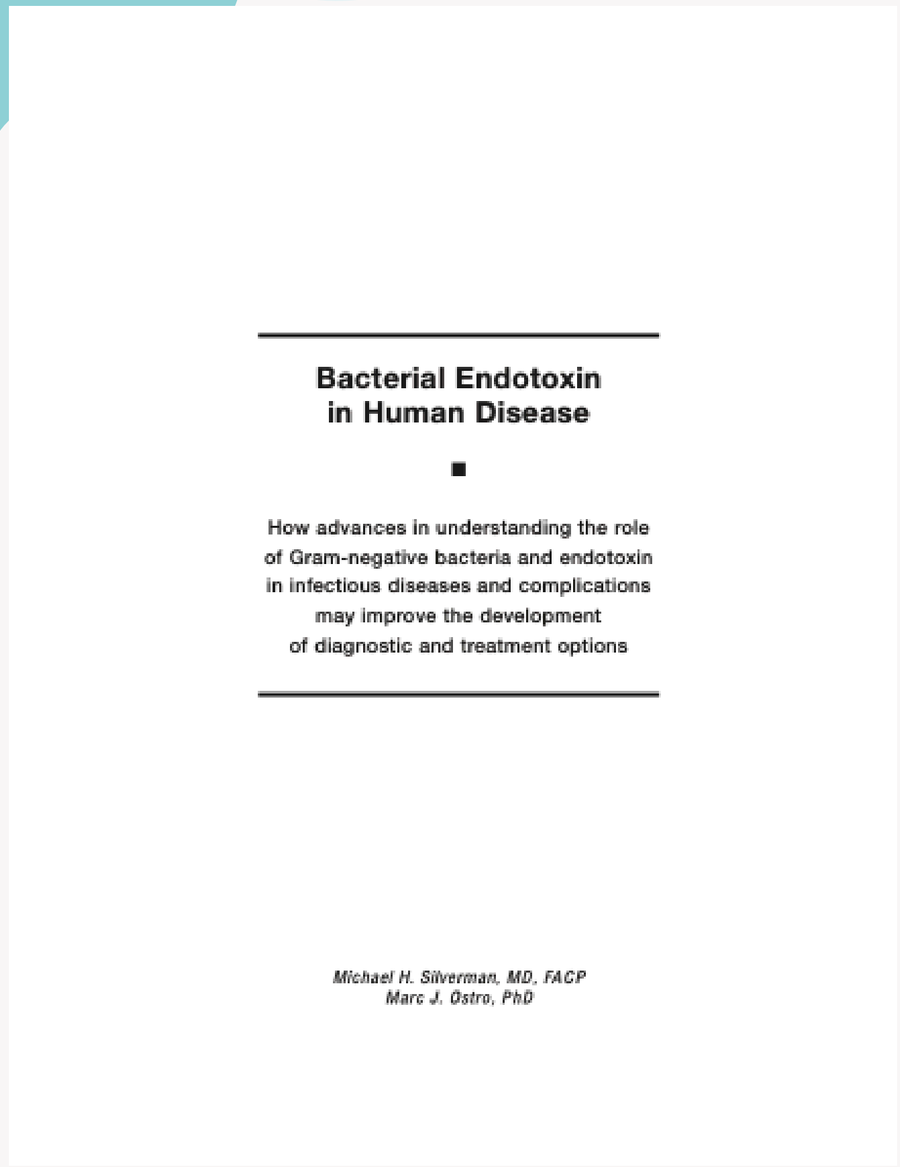


Bacterial Endotoxin in Human Disease

■

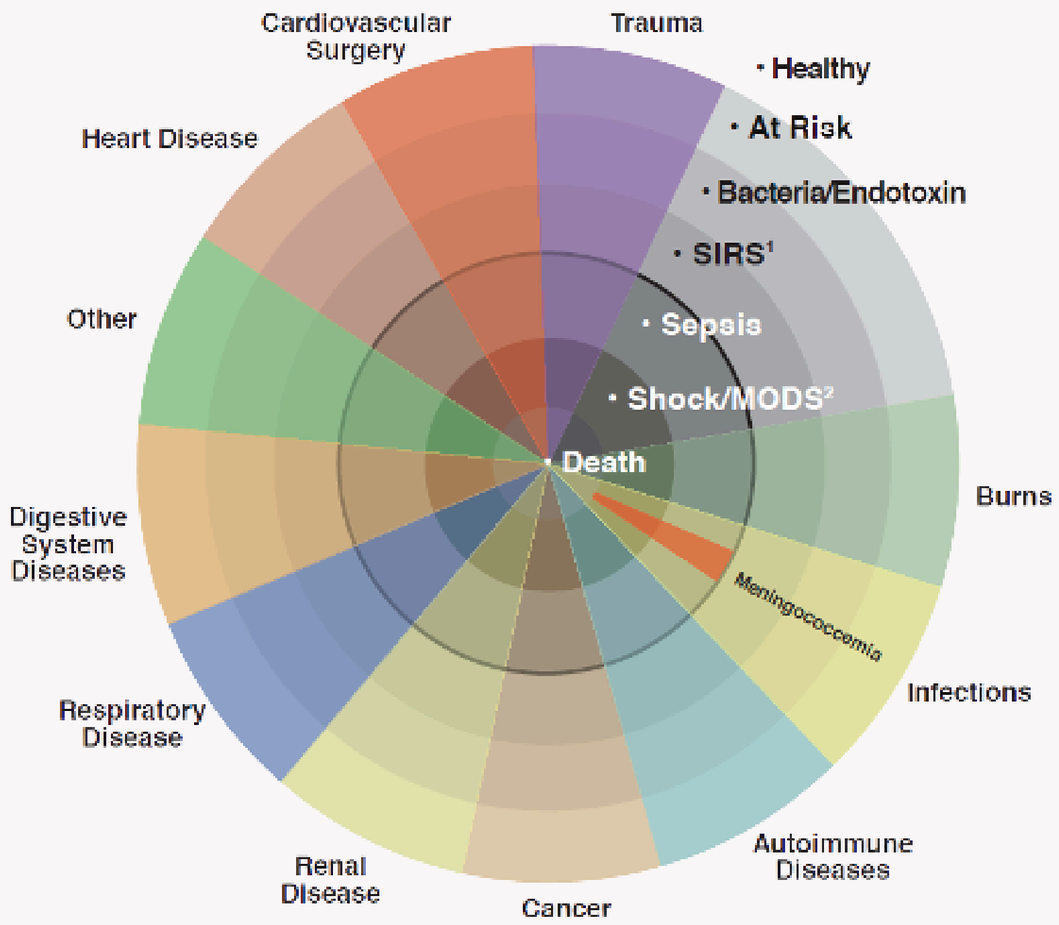
How advances in understanding the role of Gram-negative bacteria and endotoxin in infectious diseases and complications may improve the development of diagnostic and treatment options

Michael H. Silverman, MD, FACP
Marc J. Ostro, PhD

XOMA (US) LLC, Berkeley, Ca

The new model (see Figure 1) incorporates sepsis, but as a late-stage syndrome on a continuum of endotoxin-related diseases. The new map encompasses the entire inflammatory cascade and its clinical manifestations

Figure 1: A Model for Diseases Potentially Associated with Bacteria/Endotoxin



1 Systemic Inflammatory Response Syndrome

2 Multiple Organ Dysfunction Syndrome

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CLINICAL TAKEAWAY?

When LPS antibodies are elevated in a stool analysis, consider the Wheat Zoomer and the Autoimmune Zoomer

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Anti-Saccharomyces Cerevisiae Antibodies

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International Journal of *Molecular Sciences*

MDPI

Article

Correlation between Antibodies to Bacterial Lipopolysaccharides and Barrier Proteins in Sera Positive for ASCA and ANCA

Aristo Vojdani [1,2,*], Elroy Vojdani [3], Martha Herbert [4] and Datis Kharrazian [2,5,6]

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Abstract: Individuals with intestinal barrier dysfunction are more prone to autoimmunity. Lipopolysaccharides (LPS) from gut bacteria have been shown to play a role in systemic inflammation, leading to the opening of the gut and blood-brain barrier (BBB). This study aims to measure antibodies against LPS and barrier proteins in samples positive for anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA) and compare them with these same antibodies in controls to determine whether a correlation between LPS and barrier proteins could be found. We obtained 94 ASCA- and 94 ANCA-positive blood samples, as well as 188 blood samples from healthy controls. Samples were assessed for antibodies to LPS, zonulin-occludin, S100B, and aquaporin-4 (AQP4). Results show significant elevation in antibodies in about 30% of ASCA- and ANCA-positive sera and demonstrate positive linear relationships between these antibodies. The findings suggest that individuals positive for ASCA and ANCA have increased odds of developing intestinal and BBB permeability compared to healthy subjects. The levels of LPS antibodies in both ASCA- and ANCA-positive and negative specimens showed from low and moderate to high correlation with antibodies to barrier proteins. This study shows that LPS, by damaging the gut and BBBs, contribute to the extra-intestinal manifestation of IBD. We conclude that IBD patients should be screened for LPS antibodies in an effort to detect or prevent possible barrier damage at the earliest stage possible to abrogate disease symptoms in IBS and associated disorders.

Keywords: IBD; lipopolysaccharide; zonulin-occludin; aquaporin; S100B; BBB permeability

1. Introduction

Inflammatory bowel disease (IBD) is a heterogeneous group of chronic inflammatory disorders of the gastrointestinal (GI) tract that has two main distinguishable forms, Crohn's disease (CD) and ulcerative colitis (UC) [1]. According to the Centers for Disease Control and Prevention, CD can affect any part of the GI tract from the mouth to the anus, but it most often affects the portion of the small intestine before the large intestine/colon; UC, on the other hand, occurs in the large intestine and colon [2]. Another way to differentiate between CD and UC is that anti-*Saccharomyces cerevisiae* antibodies (ASCA) are associated with and used as biomarkers for CD, while anti-neutrophil cytoplasm antibodies (ANCA) are recognized as markers for UC [3].

Saccharomyces cerevisiae, also known as baker's or brewer's yeast, is the most commonly detected fungi in human fecal samples and likely originates from food. ASCA are antibodies against antigens presented by the cell wall of the yeast S. cerevisiae; they are widely recognized as test markers for Crohn's Disease.

Int. J. Mol. Sci. 2020, 21, 1381;

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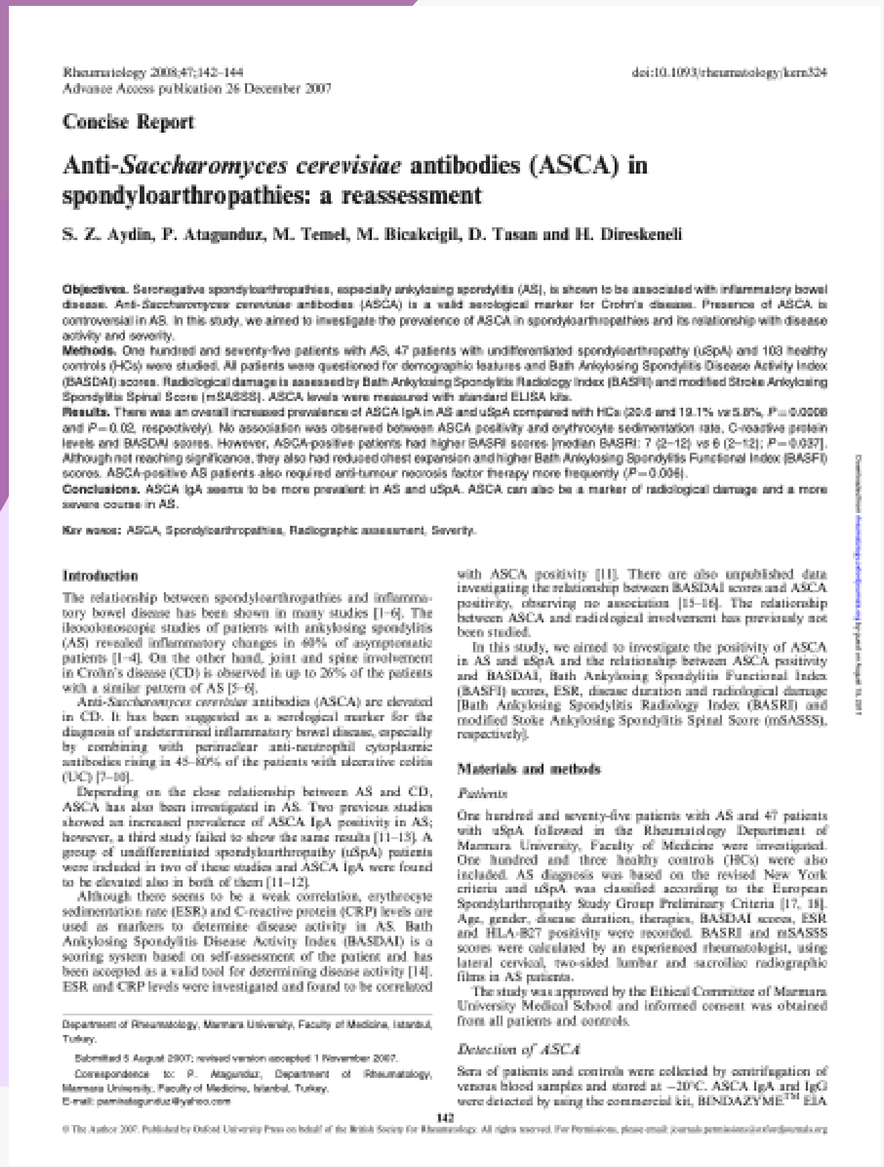

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Advance Access publication 26 December 2007

Concise Report

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) in spondyloarthropathies: a reassessment

S. Z. Aydin, P. Atagunduz, M. Temel, M. Bicakcigil, D. Tasan and H. Direskeneli

Objectives. Seronegative spondyloarthropathies, especially ankylosing spondylitis (AS), is shown to be associated with inflammatory bowel disease. Anti-*Saccharomyces cerevisiae* antibodies (ASCA) is a valid serological marker for Crohn's disease. Presence of ASCA is controversial in AS. In this study, we aimed to investigate the prevalence of ASCA in spondyloarthropathies and its relationship with disease activity and severity.

Methods. One hundred and seventy-five patients with AS, 47 patients with undifferentiated spondyloarthropathy (uSpA) and 103 healthy controls (HCs) were studied. All patients were questioned for demographic features and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) scores. Radiological damage is assessed by Bath Ankylosing Spondylitis Radiology Index (BASRI) and modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS). ASCA levels were measured with standard ELISA kits.

Results. There was an overall increased prevalence of ASCA IgA in AS and uSpA compared with HCs (20.6 and 19.1% *vs* 5.8%, $P = 0.0008$ and $P = 0.02$, respectively). No association was observed between ASCA positivity and erythrocyte sedimentation rate, C-reactive protein levels and BASDAI scores. However, ASCA-positive patients had higher BASRI scores [median BASRI: 7 (2–12) *vs* 6 (2–12); $P = 0.037$]. Although not reaching significance, they also had reduced chest expansion and higher Bath Ankylosing Spondylitis Functional Index (BASFI) scores. ASCA-positive AS patients also required anti-tumour necrosis factor therapy more frequently ($P = 0.006$).

Conclusions. ASCA IgA seems to be more prevalent in AS and uSpA. ASCA can also be a marker of radiological damage and a more severe course in AS.

Key words: ASCA, Spondyloarthropathies, Radiographic assessment, Severity.

Introduction

The relationship between spondyloarthropathies and inflammatory bowel disease has been shown in many studies [1–6]. The ileocolonoscopic studies of patients with ankylosing spondylitis (AS) revealed inflammatory changes in 60% of asymptomatic patients [1–4]. On the other hand, joint and spine involvement in Crohn's disease (CD) is observed in up to 26% of the patients with a similar pattern of AS [5–6].

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) are elevated in CD. It has been suggested as a serological marker for the diagnosis of undetermined inflammatory bowel disease, especially by combining with perinuclear anti-neutrophil cytoplasmic antibodies rising in 45–80% of the patients with ulcerative colitis (UC) [7–10].

Depending on the close relationship between AS and CD, ASCA has also been investigated in AS. Two previous studies showed an increased prevalence of ASCA IgA positivity in AS; however, a third study failed to show the same results [11–13]. A group of undifferentiated spondyloarthropathy (uSpA) patients were included in two of these studies and ASCA IgA were found to be elevated also in both of them [11–12].

Although there seems to be a weak correlation, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels are used as markers to determine disease activity in AS. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is a scoring system based on self-assessment of the patient and has been accepted as a valid tool for determining disease activity [14]. ESR and CRP levels were investigated and found to be correlated with ASCA positivity [11]. There are also unpublished data investigating the relationship between BASDAI scores and ASCA positivity, observing no association [15–16]. The relationship between ASCA and radiological involvement has previously not been studied.

In this study, we aimed to investigate the positivity of ASCA in AS and uSpA and the relationship between ASCA positivity and BASDAI, Bath Ankylosing Spondylitis Functional Index (BASFI) scores, ESR, disease duration and radiological damage [Bath Ankylosing Spondylitis Radiology Index (BASRI) and modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS), respectively].

Materials and methods

Patients

One hundred and seventy-five patients with AS and 47 patients with uSpA followed in the Rheumatology Department of Marmara University, Faculty of Medicine were investigated. One hundred and three healthy controls (HCs) were also included. AS diagnosis was based on the revised New York criteria and uSpA was classified according to the European Spondylarthropathy Study Group Preliminary Criteria [17, 18]. Age, gender, disease duration, therapies, BASDAI scores, ESR and HLA-B27 positivity were recorded. BASRI and mSASSS scores were calculated by an experienced rheumatologist, using lateral cervical, two-sided lumbar and sacroiliac radiographic films in AS patients.

The study was approved by the Ethical Committee of Marmara University Medical School and informed consent was obtained from all patients and controls.

Detection of ASCA

Sera of patients and controls were collected by centrifugation of venous blood samples and stored at −20°C. ASCA IgA and IgG were detected by using the commercial kit, BINDAZYME™ EIA

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There was an overall increased prevalence of ASCA IgA in Ankylosing Spondylitis and undifferentiated spondyloarthropathy compared with HCs (20.6 and 19.1% vs 5.8% P = 0.0008)

Rheumatology 2008;47;142–144

The Vibrant Longevity Summit

Prevalence and significance of anti-*saccharomyces cerevisiae* antibodies in primary Sjögren's syndrome

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Key words: Sjögren's syndrome, antibodies anti-Saccharomyces cerevisiae, anti-Ro52/SSA, anti-Ro60/SSB, Th17 cells

Competing interests: none declared.

ABSTRACT

Objective. *Saccharomyces cerevisiae is a common yeast used in the food industry. IgG and IgA antibodies against the phosphopeptidomannan of the S. cerevisiae cell wall (ASCA) are a well known marker of disease severity in Crohn's disease. Moreover, a number of studies assessed ASCA in several systemic and organ-specific autoimmune diseases postulating molecular mimicry as a possible link between ASCA and autoimmunity. However, since they have never been tested in primary Sjögren's syndrome (pSS), the purpose of this study was to investigate these antibodies in a large cohort of pSS patients, compared to healthy donors (HD), and their significance as potentially helpful biomarker in a clinical setting.*

Methods. *ASCA IgG+IgA were assessed with ASCA screen dot for Blue Diver instrument (Alphadia sa/nv, Belgium). The comparison between the aminoacid sequence of mannan of S. cerevisiae and well characterised auto-antigens peculiar to pSS (52kD and 60kD Ro/SSA, La/SSB) was performed with the Basic Local Alignment Search Tool (BLAST).*

Results. *The prevalence of ASCA in our pSS cohort was 4.8%. We also reported that the ASCA target protein has a high similarity with Ro60/SSA protein further supporting the molecular mimicry hypothesis. Finally, we observed that ASCA positivity is associated with pSS specific clinical and serological features. ASCA+ pSS patients displayed a triple combination of circulating anti-Ro52/SSA, anti-Ro60/SSA and anti-La/SSB antibodies, associated with low complement and cutaneous involvement.*

Conclusion. *Our data suggest a possible pathogenic/prognostic significance of ASCA in pSS.*

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ASCA IgG are a well established biomarker of Crohn's disease (CD), being detectable in 60–70% of patients. In particular, ASCA are more prevalent in adult-onset CD and appear to be linked to a more severe disease. However, their titre remains stable overtime independently of pharmacological treatment (3). ASCA IgG can also be found in patients with ulcerative colitis (UC) but with a lower prevalence, about 10–15% (4). Conversely, ASCA IgA display a higher specificity, but a lower sensitivity, for inflammatory bowel diseases (IBD).

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Our data suggest a possible pathogenic/prognostic significance of ASCA antibodies in primary Sjogren's Syndrome

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Prevalence and significance of anti-*saccharomyces cerevisiae* antibodies in primary Sjögren's syndrome

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A higher prevalence of ASCA has been also observed in:

- patients with rheumatoid arthritis,
- systemic lupus erythematosus (SLE),
- primary antiphospholipid syndrome,
- Behçet's disease,
- autoimmune thyroid diseases (ATDs),
- coeliac disease,
- autoimmune hepatitis,
- primary biliary cirrhosis,
- primary sclerosing cholangitis (PSC) and
- type 1 diabetes

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MDPI

Article

Correlation between Antibodies to Bacterial Lipopolysaccharides and Barrier Proteins in Sera Positive for ASCA and ANCA

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Abstract: Individuals with intestinal barrier dysfunction are more prone to autoimmunity. Lipopolysaccharides (LPS) from gut bacteria have been shown to play a role in systemic inflammation, leading to the opening of the gut and blood-brain barrier (BBB). This study aims to measure antibodies against LPS and barrier proteins in samples positive for anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA) and compare them with these same antibodies in controls to determine whether a correlation between LPS and barrier proteins could be found. We obtained 94 ASCA- and 94 ANCA-positive blood samples, as well as 188 blood samples from healthy controls. Samples were assessed for antibodies to LPS, zonulin+occludin, S100B, and aquaporin-4 (AQP4). Results show significant elevation in antibodies in about 30% of ASCA- and ANCA-positive sera and demonstrate positive linear relationships between these antibodies. The findings suggest that individuals positive for ASCA and ANCA have increased odds of developing intestinal and BBB permeability compared to healthy subjects. The levels of LPS antibodies in both ASCA- and ANCA-positive and negative specimens showed from low and moderate to high correlation with antibodies to barrier proteins. This study shows that LPS, by damaging the gut and BBBs, contribute to the extra-intestinal manifestation of IBD. We conclude that IBD patients should be screened for LPS antibodies in an effort to detect or prevent possible barrier damage at the earliest stage possible to abrogate disease symptoms in IBS and associated disorders.

Keywords: IBD; lipopolysaccharide; zonulin+occludin; aquaporin; S100B; BBB permeability

1. Introduction

Inflammatory bowel disease (IBD) is a heterogeneous group of chronic inflammatory disorders of the gastrointestinal (GI) tract that has two main distinguishable forms, Crohn's disease (CD) and ulcerative colitis (UC) [1]. According to the Centers for Disease Control and Prevention, CD can affect any part of the GI tract from the mouth to the anus, but it most often affects the portion of the small intestine before the large intestine/colon; UC, on the other hand, occurs in the large intestine and colon [2]. Another way to differentiate between CD and UC is that anti-*Saccharomyces cerevisiae* antibodies (ASCA) are associated with and used as biomarkers for CD, while anti-neutrophil cytoplasm antibodies (ANCA) are recognized as markers for UC [3].

Int. J. Mol. Sci. 2020, 21, 1381;

IgG, IgM and IgA positive ASCA samples showed significant <u>elevated levels of LPS antibodies</u>:

- 31% of positive IgG ASCA samples
- 33% of positive IgM ASCA samples
- 30% of positive IgA ASCA samples

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CLINICAL TAKEAWAY?

When ASCA antibodies are elevated in a stool analysis, consider the Wheat Zoomer and the Autoimmune Zoomer

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Tissue Transglutaminase Antibodies (stool)

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Coeliac disease

Cryptic genetic gluten intolerance revealed by intestinal antitransglutaminase antibodies and response to gluten-free diet

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ABSTRACT

Background and objective Antitransglutaminase (anti-TG2) antibodies are synthesised in the intestine and their presence seems predictive of future coeliac disease (CD). This study investigates whether mucosal antibodies represent an early stage of gluten intolerance even in the absence of intestinal damage and serum anti-TG2 antibodies.

Methods This study investigated 22 relatives of patients with CD genetically predisposed to gluten intolerance but negative for both serum anti-TG2 antibodies and intestinal abnormalities. Fifteen subjects were symptomatic and seven were asymptomatic. The presence of immunoglobulin A anti-TG2 antibodies in the intestine was studied by creating phage-antibody libraries against TG-2. The presence of intestinal anti-TG2 antibodies was compared with the serum concentration of the intestinal fatty acid-binding protein (I-FABP), a marker for early intestinal mucosal damage. The effects of a 12-month gluten-free diet on anti-TG2 antibody production and the subjects' clinical condition was monitored. Twelve subjects entered the study as controls.

Results The intestinal mucosa appeared normal in 18/22; 4 had a slight increase in intraepithelial lymphocytes. Mucosal anti-TG2 antibodies were isolated in 15/22 subjects (68%); in particular symptomatic subjects were positive in 13/15 cases and asymptomatic subjects in 2/7 cases ($p=0.01$). No mucosal antibodies were selected from the controls' biopsies. There was significant correlation between the presence of intestinal anti-TG2 antibodies and positive concentrations of I-FABP ($p=0.0003$). After a gluten-free diet, 19/22 subjects underwent a second intestinal biopsy, which showed that anti-TG2 antibodies had disappeared in 12/15 ($p=0.002$), while I-FABP decreased significantly ($p<0.0001$). The diet resolved both extraintestinal and intestinal symptoms.

Conclusions A new form of genetic-dependent gluten intolerance has been described in which none of the usual diagnostic markers is present. Symptoms and intestinal anti-TG2 antibodies respond to a gluten free-diet. The detection of intestinal anti-TG2 antibodies by the phage-antibody libraries has an important diagnostic and therapeutic impact for the subjects with gluten-dependent intestinal or extraintestinal symptoms.

Clinical trial number NCT00677495.

Significance of this study

What is already known about this subject?

- Immunoglobulin A antitranglutaminase antibodies are synthesised in the small bowel mucosa and seem predictive of future overt coeliac disease (CD).
- The heavy chain variable regions of these antibodies are primarily derived from the IGHV5-51 gene from the VH5 antibody variable gene family. No particular light chain is preferred.
- Early studies demonstrated that the presence of both serum and intestinal mucosa antitransglutaminase antibodies are predictive of CD even in the absence of intestinal damage.

What are the new findings?

- Relatives of patients with CD genetically predisposed to gluten intolerance produce IGVH5-51-dependent antitranglutaminase antibodies at the intestinal level in the absence of serum antitransglutaminase antibodies and intestinal damage.
- The presence of mucosal antitranglutaminase antibodies is significantly related to the presence of gluten-dependent symptoms and to serum levels of intestinal fatty acid-binding protein, a marker of early enterocyte damage.
- Many of these subjects had both extraintestinal (eg, anaemia, elbow arthritis, pancytopenia) and intestinal (eg, explosive diarrhoea, severe constipation) symptoms that were resolved on a gluten-free diet.

How might it impact on clinical practice in the foreseeable future?

- The physician should be informed that cryptic genetic gluten intolerance may manifest itself without intestinal damage or serum antitransglutaminase antibodies and that these subjects can be diagnosed by measuring the levels of antitransglutaminase antibodies in the intestinal mucosa. The symptomatic subjects diagnosed by the presence of these mucosal antibodies will benefit from gluten-free diets, which should resolve their intestinal and extraintestinal symptoms.

Immunoglobulin A anti-tranglutaminase antibodies are synthesised in the small bowel mucosa and seem predictive of future overt coeliac disease (CD)

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The Vibrant Longevity Summit

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ORIGINAL ARTICLE

Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: A prospective and randomized clinical study

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Abstract

Objective. In coeliac disease, autoantibodies directed against transglutaminase 2 are produced in small-bowel mucosa, and they have been found to be deposited extracellularly. The aim of this study was to investigate whether such mucosal IgA deposits are important in the diagnostic work-up of early-stage coeliac disease without small-bowel mucosal villous atrophy. ***Material and methods***. Forty-one adults suspected of coeliac disease owing to increased density of mucosal γδ+ intraepithelial lymphocytes but normal villous morphology were randomized to gluten challenge or a gluten-free diet for 6 months. Clinically and histologically verified gluten dependency was compared with existence of small-bowel mucosal transglutaminase 2-specific extracellular IgA deposits and (coeliac disease-type) HLA DQ2 and DQ8; 34 non-coeliac subjects and 18 patients with classical coeliac disease served as controls. ***Results***. Of the 41 patients, 5 in the challenge group and 6 in the gluten-free diet group were clinically gluten sensitive; all 11 had HLA DQ2 or DQ8. Ten of these 11 patients showed transglutaminase 2-targeted mucosal IgA deposits, which were dependent on gluten consumption. Minimal IgA deposits were seen in only 3 out of 30 patients with suspected coeliac disease without any clinically detected gluten dependency. The deposits were found in all classical coeliac patients and in none of the non-coeliac control subjects. ***Conclusions***. Clinically pertinent coeliac disease exists despite normal small-bowel mucosal villous architecture. Mucosal transglutaminase 2-specific IgA deposits can be utilized in detecting such patients with genetic gluten intolerance.

Key Words: *Coeliac disease, IgA-deposit, intraepitehlial lymphocytes, latency, transglutaminase antibodies*

Introduction

The current diagnostic criteria for coeliac disease require small-bowel mucosal villous atrophy that recovers on a gluten-free diet [1]. Clearly, the spectrum of the disease is wider: the mucosal damage develops gradually from inflammation to crypt hyperplasia and finally to villous atrophy [2]. The mucosal inflammation is unspecific, and can be found in a variety of disorders [3,4]; therefore, it is difficult to tell whether minor mucosal changes are due to early development of coeliac disease. Increased density of γδ T-cell-receptor-bearing intraepithelial lymphocytes (IELs) is considered to be typical for coeliac disease [5]. These cells have been found in the early stage of the disease, even before the development of villous atrophy [6–8], but unfortunately also in conditions other than coeliac disease [9]. In some patients evincing normal small-bowel mucosal villous morphology positive serum endomysial (EmA) [10–12] or jejunal fluid coeliac disease-associated antibodies (IgA- and IgM-class gliadin and tissue transglutaminase antibodies) [13,14] have predicted impending coeliac disease. Nonetheless, the concept of early coeliac disease is poorly understood.

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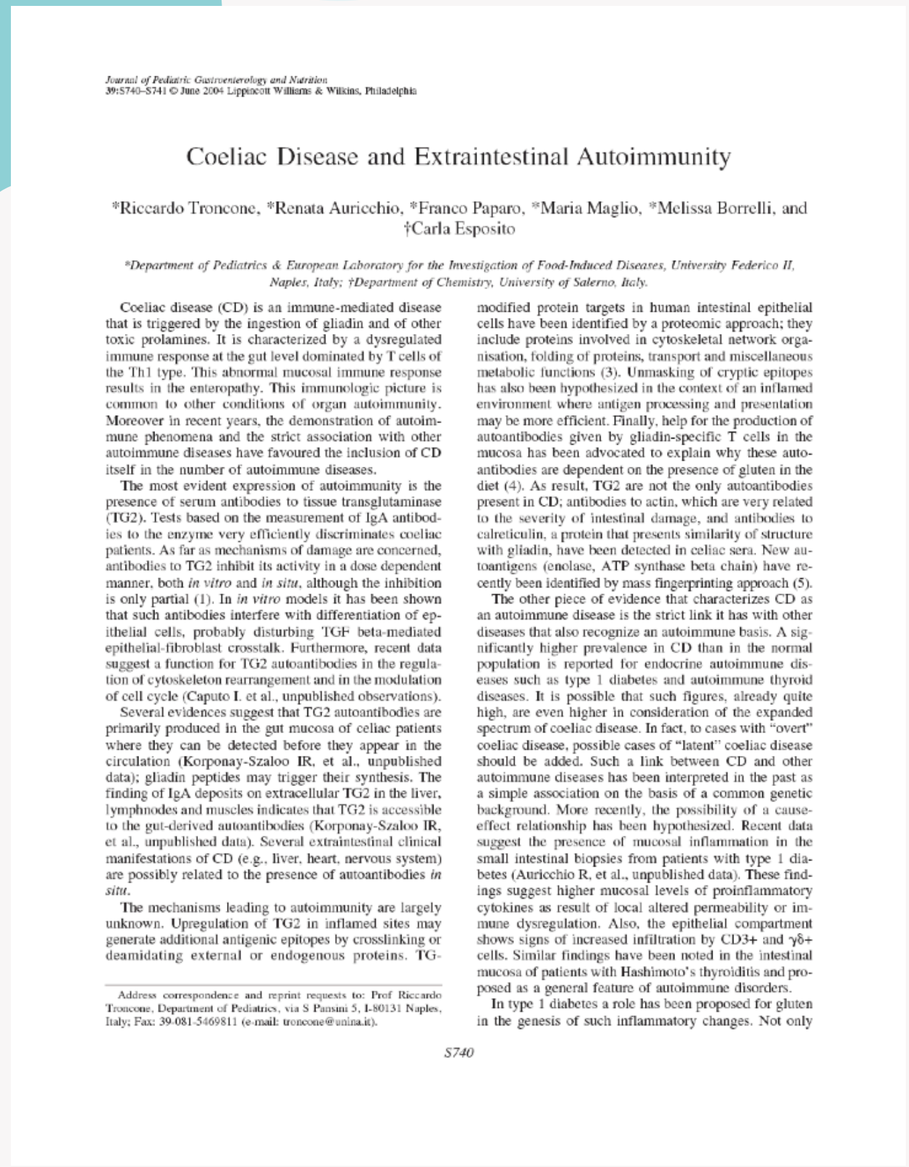
Coeliac disease autoantibodies against tissue transglutaminase (TG2) are produced in the intestinal mucosa and the antibodies can deposit on extracellular TG2 in the small-bowel mucosa <u>even when not measurable in serum</u>

Scandinavian Journal of Gastroenterology, 2005; 40: 564/572

The Vibrant Longevity Summit



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Coeliac Disease and Extraintestinal Autoimmunity

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Coeliac disease (CD) is an immune-mediated disease that is triggered by the ingestion of gliadin and of other toxic prolamines. It is characterized by a dysregulated immune response at the gut level dominated by T cells of the Th1 type. This abnormal mucosal immune response results in the enteropathy. This immunologic picture is common to other conditions of organ autoimmunity. Moreover in recent years, the demonstration of autoimmune phenomena and the strict association with other autoimmune diseases have favoured the inclusion of CD itself in the number of autoimmune diseases.

The most evident expression of autoimmunity is the presence of serum antibodies to tissue transglutaminase (TG2). Tests based on the measurement of IgA antibodies to the enzyme very efficiently discriminates coeliac patients. As far as mechanisms of damage are concerned, antibodies to TG2 inhibit its activity in a dose dependent manner, both *in vitro* and *in situ*, although the inhibition is only partial (1). In *in vitro* models it has been shown that such antibodies interfere with differentiation of epithelial cells, probably disturbing TGF beta-mediated epithelial-fibroblast crosstalk. Furthermore, recent data suggest a function for TG2 autoantibodies in the regulation of cytoskeleton rearrangement and in the modulation of cell cycle (Caputo I. et al., unpublished observations).

Several evidences suggest that TG2 autoantibodies are primarily produced in the gut mucosa of celiac patients where they can be detected before they appear in the circulation (Korponay-Szaloo IR, et al., unpublished data); gliadin peptides may trigger their synthesis. The finding of IgA deposits on extracellular TG2 in the liver, lymphnodes and muscles indicates that TG2 is accessible to the gut-derived autoantibodies (Korponay-Szaloo IR, et al., unpublished data). Several extraintestinal clinical manifestations of CD (e.g., liver, heart, nervous system) are possibly related to the presence of autoantibodies *in situ*.

The mechanisms leading to autoimmunity are largely unknown. Upregulation of TG2 in inflamed sites may generate additional antigenic epitopes by crosslinking or deamidating external or endogenous proteins. TG-modified protein targets in human intestinal epithelial cells have been identified by a proteomic approach; they include proteins involved in cytoskeletal network organisation, folding of proteins, transport and miscellaneous metabolic functions (3). Unmasking of cryptic epitopes has also been hypothesized in the context of an inflamed environment where antigen processing and presentation may be more efficient. Finally, help for the production of autoantibodies given by gliadin-specific T cells in the mucosa has been advocated to explain why these autoantibodies are dependent on the presence of gluten in the diet (4). As result, TG2 are not the only autoantibodies present in CD; antibodies to actin, which are very related to the severity of intestinal damage, and antibodies to calreticulin, a protein that presents similarity of structure with gliadin, have been detected in celiac sera. New autoantigens (enolase, ATP synthase beta chain) have recently been identified by mass fingerprinting approach (5).

The other piece of evidence that characterizes CD as an autoimmune disease is the strict link it has with other diseases that also recognize an autoimmune basis. A significantly higher prevalence in CD than in the normal population is reported for endocrine autoimmune diseases such as type 1 diabetes and autoimmune thyroid diseases. It is possible that such figures, already quite high, are even higher in consideration of the expanded spectrum of coeliac disease. In fact, to cases with "overt" coeliac disease, possible cases of "latent" coeliac disease should be added. Such a link between CD and other autoimmune diseases has been interpreted in the past as a simple association on the basis of a common genetic background. More recently, the possibility of a cause-effect relationship has been hypothesized. Recent data suggest the presence of mucosal inflammation in the small intestinal biopsies from patients with type 1 diabetes (Auricchio R, et al., unpublished data). These findings suggest higher mucosal levels of proinflammatory cytokines as result of local altered permeability or immune dysregulation. Also, the epithelial compartment shows signs of increased infiltration by CD3+ and γδ+ cells. Similar findings have been noted in the intestinal mucosa of patients with Hashimoto's thyroiditis and proposed as a general feature of autoimmune disorders.

In type 1 diabetes a role has been proposed for gluten in the genesis of such inflammatory changes. Not only

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TG2 autoantibodies are primarily produced in the gut mucosa of celiac patients where they can be detected before they appear in the circulation

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The Vibrant Longevity Summit

Cryptic genetic gluten intolerance revealed by intestinal antitransglutaminase antibodies and response to gluten-free diet

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ABSTRACT

Background and objective Antitransglutaminase (anti-TG2) antibodies are synthesised in the intestine and their presence seems predictive of future coeliac disease (CD). This study investigates whether mucosal antibodies represent an early stage of gluten intolerance even in the absence of intestinal damage and serum anti-TG2 antibodies.

Methods This study investigated 22 relatives of patients with CD genetically predisposed to gluten intolerance but negative for both serum anti-TG2 antibodies and intestinal abnormalities. Fifteen subjects were symptomatic and seven were asymptomatic. The presence of immunoglobulin A anti-TG2 antibodies in the intestine was studied by creating phage-antibody libraries against TG-2. The presence of intestinal anti-TG2 antibodies was compared with the serum concentration of the intestinal fatty acid-binding protein (I-FABP), a marker for early intestinal mucosal damage. The effects of a 12-month gluten-free diet on anti-TG2 antibody production and the subjects' clinical condition was monitored. Twelve subjects entered the study as controls.

Results The intestinal mucosa appeared normal in 18/22; 4 had a slight increase in intraepithelial lymphocytes. Mucosal anti-TG2 antibodies were isolated in 15/22 subjects (68%); in particular symptomatic subjects were positive in 13/15 cases and asymptomatic subjects in 2/7 cases ($p=0.01$). No mucosal antibodies were selected from the controls' biopsies. There was significant correlation between the presence of intestinal anti-TG2 antibodies and positive concentrations of I-FABP ($p=0.0008$). After a gluten-free diet, 19/22 subjects underwent a second intestinal biopsy, which showed that anti-TG2 antibodies had disappeared in 12/15 ($p=0.002$), while I-FABP decreased significantly ($p<0.0001$). The diet resolved both extraintestinal and intestinal symptoms.

Conclusions A new form of genetic-dependent gluten intolerance has been described in which none of the usual diagnostic markers is present. Symptoms and intestinal anti-TG2 antibodies respond to a gluten free-diet. The detection of intestinal anti-TG2 antibodies by the phage-antibody libraries has an important diagnostic and therapeutic impact for the subjects with gluten-dependent intestinal or extraintestinal symptoms.

Clinical trial number NCT00877695.

Significance of this study

What is already known about this subject?
- Immunoglobulin A antitransglutaminase antibodies are synthesised in the small bowel mucosa and seem predictive of future overt coeliac disease (CD).
- The heavy chain variable regions of these antibodies are primarily derived from the IGHV5-51 gene from the VH5 antibody variable gene family. No particular light chain is preferred.
- Early studies demonstrated that the presence of both serum and intestinal mucosa antitransglutaminase antibodies are predictive of CD even in the absence of intestinal damage.

What are the new findings?
- Relatives of patients with CD genetically predisposed to gluten intolerance produce IGHV5-51-dependent antitransglutaminase antibodies at the intestinal level in the absence of serum antitransglutaminase antibodies and intestinal damage.
- The presence of mucosal antitransglutaminase antibodies is significantly related to the presence of gluten-dependent symptoms and to serum levels of intestinal fatty acid-binding protein, a marker of early enterocyte damage.
- Many of these subjects had both extraintestinal (eg, anaemia, elbow arthritis, pancytopenia) and intestinal (eg, explosive diarrhoea, severe constipation) symptoms that were resolved on a gluten-free diet.

How might it impact on clinical practice in the foreseeable future?
- The physician should be informed that cryptic genetic gluten intolerance may manifest itself without intestinal damage or serum antitransglutaminase antibodies and that these subjects can be diagnosed by measuring the levels of antitransglutaminase antibodies in the intestinal mucosa. The symptomatic subjects diagnosed by the presence of these mucosal antibodies will benefit from gluten-free diets, which should resolve their intestinal and extraintestinal symptoms.

Gut 2011;60:1487e1493

IgA anti-TG2 antibodies, negative in the blood, were studied in the intestine of 22 relatives of patients with CD genetically predisposed to gluten intolerance but negative for both serum anti-TG2 antibodies and intestinal abnormalities.

- Mucosal anti-TG2 antibodies were isolated in 15/22 subjects (68%)
- in particular symptomatic subjects were positive in 13/15 (87%)
- asymptomatic subjects were positive in 2/7 cases

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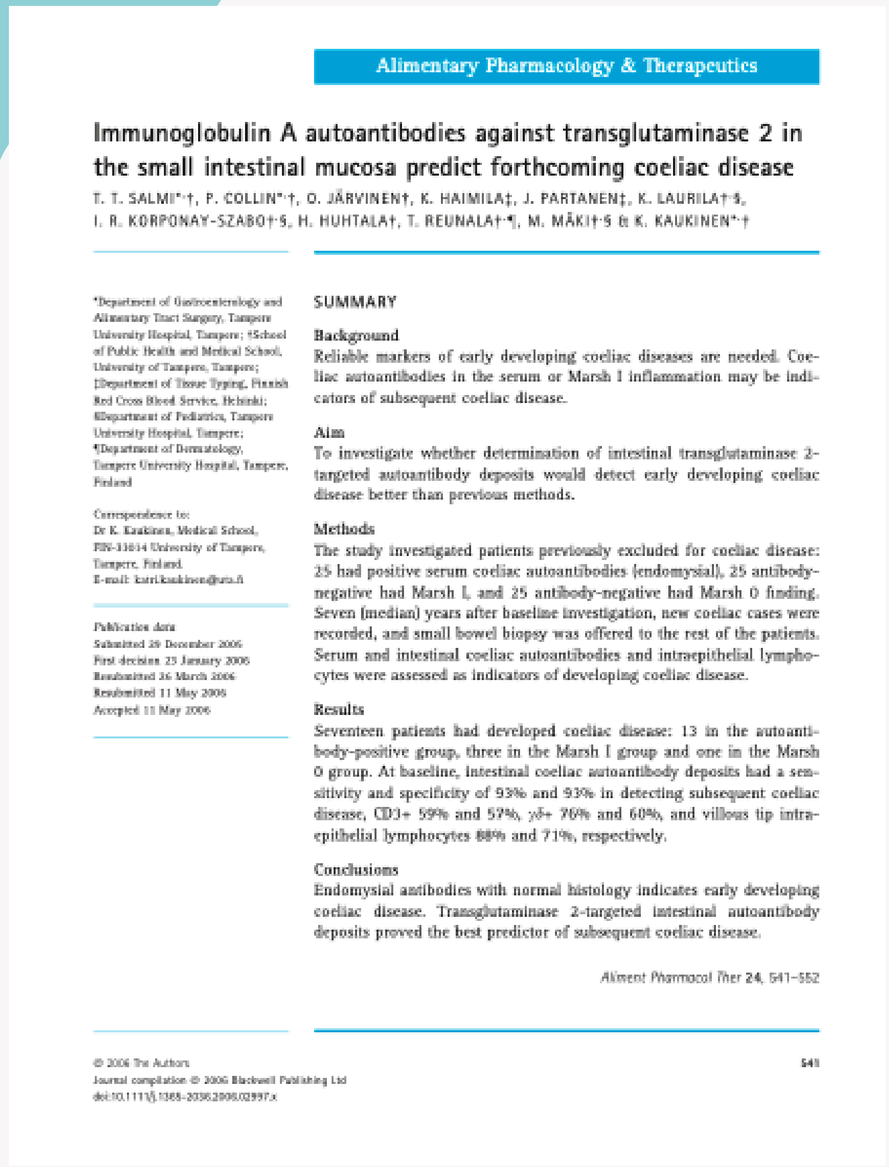

Alimentary Pharmacology & Therapeutics

Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease

T. T. SALMI*·†, P. COLLIN*·†, O. JÄRVINEN†, K. HAIMILA‡, J. PARTANEN‡, K. LAURILA†·§, I. R. KORPONAY-SZABO†·§, H. HUHTALA†, T. REUNALA†·¶, M. MÄKI†·§ & K. KAUKINEN*·†

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SUMMARY

Background
Reliable markers of early developing coeliac diseases are needed. Coeliac autoantibodies in the serum or Marsh I inflammation may be indicators of subsequent coeliac disease.

Aim
To investigate whether determination of intestinal transglutaminase 2-targeted autoantibody deposits would detect early developing coeliac disease better than previous methods.

Methods
The study investigated patients previously excluded for coeliac disease: 25 had positive serum coeliac autoantibodies (endomysial), 25 antibody-negative had Marsh I, and 25 antibody-negative had Marsh 0 finding. Seven (median) years after baseline investigation, new coeliac cases were recorded, and small bowel biopsy was offered to the rest of the patients. Serum and intestinal coeliac autoantibodies and intraepithelial lymphocytes were assessed as indicators of developing coeliac disease.

Results
Seventeen patients had developed coeliac disease: 13 in the autoantibody-positive group, three in the Marsh I group and one in the Marsh 0 group. At baseline, intestinal coeliac autoantibody deposits had a sensitivity and specificity of 93% and 93% in detecting subsequent coeliac disease, CD3+ 59% and 57%, γδ+ 76% and 60%, and villous tip intraepithelial lymphocytes 88% and 71%, respectively.

Conclusions
Endomysial antibodies with normal histology indicates early developing coeliac disease. Transglutaminase 2-targeted intestinal autoantibody deposits proved the best predictor of subsequent coeliac disease.

doi:10.1111/j.1365-2036.2006.02997.x

When these TG2-targeted autoantibodies were examined where they are produced, in the small bowel mucosa, 93% of all patients with early developing coeliac disease were identified in the absence of villous atrophy 7 years in advance of diagnosis.

Aliment Pharmacol Ther 24, 541–552

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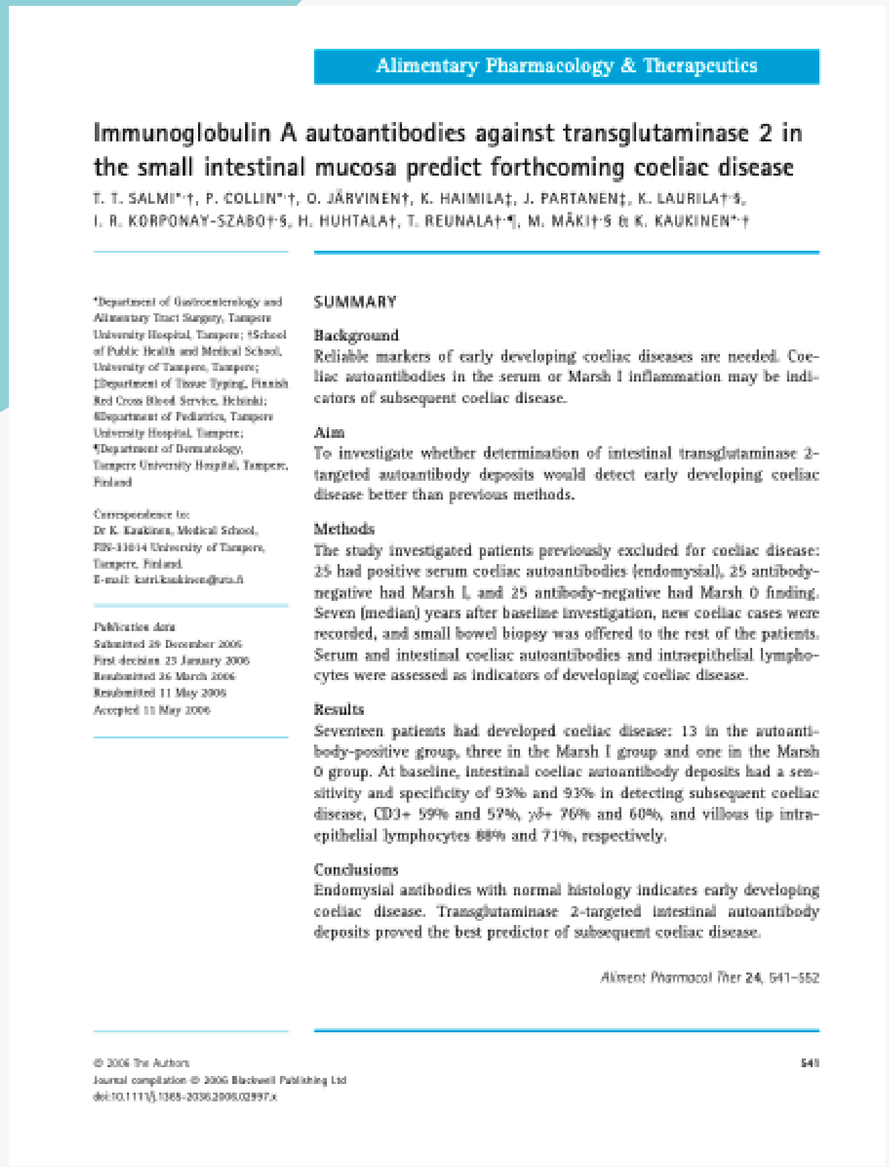

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Aliment Pharmacol Ther 24, 541–552

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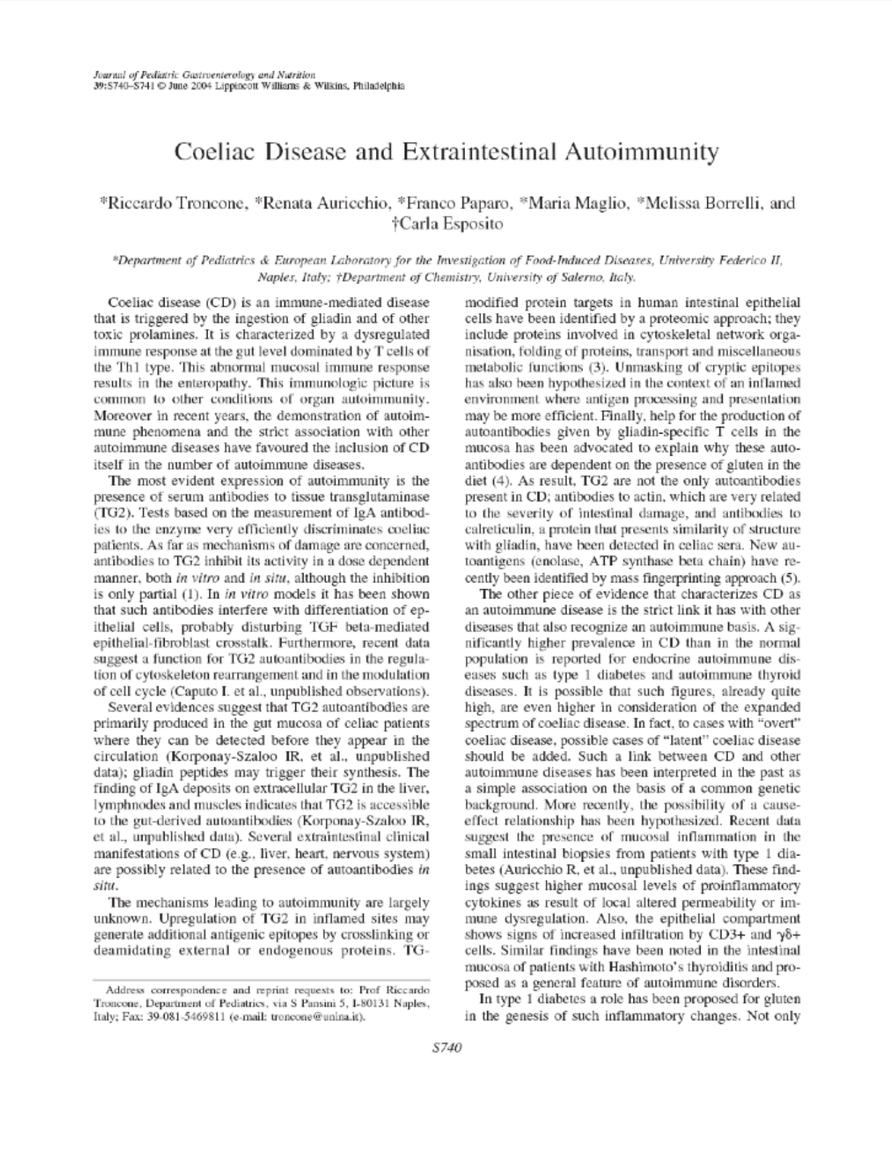
Aliment Pharmacol Ther 24, 2006 541–552

We consider that intestinal IgA deposits targeted against TG2 are currently the best method in revealing early developing coeliac disease. (identified median 7.1 years before diagnosis)



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Coeliac Disease and Extraintestinal Autoimmunity

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*Department of Pediatrics & European Laboratory for the Investigation of Food-Induced Diseases, University Federico II, Naples, Italy; †Department of Chemistry, University of Salerno, Italy.

Coeliac disease (CD) is an immune-mediated disease that is triggered by the ingestion of gliadin and of other toxic prolamines. It is characterized by a dysregulated immune response at the gut level dominated by T cells of the Th1 type. This abnormal mucosal immune response results in the enteropathy. This immunologic picture is common to other conditions of organ autoimmunity. Moreover in recent years, the demonstration of autoimmune phenomena and the strict association with other autoimmune diseases have favoured the inclusion of CD itself in the number of autoimmune diseases.

The most evident expression of autoimmunity is the presence of serum antibodies to tissue transglutaminase (TG2). Tests based on the measurement of IgA antibodies to the enzyme very efficiently discriminates coeliac patients. As far as mechanisms of damage are concerned, antibodies to TG2 inhibit its activity in a dose dependent manner, both *in vitro* and *in situ*, although the inhibition is only partial (1). In *in vitro* models it has been shown that such antibodies interfere with differentiation of epithelial cells, probably disturbing TGF beta-mediated epithelial-fibroblast crosstalk. Furthermore, recent data suggest a function for TG2 autoantibodies in the regulation of cytoskeleton rearrangement and in the modulation of cell cycle (Caputo I. et al., unpublished observations).

Several evidences suggest that TG2 autoantibodies are primarily produced in the gut mucosa of celiac patients where they can be detected before they appear in the circulation (Korponay-Szaloo IR, et al., unpublished data); gliadin peptides may trigger their synthesis. The finding of IgA deposits on extracellular TG2 in the liver, lymphnodes and muscles indicates that TG2 is accessible to the gut-derived autoantibodies (Korponay-Szaloo IR, et al., unpublished data). Several extraintestinal clinical manifestations of CD (e.g., liver, heart, nervous system) are possibly related to the presence of autoantibodies *in situ*.

The mechanisms leading to autoimmunity are largely unknown. Upregulation of TG2 in inflamed sites may generate additional antigenic epitopes by crosslinking or deamidating external or endogenous proteins. TG-modified protein targets in human intestinal epithelial cells have been identified by a proteomic approach; they include proteins involved in cytoskeletal network organisation, folding of proteins, transport and miscellaneous metabolic functions (3). Unmasking of cryptic epitopes has also been hypothesized in the context of an inflamed environment where antigen processing and presentation may be more efficient. Finally, help for the production of autoantibodies given by gliadin-specific T cells in the mucosa has been advocated to explain why these autoantibodies are dependent on the presence of gluten in the diet (4). As result, TG2 are not the only autoantibodies present in CD; antibodies to actin, which are very related to the severity of intestinal damage, and antibodies to calreticulin, a protein that presents similarity of structure with gliadin, have been detected in celiac sera. New autoantigens (enolase, ATP synthase beta chain) have recently been identified by mass fingerprinting approach (5).

The other piece of evidence that characterizes CD as an autoimmune disease is the strict link it has with other diseases that also recognize an autoimmune basis. A significantly higher prevalence in CD than in the normal population is reported for endocrine autoimmune diseases such as type 1 diabetes and autoimmune thyroid diseases. It is possible that such figures, already quite high, are even higher in consideration of the expanded spectrum of coeliac disease. In fact, to cases with "overt" coeliac disease, possible cases of "latent" coeliac disease should be added. Such a link between CD and other autoimmune diseases has been interpreted in the past as a simple association on the basis of a common genetic background. More recently, the possibility of a cause-effect relationship has been hypothesized. Recent data suggest the presence of mucosal inflammation in the small intestinal biopsies from patients with type 1 diabetes (Auricchio R, et al., unpublished data). These findings suggest higher mucosal levels of proinflammatory cytokines as result of local altered permeability or immune dysregulation. Also, the epithelial compartment shows signs of increased infiltration by CD3+ and γδ+ cells. Similar findings have been noted in the intestinal mucosa of patients with Hashimoto's thyroiditis and proposed as a general feature of autoimmune disorders.

In type 1 diabetes a role has been proposed for gluten in the genesis of such inflammatory changes. Not only

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The finding of IgA TG2 deposits on extracellular TG2 in the liver, lymph nodes and muscles indicates that TG2 (in other tissues) is accessible to the gut-derived autoantibodies.

Jour of Ped Gastro Nut 39:S740–S741,2004

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CLINICAL TAKEAWAY?

When zonulin antibodies are elevated in a stool analysis, consider the Wheat Zoomer and the Autoimmune Zoomer

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Anti-Gliadin Antibodies

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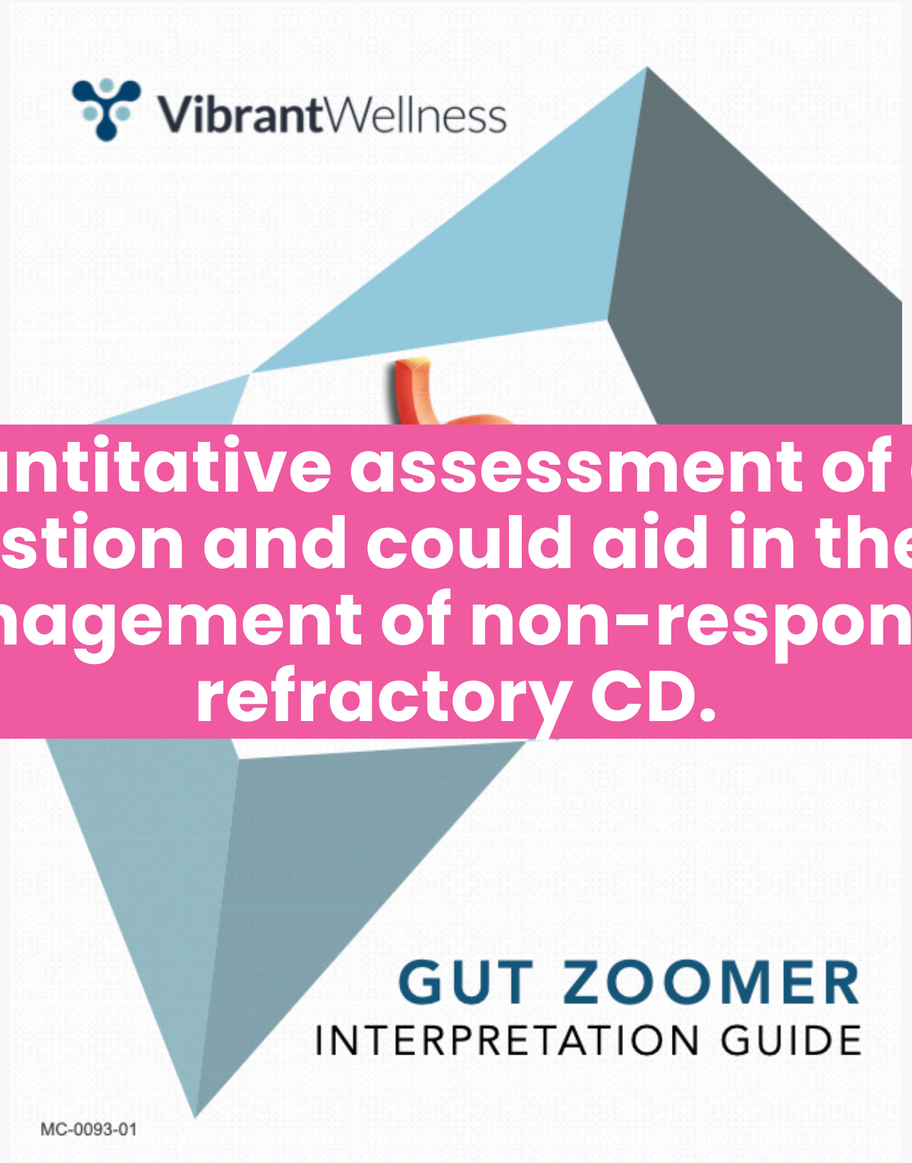

A direct and quantitative assessment of gluten exposure early after ingestion and could aid in the diagnosis and clinical management of non-responsive CD and refractory CD.

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What it measures: Mucosal immune response in the gut to gluten peptides.

Benefits:

- Suggests wheat exposure in the gut
- Reflects local gut immune activation,
  not just exposure-critical to differentiate = loss of oral tolerance.
- May detect immune responses before serum antibodies develop.
- Can help identify “leaky gut” physiology (mucosal antibodies in stool suggest barrier activation).
- Useful for functional/early detection NCGS or mucosal inflammation contexts.

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BUT REMEMBER

- Because the gut immune system is exposed to a broad range of dietary antigens, stool alpha-gliadin antibody assays may capture not only direct wheat exposure but also immune responses to cross-reactive proteins.
- This means that in sensitive individuals, a positive stool alpha-gliadin antibody test could, in theory, reflect exposure to corn or other cross-reactive foods if immune cross-recognition occurs.

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Cross-Reaction between Gliadin and Different Food and Tissue Antigens

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ABSTRACT

A subgroup of coeliac disease patients continues to experience symptoms even on a gluten-free diet (GFD). We attempted to determine whether these symptoms could be due to either cross-contamination with gluten-containing foods or cross-reactivity between α-gliadin and non-gluten foods consumed on a GFD. We measured the reactivity of affinity-purified polyclonal and monoclonal α-gliadin 33-mer peptide antibodies against gliadin and additional food antigens commonly consumed by patients on a GFD using ELISA and dot-blot. We also examined the immune reactivity of these antibodies with various tissue antigens. We observed significant immune reactivity when these antibodies were applied to cow's milk, milk chocolate, milk butyrophilin, whey protein, casein, yeast, oats, corn, millet, instant coffee and rice. To investigate whether there was cross-reactivity between α-gliadin antibody and different tissue antigens, we measured the degree to which this antibody bound to these antigens. The most significant binding occurred with asialoganglioside, hepatocyte, glutamic acid decarboxylase 65, adrenal 21-hydroxylase, and various neural antigens. The specificity of anti-α-gliadin binding to different food and tissue antigens was demonstrated by absorption and inhibition studies. We also observed significant cross-reactivity between α-gliadin 33-mer and various food antigens, but some of these reactions were associated with the contamination of non-gluten foods with traces of gluten. The consumption of cross-reactive foods as well as gluten-contaminated foods may be responsible for the continuing symptoms presented by a subgroup of patients with coeliac disease. The lack of response of some CD patients may also be due to antibody cross-reactivity with non-gliadin foods. These should then be treated as gluten-like peptides and should also be excluded from the diet when the GFD seems to fail.

Keywords: Cross-Reaction; Gliadin; Food Antigens; Tissue Antigens; Celiac Disease; Gluten-Free Diet

1. Introduction

Gluten sensitivity and celiac disease (CD) are gastrointestinal disorders resulting from a breakdown in oral tolerance and a subsequent inappropriate immune response against wheat proteins [1,2]. A majority of these patients have specific antibodies directed against tissue transglutaminase, various gliadins, glutenins, gluteomorphins, wheat germ agglutinin protein and peptides [3]. If left untreated, individuals may develop autoimmune injury to the gut, skin, brain, joints, liver, thyroid, bone, reproductive organs and other parts of the body [4].

The commonly recognized therapy for these disorders is a gluten-free diet (GFD). However, the response to a GFD is poor in up to 30% of patients, and patients may exhibit persistent or recurrent symptoms [5]. In fact, when histological response was assessed in celiac patients after 6 months of following a GFD, complete normalization and reconstruction of villous architecture was observed only in 8% of individuals, while 65% of these patients were in remission and 27% did not respond to GFD and had no observable change in their clinical symptoms [6]. The lack of improvement in histopathology and clinical symptomatology in a subgroup of patients on a GFD may be associated with dietary non-adherence or cross-reactive epitopes triggering a state of heightened immunological reactivity in gluten-sensitive individuals [7]. Indeed, celiac peptides that are recognized by sera from patients with active disease share homology with various self-microbial and food antigens [8]. These include Rotavirus major neutralizing protein VP-7, human heat shock protein-60, desmoglein-1 or myotubularin-related protein-2, collagen type VII, toll-like receptor-4, *Saccharomyces cerevisiae*, and milk proteins [8,9-13]. In one study, because patients with CD still had GI symptoms, researchers suspected that cow's milk protein may have been involved. Therefore, they used rectal protein challenge to investigate the inflammatory reaction to gluten and milk proteins in 20 adult

*Corresponding author.

This immune reaction (alpha-gliadin antibodies) against various food antigens was the greatest against α- + β-casein (1.45), followed by yeast (0.94), casomorphin (0.86), oat cultivar #2 (0.68), fresh corn (0.68), milk (0.61), millet (0.51), milk chocolate(0.49), instant coffee (0.46), rice (0.45), milk butyrophilin (0.39), and whey protein (0.36).

Food and Nutrition Sciences, 2013, 4, 20-32

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Scientific Research

Cross-Reaction between Gliadin and Different Food and Tissue Antigens

Aristo Vojdani[1,2*], **Igal Tarash**[1]

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Second to casein, gliadin antibody reacted considerably with brewer's yeast antigens (Saccharomyces cerevisiae).

Food and Nutrition Sciences, 2013, 4, 20-32

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AND, is an early biomarker of an activated immune response to wheat and validates the need for further exploration with a Wheat Zoomer.

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218

COELIAC DISEASE

Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function

M G Clemente, S De Virgiliis, J S Kang, R Macatagney, M P Musu, M R Di Pierro, S Drago, M Congia, A Fasano

Gut 2003;52:218–223

See end of article for authors' affiliations

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Accepted for publication 9 September 2002

Background and aims: Despite the progress made in understanding the immunological aspects of the pathogenesis of coeliac disease (CD), the early steps that allow gliadin to cross the intestinal barrier are still largely unknown. The aim of this study was to establish whether gliadin activates a zonulin dependent enterocyte intracellular signalling pathway(s) leading to increased intestinal permeability.
Methods: The effect of gliadin on the enterocyte actin cytoskeleton was studied on rat intestinal epithelial (IEC-6) cell cultures by fluorescence microscopy and spectrofluorimetry. Zonulin concentration was measured on cell culture supernatants by enzyme linked immunosorbent assay. Transepithelial intestinal resistance (Rt) was measured on ex vivo intestinal tissues mounted in Ussing chambers.
Results: Incubation of cells with gliadin led to a reversible protein kinase C (PKC) mediated actin polymerisation temporarily coincident with zonulin release. A significant reduction in Rt was observed after gliadin addition on rabbit intestinal mucosa mounted in Ussing chambers. Pretreatment with the zonulin inhibitor FZI/0 abolished the gliadin induced actin polymerisation and Rt reduction but not zonulin release.
Conclusions: Gliadin induces zonulin release in intestinal epithelial cells in vitro. Activation of the zonulin pathway by PKC mediated cytoskeleton reorganisation and tight junction opening leads to a rapid increase in intestinal permeability.

Coeliac disease (CD) is an autoimmune enteropathy triggered by ingestion of gluten containing grains in genetically susceptible individuals. The gliadin fraction of wheat gluten represents the environmental factor responsible for the development of the intestinal damage typical of the disease.[1] While in recent years we have witnessed significant progress on the immunological aspects of CD pathogenesis,[2] no major achievements have been made in understanding the early steps that allow gliadin to cross the intestinal epithelial barrier to be recognised by the intestinal immune system.[3] Gliadin deamidation by tissue transglutaminase has been demonstrated to enhance the recognition of gliadin peptides by HLA DQ2/DQ8 T cells in genetically predisposed subjects and it might initiate the cascade of autoimmune reactions which are finally responsible for mucosal destruction through production of cytokines and matrix metalloproteinases.[4 5] These reactions imply that gliadin and/or its breakdown peptides in someway cross the intestinal epithelial barrier and reach the lamina propria of the intestinal mucosa where they are recognised by antigen presenting cells. Under physiological circumstances the intestinal epithelial barrier is described as being almost impermeable to macromolecules.[3] However, CD is characterised by enhanced paracellular permeability across intestinal epithelium—that is, "leaky gut", a condition that would allow passage of macromolecules through the paracellular space.[6–8] We have recently reported that zonulin, a modulator of tight junction (tj) permeability,[9] is upregulated during the acute phase of CD.[10] Following binding to its surface receptor, zonulin induces a protein kinase C (PKC) mediated polymerisation of intracellular actin filaments which are directly connected to structural proteins of the tj hence regulating epithelial permeability.[11 12] The complex actin cytoskeleton network of the enterocyte is known to be involved in the intracellular trafficking of molecules as well as in the regulation of paracellular permeability by its direct interaction with the tj structural proteins.[13–15] This study was aimed at establishing the interplay between gliadin and the enterocyte, with specific emphasis on the effect of gliadin on zonulin release and subsequent activation of intracellular signalling leading to the disassembly of intercellular tj.

METHODS

IEC-6 cell cultures

Rat intestinal epithelial cells (IEC-6 cells) were grown in cell culture flasks (Falcon Labware, Reston, Virginia, USA) at 37°C in an atmosphere of 95% air and 5% CO_2. The medium consisted of Dulbecco's modified Eagle's medium (Gibco, Rockville, Maryland, USA) containing 4500 mg/l D-glucose, pyridoxine hydrochloride, 5% heat inactivated (56°C, 30 minute) fetal bovine serum, 0.1 U/ml bovine insulin, 4 mM L-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin.

Gliadin peptides

Gliadin (Sigma, St Louis, Missouri, USA) was freshly prepared in a 70% ethanol solution (20 mg/ml) and used at serial dilutions in the cell culture medium, ranging from the 1:20 dilution (final concentration: gliadin 1 mg/ml; ethanol 3.5%) to the 1:200 dilution (final concentration: gliadin 0.1 mg/ml; ethanol 0.35%). The pH was adjusted to 7.4 when necessary by 1 M NaOH buffer. Similar ethanol concentrations were added to the final concentration of bovine serum albumin (BSA) and zein from maize (Sigma) used as negative controls. Ethanol concentration was never more than 3.5% in the final solution in order to avoid any direct effect of ethanol on cultured cells. Synthetic peptides 31–55 and 22–59 (Biopolymer Laboratories, University of Maryland, Baltimore, Maryland, USA) were

Abbreviations: CD, coeliac disease; Rt, transepithelial electrical resistance; Zot, zonula occludens toxin; tj, tight junctions; PKC, protein kinase C; BSA, bovine serum albumin; PBS, phosphate buffered saline; CV, coefficient of variation.

The results of our study indicate that gliadin activates the zonulin signaling pathway in normal intestinal epithelial cells.

Gut 2003;52:218–223

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Review

Intestinal Barrier Function in Gluten-Related Disorders

Danielle Cardoso-Silva [1,†], Deborah Delbue [1,†], Alice Itzlinger [1], Renée Moerkens [2], Sebo Withoff [2], Federica Branchi [1] and Michael Schumann [1,*]

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Abstract: Gluten-related disorders include distinct disease entities, namely celiac disease, wheat-associated allergy and non-celiac gluten/wheat sensitivity. Despite having in common the contact of the gastrointestinal mucosa with components of wheat and other cereals as a causative factor, these clinical entities have distinct pathophysiological pathways. In celiac disease, a T-cell mediate immune reaction triggered by gluten ingestion is central in the pathogenesis of the enteropathy, while wheat allergy develops as a rapid immunoglobulin E- or non-immunoglobulin E-mediated immune response. In non-celiac wheat sensitivity, classical adaptive immune responses are not involved. Instead, recent research has revealed that an innate immune response to a yet-to-be-defined antigen, as well as the gut microbiota, are pivotal in the development in this disorder. Although impairment of the epithelial barrier has been described in all three clinical conditions, its role as a potential pathogenetic co-factor, specifically in celiac disease and non-celiac wheat sensitivity, is still a matter of investigation. This article gives a short overview of the mucosal barrier of the small intestine, summarizes the aspects of barrier dysfunction observed in all three gluten-related disorders and reviews literature data in favor of a primary involvement of the epithelial barrier in the development of celiac disease and non-celiac wheat sensitivity.

Keywords: epithelial barrier; permeability; celiac disease; non-celiac gluten sensitivity; non-celiac wheat sensitivity; wheat allergy

1. The Intestinal Barrier

The intestinal barrier has a crucial role in protecting the organism against pathogens and possible harmful substances derived from the external environment (Figure 1). It is formed by a mucus and epithelial layer and by the lamina propria underneath. Immune cells, components of the intestinal microbiota and anti-microbial peptides have crucial functions in maintaining the intestinal barrier function [1,2].

There is a barrier-impairing effect exerted by gliadin in NCWS similarly to CeD

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ORCID number: Laura Coto 0000-0002-3883-2027; Iratí Mendia 0000-0001-8584-7985; Carolina Sousa 0000-0002-9235-9486; Julio César Bai 0000-0003-4159-8185; Angel Cebolla 0000-0002-6976-4522.

Author contributions: Coto L, Mendia I, Sousa C, Bai JC and Cebolla A contributed equally to the revision of the literature, wrote the draft, and/or revised the final manuscript for intellectual content.

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See corresponding editorial on page 537.

Monitoring of gluten-free diet compliance in celiac patients by assessment of gliadin 33-mer equivalent epitopes in feces[1–3]

Isabel Comino, Ana Real, Sanjiago Vivas, Miguel Ángel Sáfez, Alberto Caminero, Esther Nistal, Javier Casqueiro, Alfonso Rodríguez-Herrera, Angel Cebolla, and Carolina Sousa

ABSTRACT

Background: Certain immunotoxic peptides from gluten are resistant to gastrointestinal digestion and can interact with celiac-patient factors to trigger an immunologic response. A gluten-free diet (GFD) is the only effective treatment for celiac disease (CD), and its compliance should be monitored to avoid cumulative damage. However, practical methods to monitor diet compliance and to detect the origin of an outbreak of celiac clinical symptoms are not available.

Objective: We assessed the capacity to determine the gluten ingestion and monitor GFD compliance in celiac patients by the detection of gluten and gliadin 33-mer equivalent peptidic epitopes (33EPs) in human feces.

Design: Fecal samples were obtained from healthy subjects, celiac patients, and subjects with other intestinal pathologies with different diet conditions. Gluten and 33EPs were analyzed by using immunochromatography and competitive ELISA with a highly sensitive antigliadin 33-mer monoclonal antibody.

Results: The resistance of a significant part of 33EPs to gastrointestinal digestion was shown in vitro and in vivo. We were able to detect gluten peptides in feces of healthy individuals after consumption of a normal gluten-containing diet, after consumption of a GFD combined with controlled ingestion of a fixed amount of gluten, and after ingestion of <100 mg gluten/d. These methods also allowed us to detect GFD infringement in CD patients.

Conclusions: Gluten-derived peptides could be sensitively detected in human feces in positive correlation with the amount of gluten intake. These techniques may serve to show GFD compliance or infringement and be used in clinical research in strategies to eliminate gluten immunotoxic peptides during digestion. This trial was registered at clinicaltrials.gov as NCT01478867. *Am J Clin Nutr* 2012;95:670–7.

INTRODUCTION

Gluten is the storage protein of wheat, rye, barley, and oats and is not well tolerated in genetically predisposed individuals who suffer from CD[4]. Although most dietary proteins are digested into simple amino acids, dipeptides, and tripeptides by gastrointestinal proteases, gluten proteins are not completely digested and remain in the gastrointestinal tract (1, 2). The α-gliadin 33-mer is one of the digestion-resistant gluten peptides that is highly reactive to isolated celiac T cells and is the main immunodominant toxic peptide in celiac patients (3–5).

A lifelong GFD is currently the only available treatment for CD patients. Clinical manifestations associated with untreated CD, such as osteoporosis, anemia, depression, and infertility, can ameliorate with a GFD. Therefore, strict adherence to a GFD is essential to reduce symptoms, avoid nutritional deficiencies, and improve quality of life. However, according to several reports, dietary transgression is relatively frequent (32.6–55.4%) in celiac patients (6). In addition, a part of the celiac population (5–10%) does not respond to a GFD and has persistent villous atrophy with continued malabsorption. Although it is possible for patients to relapse despite a strict GFD, involuntary infringement or hypersensitivity to small amounts of gluten can also trigger the symptoms of the disease. Thus, an accurate marker that allows short-term monitoring of GFD compliance is needed. Approximately 1–2% of patients, mainly adults, can develop RCD, which is characterized by symptomatic malabsorption and persistent villous atrophy despite a strict GFD (7, 8). A demonstration of a lack of gluten in the diet of RCD patients would help in the differential diagnosis (9). Direct measures of dietary transgressions, such as mucosal inflammation and antitissue transglutaminase or antigliadin antibody concentrations in serum, could be used to confirm GFD compliance. However, because a decrease in antibody titers may take years to achieve even when

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Since all of the methods used so far fail to offer a completely reliable measure of dietary compliance, or are impractical, it was recently proposed to assay gluten immunogenic peptides (ie. Alpha-gliadin), detectable in feces as a result of the incomplete breakdown of gluten in the gastrointestinal tract.

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See corresponding editorial on page 537.

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Determination of gluten immunogenic peptides for the management of the treatment adherence of celiac disease: A systematic review

Laura Coto, Iratí Mendia, Carolina Sousa, Julio César Bai, Angel Cebolla

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Author contributions: Coto L, Mendia I, Sousa C, Bai JC and Cebolla A contributed equally to the revision of the literature, wrote the draft, and/or revised the final manuscript for intellectual content.

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Conflict-of-interest statement: Angel Cebolla is the founder and current CEO of Biomedal S.L., Angel Cebolla and Carolina Sousa are inventors of the patent "Detecting gluten peptides in human fluids" (No. WO/2016/085643). Laura Coto and Iratí Mendia are employees at Biomedal S.L., Julio César Bai declares no conflict of interest.

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Abstract

BACKGROUND

Gluten is a complex mixture of proteins with immunogenic peptide sequences triggering the autoimmune activity in patients with celiac disease (CeD). Gluten immunogenic peptides (GIP) are resistant to gastrointestinal digestion and are then excreted via the stool and urine. Most common detection methods applied in the follow-up visits for CeD patients such as serology tests, diabetic interviews, questionnaires, and duodenal biopsy have been proved to be inefficient, invasive, or inaccurate for evaluating gluten-free diet (GFD) compliance. Determination of excreted GIP in stool and urine has been developed as a non-invasive, direct, and specific test for GFD monitoring.

AIM

To summarize published literature about the clinical utility of GIP determination in comparison to the tools employed for GFD monitoring.

METHODS

PubMed and Web of Science searches were performed using the keywords "gluten immunogenic peptides" or "gluten immunogenic peptide" and a

This novel technique was highly sensitive for the detection of GFD transgressions and therefore could facilitate the follow-up of patients with CD.

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Table 1 Studies that have used gluten immunogenic peptides determination in stool and/or urine for gluten-free diet monitoring

Ref.	Design	Study population	Intervention	Main results
Comino *et al* [8]	Prospective, multicenter, observational study	184 adult and pediatric CeD patients	Fecal GIP ELISA, serology, questionnaires, and symptoms to evaluate adherence to the GFD	GIP-positive results were found in 12%-28% of children < 12 years-old, 30% in > 13 years-old females and 60% in > 13 years-old males. Low correlation of anti-tTG and anti-DGP markers and poor adherence to the GFD
Moreno *et al* [9]	Randomized controlled study	58 adult and pediatric CeD patients and 76 healthy controls	Urine GIP LFIA test, serology, and duodenal biopsy to evaluate adherence to the GFD	About 50% CeD patients were GIP-positive. High correlation of GIP quantifiable concentration in urine with persistent villus atrophy in treated CeD patients (n = 25). No correlation between serology and mucosal damage
Gerasimidis *et al*[39]	Cross-sectional study cohort for a subgroup	63 pediatric CeD patients	Fecal ELISA GIP test, serology, and questionnaires to evaluate gluten intake during diagnosis and adherence to the GFD after diagnosis	GIP-positive results in 95% of de novo patients with CeD during diagnosis. GIP-positive results were found in 17% and 27% of patients after 6 and 12 months of the beginning of the GFD, respectively. GIP-positive results were found in 16%, 16%, and 14% of patients considered compliant according to the Biagi score, tTG, and clinical assessment, respectively
Comino *et al* [40]	Prospective, multicenter, observational study	64 pediatric CeD patients	Fecal GIP ELISA, serology, questionnaires, and symptoms to evaluate adherence to the GFD after diagnosis	Most children (97%) were GIP-positive at diagnosis. A decrease of GIP detection was observed on a GFD, but the rate of GIP-positive results increased from 13% at 6 months to 25% at 24 months. Anti-tTG antibody levels showed low sensitivity to identify patients with GIP-positive results. Dietitian assessment was only moderately correlated with GIP detection
Costa *et al*[41]	Cross-sectional study and prospective cohort	44 adult CeD patients	Fecal GIP ELISA, stool and urine LFIA GIP tests, serology, questionnaires, and symptoms to evaluate adherence to the GFD	25% of patients had at least one GIP-positive test, 32% in asymptomatic patients and 15.8% in symptomatic patients. Dietary assessment estimated gluten intake in only 50% of GIP-positive samples. Anti-tTG and anti-DGP positive results in 3/12 and 6/12 of GIP-positive cases, respectively
Silvester *et al* [29,30]	Prospective longitudinal study	18 adult CeD patients	Monitoring GFD adherence by collection of daily food, stool, and urine samples for the analysis of GIP content, and relationship with duodenal biopsy, serology, questionnaires, and symptoms	GIP were detected in 66,7% patients. No significant correlation was found between gluten ingestion and non-invasive measures of GFD adherence. Most patients with normal anti-tTG had ≥ 1 GIP-positive sample (64%), 2/3 of these had persistent villous atrophy (Marsh 3a) and 2/3 of those with all GIP-negative samples had normal villous architecture (Marsh 0-1) but 4/6 with Marsh 0 had detectable gluten in ≥ 1 sample
Ruiz-Carnicer *et al* [23]	Prospective observational study	22 newly diagnosed CeD patients, 77 CeD patients following a GFD and 13 healthy volunteers	Urine LFIA GIP test to evaluate adherence to the GFD and comparison with serology, clinical manifestations, dietary questionnaire, and histological results	Mucosal damage (Marsh II-III) was found in 24% of CeD patients, 94%of these had ≥ 1 GIP urine sample. 60-80% of these were asymptomatic, had negative serologic results and were compliant with treatment regarding the dietary questionnaire. GIP-negative results were found in 97% of the patients without mucosal damage
Fernandez-Miaja *et al*[22]	Cross-sectional study	80 pediatric CeD patients	Relationship of fecal LFIA GIP for GFD monitoring GFD with CDAT, serology and sociodemographic and clinical data	Acceptable agreement was found between GIP detection and CDAT questionnaire (92.5% and 86.3% adherence rate, respectively). Most patients (83.3%) with GIP-positive results had negative anti-tTG antibodies
Porcelli *et al* [42]	Cross-sectional study	25 CeD patients	Assessment of compliance with the GFD using Fecal GIP ELISA testing, the Biagi questionnaire, evaluation of symptoms and serology	GIP-positive results were found in 4 patients, 2 of these complied with the GFD according to the Biagi questionnaire. All GIP-negative patients were asymptomatic. Levels of anti-tTG antibodies were significantly higher in GIP-positive patients than in GIP-negative patients
Roca *et al*[43]	Prospective, cross-sectional study	43 pediatric CeD patients at follow-up (Group 1) and 18 at diagnosis (Group 2)	Fecal GIP ELISA and LFIA analysis to monitor in real life the adherence to GFD Comparison to food record questionnaire and serology	Group 1: GIP-positive results were found in of 34.9% patients by ELISA (46.7% also by LFIA). 48.8% of patients had positive anti-tTG antibodies (4 reported symptoms) and 10 of these had GIP-positive results by ELISA (70% also by LFIA) (2 reported symptoms). All the transgressions detected by food record were also detected with GIP
Porcelli *et al* [44]	Cross-sectional study	55 CeD patients: 27 adults and 28 children	Assessment of compliance with the GFD using Fecal GIP ELISA, the Biagi questionnaire, evaluation of symptoms and serology	GIP-positive results were found in 8 patients, 71.4% of these were asymptomatic and 37.5% had raised anti-tTG antibodies. A significant association was found between the Biagi score and GIP-positive results but according to the Biagi score, 57.1% of GIP-positive patients followed the diet strictly and 5.4% of GIP-negative subjects did not comply with the diet

GIP-positive (stool) results were found in:

- 12%-28% of children < 12 years-old,
- 30% in > 13 years-old females and
- 60% in > 13 years-old males. Low correlation of anti-tTG and anti- DGP markers and poor adherence to the GFD

About 50% CD patients were GIP-positive.

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- Most children (97%) were GIP-positive at diagnosis.
- A decrease of GIP detection was observed on a GFD, but
- the rate of GIP-positive results increased from 13% at 6 months to 25% at 24 months. (suggesting repeated exposures)

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If ever Clinicians needed the science of why you MUST have an affiliation with a Nutritionist, Certified Health Coach, Certified Gluten-free Practitioner, or well-trained Staff, Clinicians are unknowingly setting up these patients for early mortality (excuse me, but WAKE UP)

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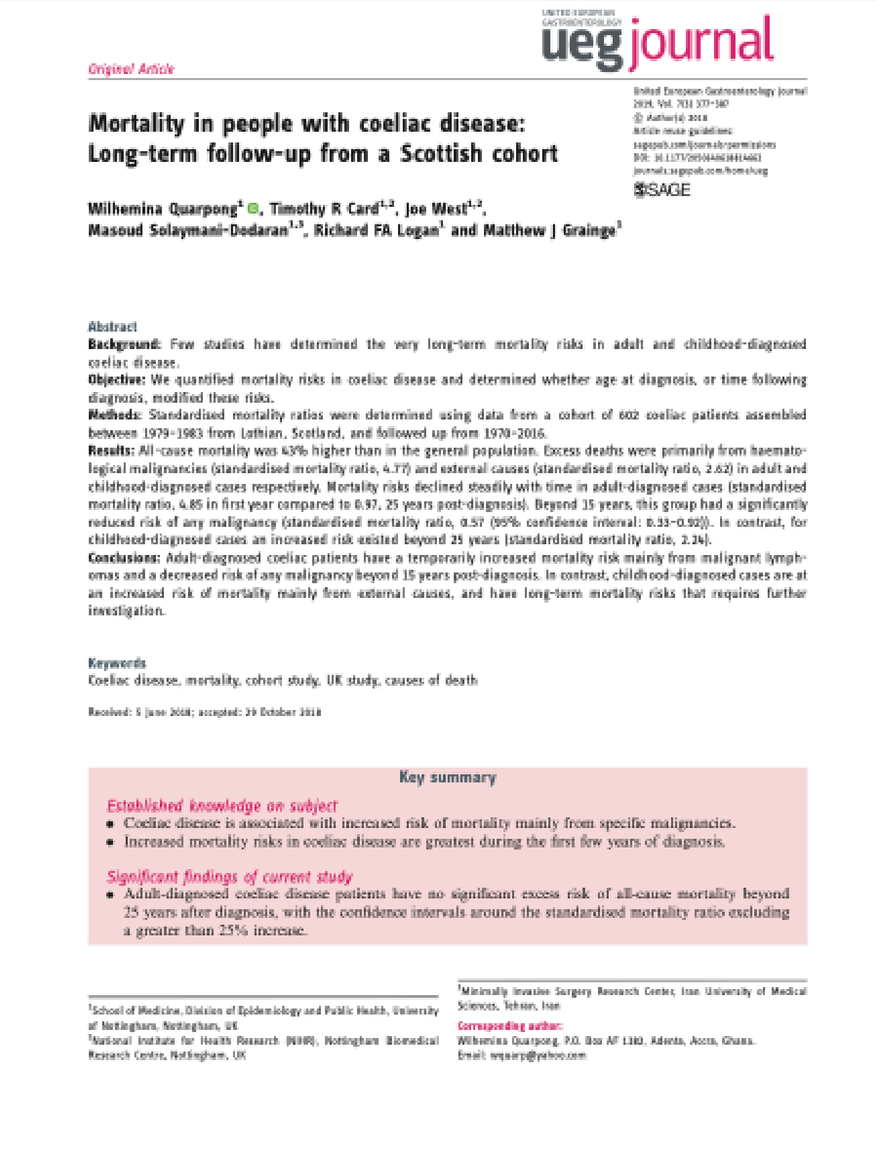

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Original Article

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Abstract

Background: Few studies have determined the very long-term mortality risks in adult and childhood-diagnosed coeliac disease.

Objective: We quantified mortality risks in coeliac disease and determined whether age at diagnosis, or time following diagnosis, modified these risks.

Methods: Standardised mortality ratios were determined using data from a cohort of 602 coeliac patients assembled between 1979–1983 from Lothian, Scotland, and followed up from 1970–2016.

Results: All-cause mortality was 43% higher than in the general population. Excess deaths were primarily from haematological malignancies (standardised mortality ratio, 4.77) and external causes (standardised mortality ratio, 2.62) in adult and childhood-diagnosed cases respectively. Mortality risks declined steadily with time in adult-diagnosed cases (standardised mortality ratio, 4.85 in first year compared to 0.97, 25 years post-diagnosis). Beyond 15 years, this group had a significantly reduced risk of any malignancy (standardised mortality ratio, 0.57 (95% confidence interval: 0.33–0.92)). In contrast, for childhood-diagnosed cases an increased risk existed beyond 25 years (standardised mortality ratio, 2.24).

Conclusions: Adult-diagnosed coeliac patients have a temporarily increased mortality risk mainly from malignant lymphomas and a decreased risk of any malignancy beyond 15 years post-diagnosis. In contrast, childhood-diagnosed cases are at an increased risk of mortality mainly from external causes, and have long-term mortality risks that requires further investigation.

Keywords
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The highest mortality risk for this group (dx'd CD) was observed in the year following diagnosis (SMR 4.85; 95% CI 2.42–8.68). (That's a 385% higher mortality than expected)

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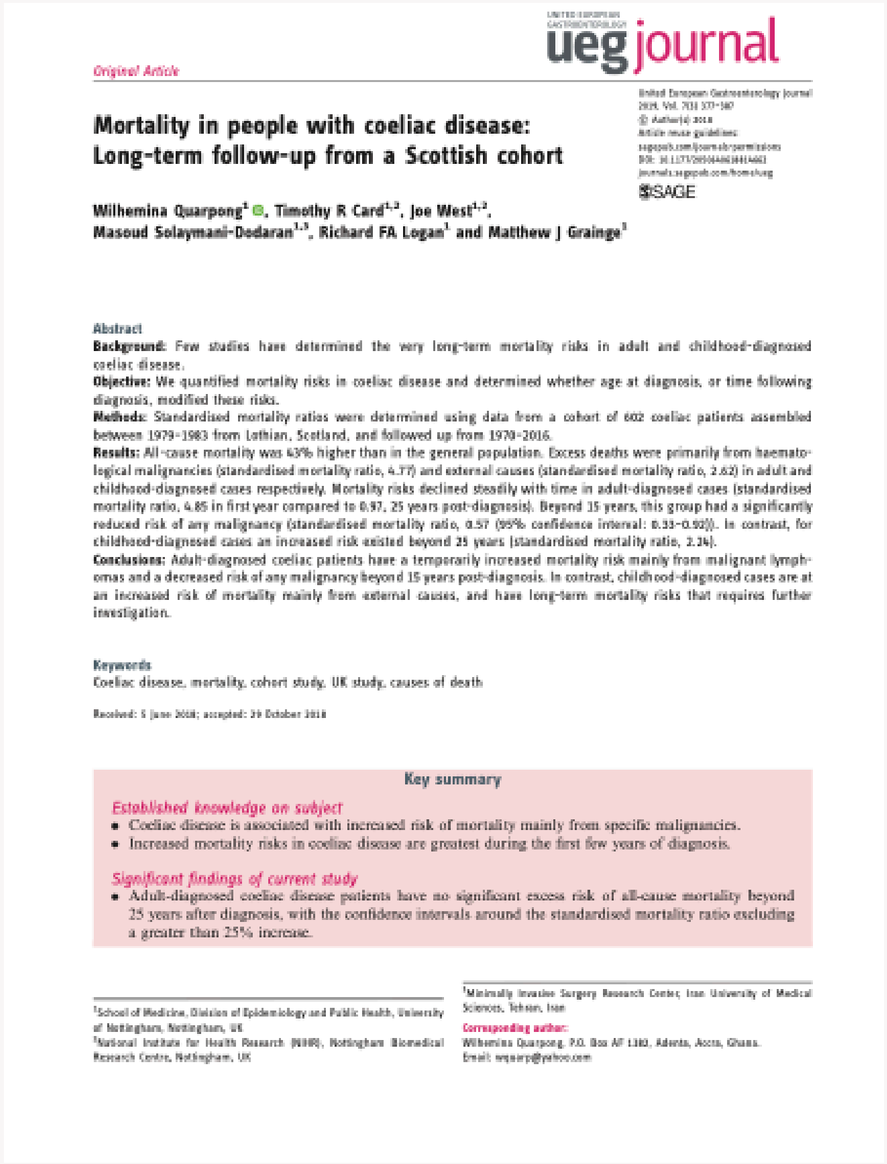

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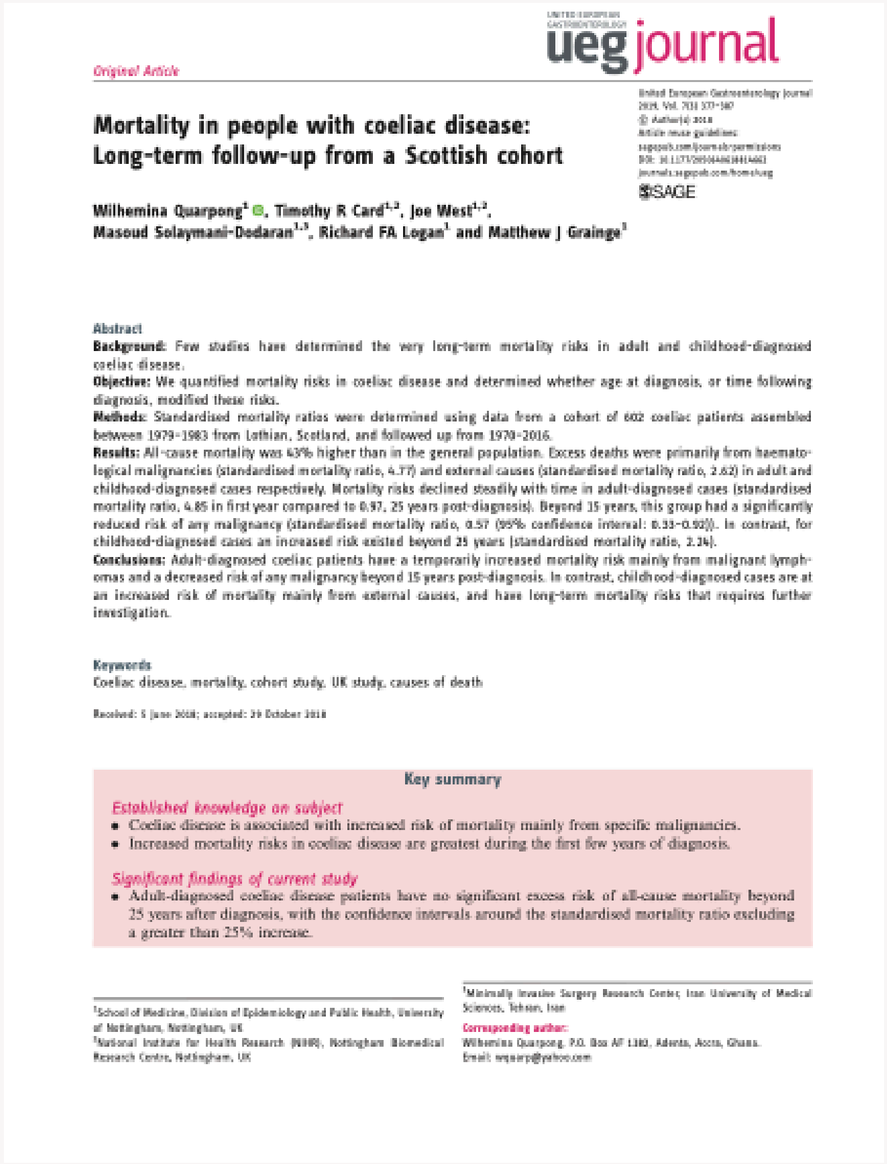
Within 5–9 years post-diagnosis, the excess risks had decreased to 49% (SMR 41.49; 95% CI 0.97–2.18).



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Beyond 25 years after diagnosis, adulthood-diagnosed CD patients had no significant excess risk,

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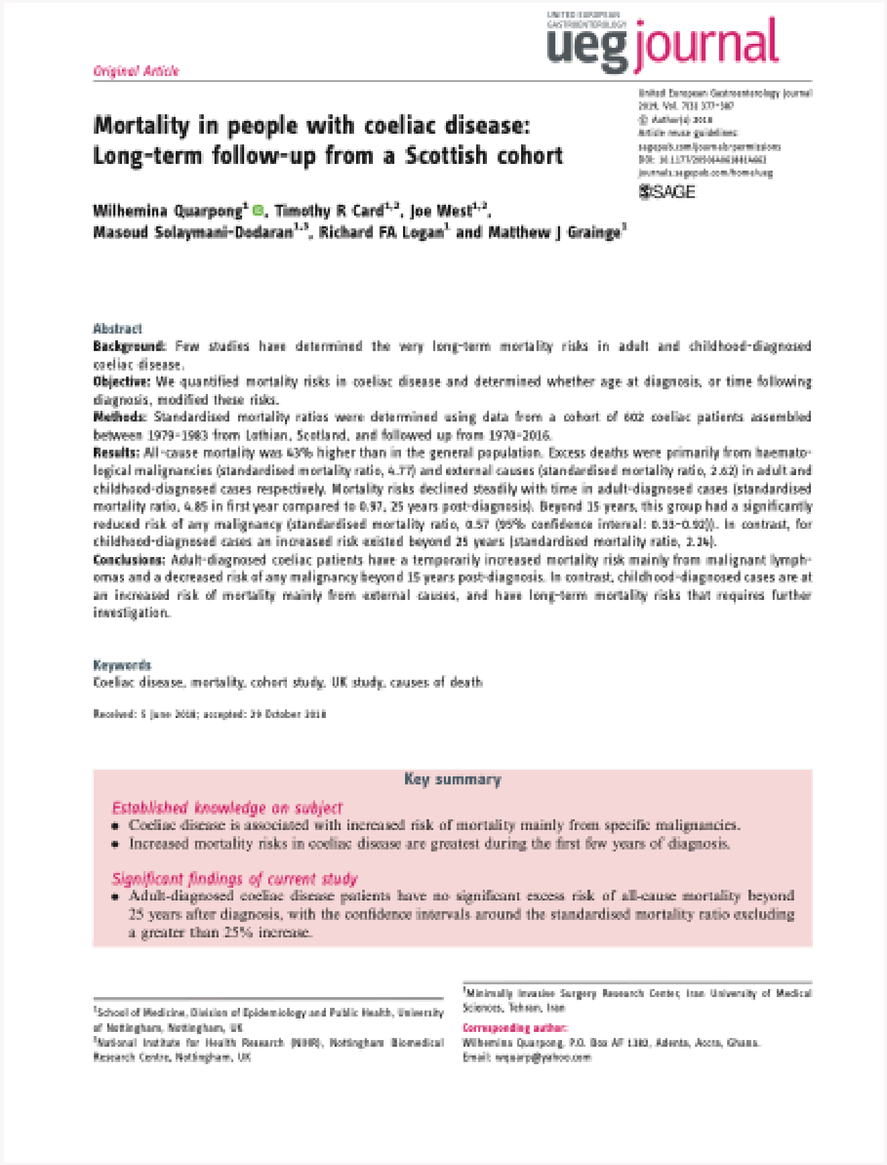

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Beyond 25 years after diagnosis, those diagnosed in childhood had more than double the mortality risk (SMR = 2.24; 95% CI 1.45–3.30).

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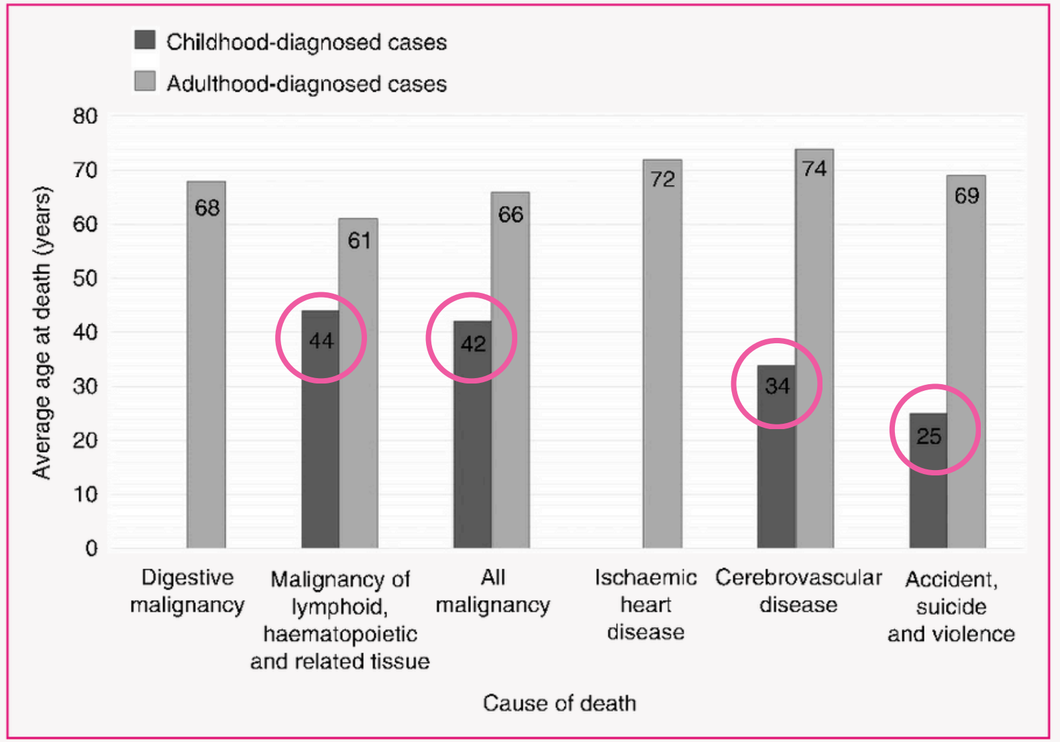

Figure 2. Average ages at death from specific causes.
Average age of death among participants who died by cause of death.

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CLINICAL TAKEAWAY?

When anti-gliadin antibodies are elevated in a stool analysis, consider the Wheat Zoomer, other food zoomers and the Autoimmune Zoomer

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Secretory IgA

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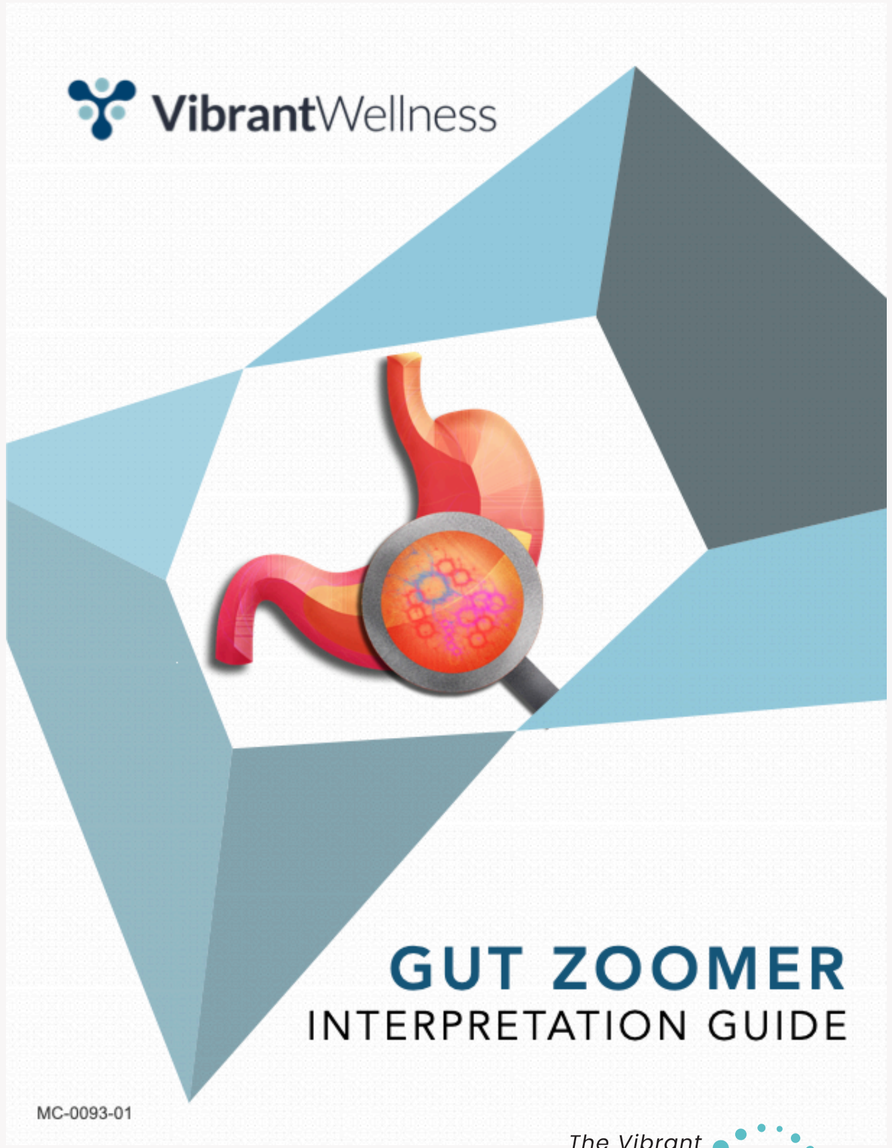
Secretory IgA is the primary antibody that protects us from pathogens and from toxins penetrating mucosal surfaces. The mucus layer that lines the intestinal wall is rich in SIgA, and there SIgA plays a crucial role in protecting the integrity of the intestinal epithelium.



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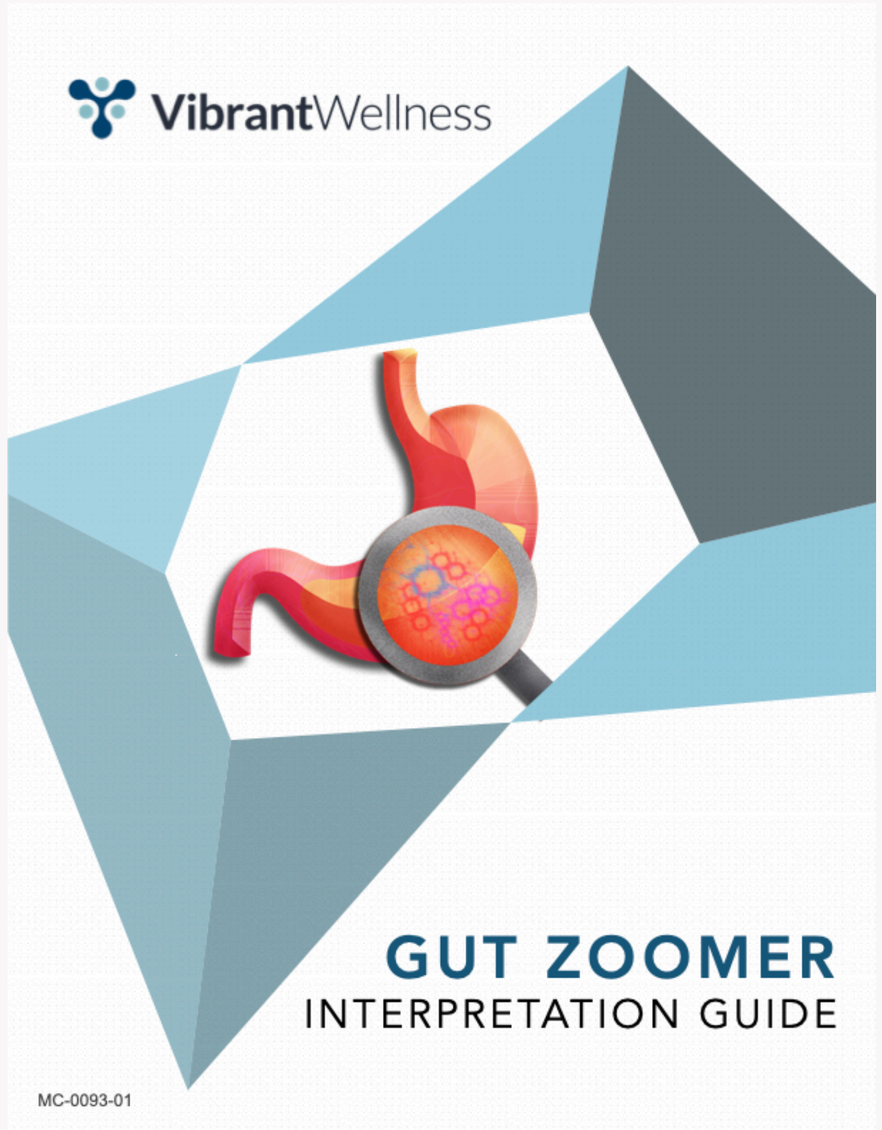
Marked elevations in SIgA are indicative of immune upregulation in the gut. Causes could be due to food sensitivities, intestinal permeability, inflammation, or infections.



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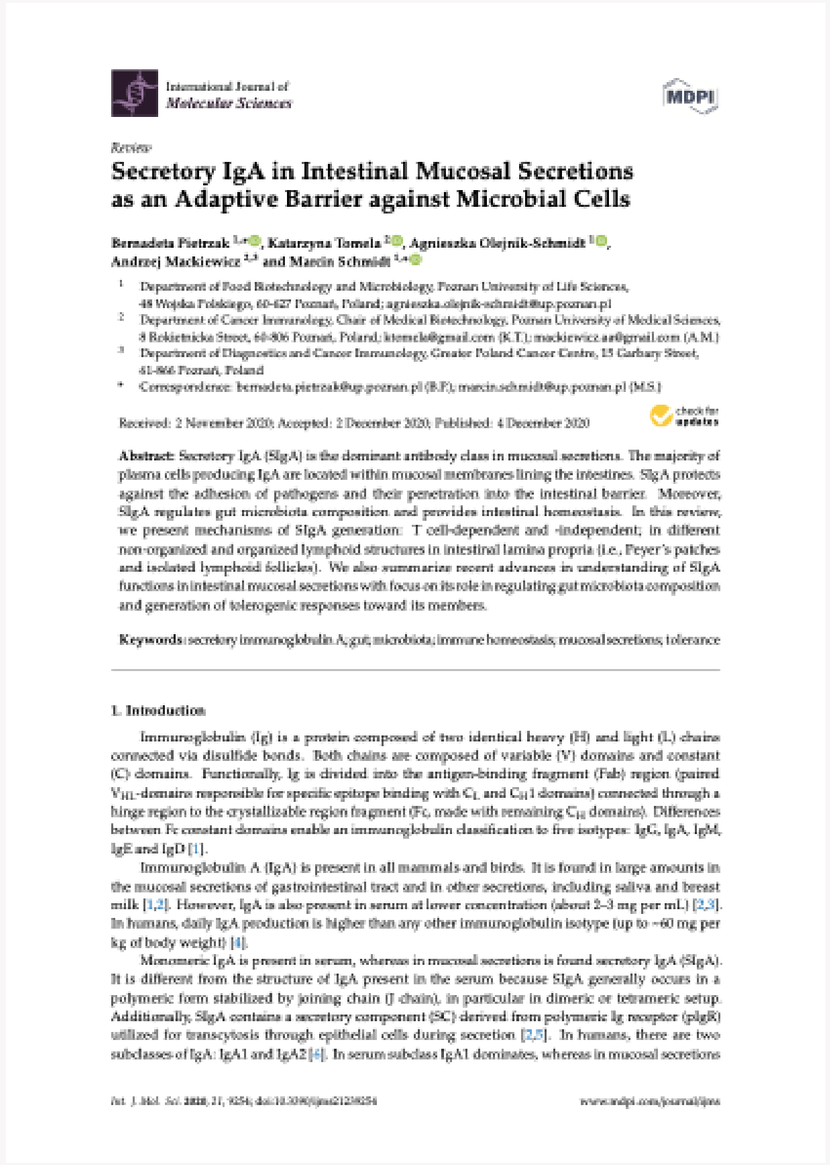

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Review

Secretory IgA in Intestinal Mucosal Secretions as an Adaptive Barrier against Microbial Cells

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Abstract: Secretory IgA (SIgA) is the dominant antibody class in mucosal secretions. The majority of plasma cells producing IgA are located within mucosal membranes lining the intestines. SIgA protects against the adhesion of pathogens and their penetration into the intestinal barrier. Moreover, SIgA regulates gut microbiota composition and provides intestinal homeostasis. In this review, we present mechanisms of SIgA generation: T cell-dependent and -independent; in different non-organized and organized lymphoid structures in intestinal lamina propria (i.e., Peyer's patches and isolated lymphoid follicles). We also summarize recent advances in understanding of SIgA functions in intestinal mucosal secretions with focus on its role in regulating gut microbiota composition and generation of tolerogenic responses toward its members.

Keywords: secretory immunoglobulin A; gut; microbiota; immune homeostasis; mucosal secretions; tolerance

1. Introduction

Immunoglobulin (Ig) is a protein composed of two identical heavy (H) and light (L) chains connected via disulfide bonds. Both chains are composed of variable (V) domains and constant (C) domains. Functionally, Ig is divided into the antigen-binding fragment (Fab) region (paired $V_{H/L}$-domains responsible for specific epitope binding with C_L and C_H1 domains) connected through a hinge region to the crystallizable region fragment (Fc, made with remaining C_H domains). Differences between Fc constant domains enable an immunoglobulin classification to five isotypes: IgG, IgA, IgM, IgE and IgD [1].

Immunoglobulin A (IgA) is present in all mammals and birds. It is found in large amounts in the mucosal secretions of gastrointestinal tract and in other secretions, including saliva and breast milk [1,2]. However, IgA is also present in serum at lower concentration (about 2–3 mg per mL) [2,3]. In humans, daily IgA production is higher than any other immunoglobulin isotype (up to ~60 mg per kg of body weight) [4].

Monomeric IgA is present in serum, whereas in mucosal secretions is found secretory IgA (SIgA). It is different from the structure of IgA present in the serum because SIgA generally occurs in a polymeric form stabilized by joining chain (J chain), in particular in dimeric or tetrameric setup. Additionally, SIgA contains a secretory component (SC) derived from polymeric Ig receptor (pIgR) utilized for transcytosis through epithelial cells during secretion [2,5]. In humans, there are two subclasses of IgA: IgA1 and IgA2 [6]. In serum subclass IgA1 dominates, whereas in mucosal secretions

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In steady-state conditions, approximately 36% of the gut microbiota is coated with SIgA, whereas during inflammation, this number can increase up to 69%

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IgA deficiency destabilizes homeostasis towards intestinal microbes and increases systemic immune dysregulation

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Abstract

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Competing interests
The authors declare no competing interests.

Selective IgA deficiency (SIgAD) is the most common primary immune deficiency, affecting ~1 in 600 Caucasian individuals

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Competing interests
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While SIgAD is clinically defined by absence of serum IgA, the symptomatology and immune dysregulation were concentrated in the subjects who were also fecal IgA-deficient.

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Competing interests
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SIgAD subjects who possess fecal IgA have less immune dysregulation and clinical symptoms than those SIgAD subjects without fecal IgA

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Competing interests
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We found a pattern of more symptomatic disease in SIgAD subjects lacking fecal IgA compared to SIgAD subjects possessing fecal IgA, including:

- more autoimmune disease (29% vs 0%)
- more allergy (64% vs 40%)

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And remember, this study was done in children. If 29% of SIgAD children have already developed an autoimmune disease, what percentage of SIgAD children may develop an autoimmune disease within the next 10-20 years?

Solid rationale to do the Autoimmune Zoomer every few years

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Fecal Zonulin Antibodies

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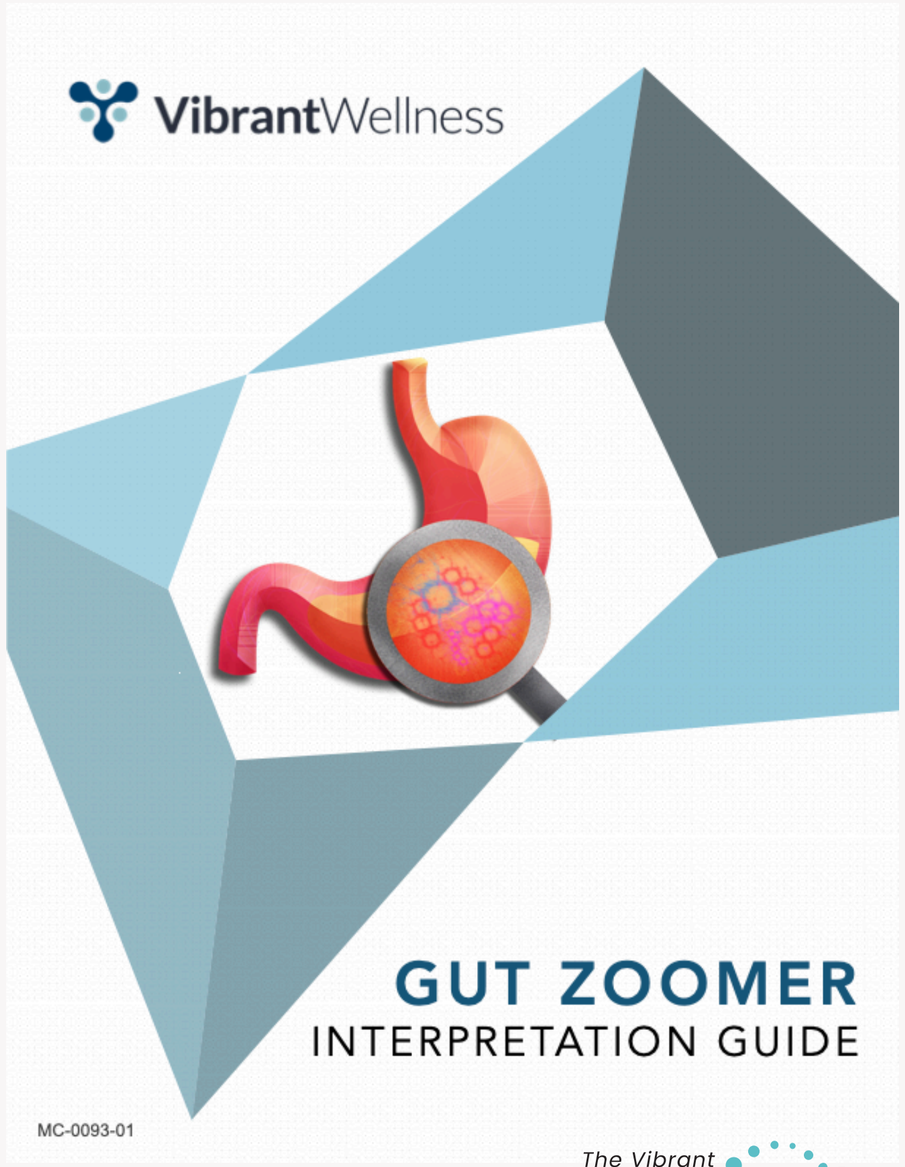
Fecal zonulin measurement may be advantageous, compared to serum zonulin, when assessing intestinal permeability, as serum zonulin may constitute secretion not only from intestinal cells, but also from extraintestinal tissues such as the liver, heart and brain.



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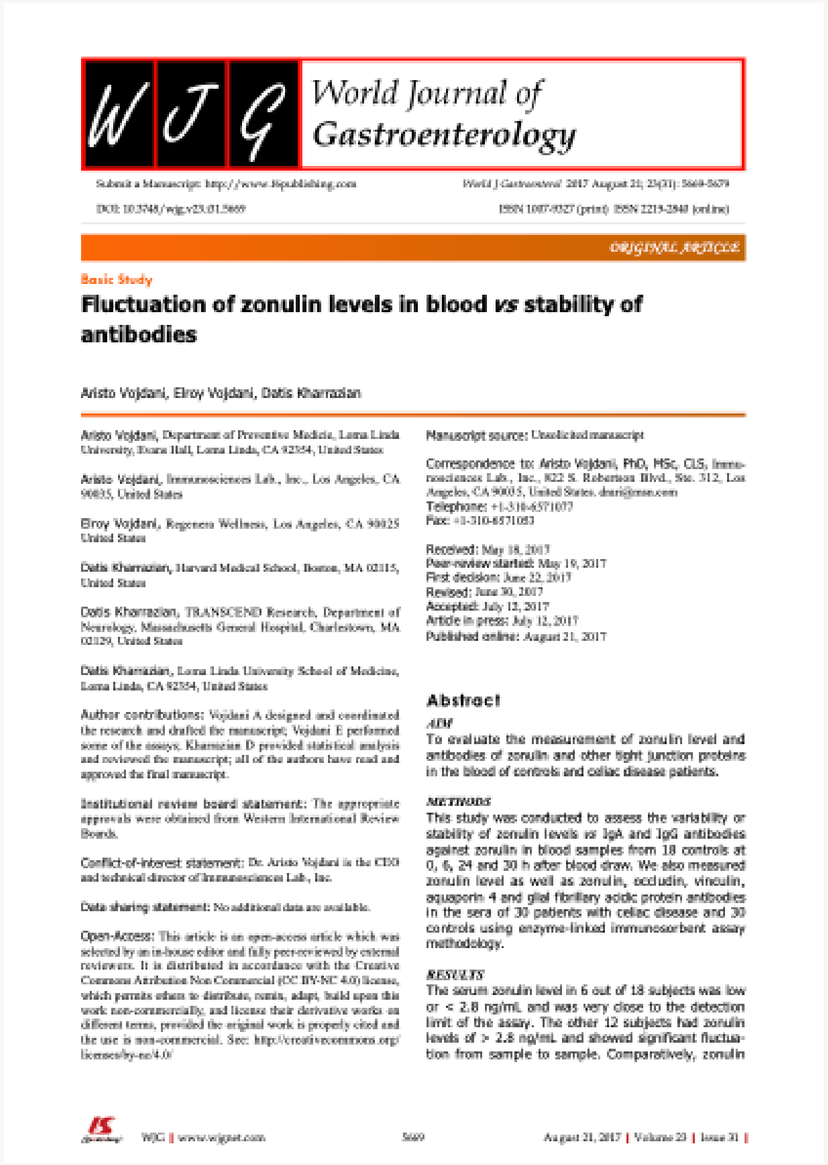
Zonulin Antibodies

- Marker of Local Mucosal Immune Activation
- Antibodies to zonulin in stool suggest the gut immune system is actively targeting zonulin proteins or zonulin-like proteins in the lumen.
- This indicates immune dysregulation at the epithelial barrier, not just transient zonulin release.
- Association With Chronic Barrier Dysfunction
- Elevated stool anti-zonulin antibodies may indicate that zonulin release has been persistent enough to trigger mucosal antibody production.
- This implies chronic intestinal permeability rather than a short-term fluctuation.
- Potential Cross-Reactivity: Zonulin is pre-haptoglobin-2; antibodies may sometimes cross-react with related proteins. Thus, positivity could reflect broader barrier protein autoimmunity beyond zonulin itself.



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ORIGINAL ARTICLE

Basic Study

Fluctuation of zonulin levels in blood *vs* stability of antibodies

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Conflict-of-interest statement: Dr. Aristo Vojdani is the CEO and technical director of Immunosciences Lab., Inc.

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Abstract

AIM

To evaluate the measurement of zonulin level and antibodies of zonulin and other tight junction proteins in the blood of controls and celiac disease patients.

METHODS

This study was conducted to assess the variability or stability of zonulin levels *vs* IgA and IgG antibodies against zonulin in blood samples from 18 controls at 0, 6, 24 and 30 h after blood draw. We also measured zonulin level as well as zonulin, occludin, vinculin, aquaporin 4 and glial fibrillary acidic protein antibodies in the sera of 30 patients with celiac disease and 30 controls using enzyme-linked immunosorbent assay methodology.

RESULTS

The serum zonulin level in 6 out of 18 subjects was low or < 2.8 ng/mL and was very close to the detection limit of the assay. The other 12 subjects had zonulin levels of > 2.8 ng/mL and showed significant fluctuation from sample to sample. Comparatively, zonulin

Zonulin levels will fluctuate in the blood stream. The half-life of these molecules, in the blood stream, ranges from 4 min to 4 hours

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Indeed, this fluctuation in blood zonulin level was studied for a period of 6 d in ICU patients with sepsis, and values were varied by a factor of 2-10 from day to day

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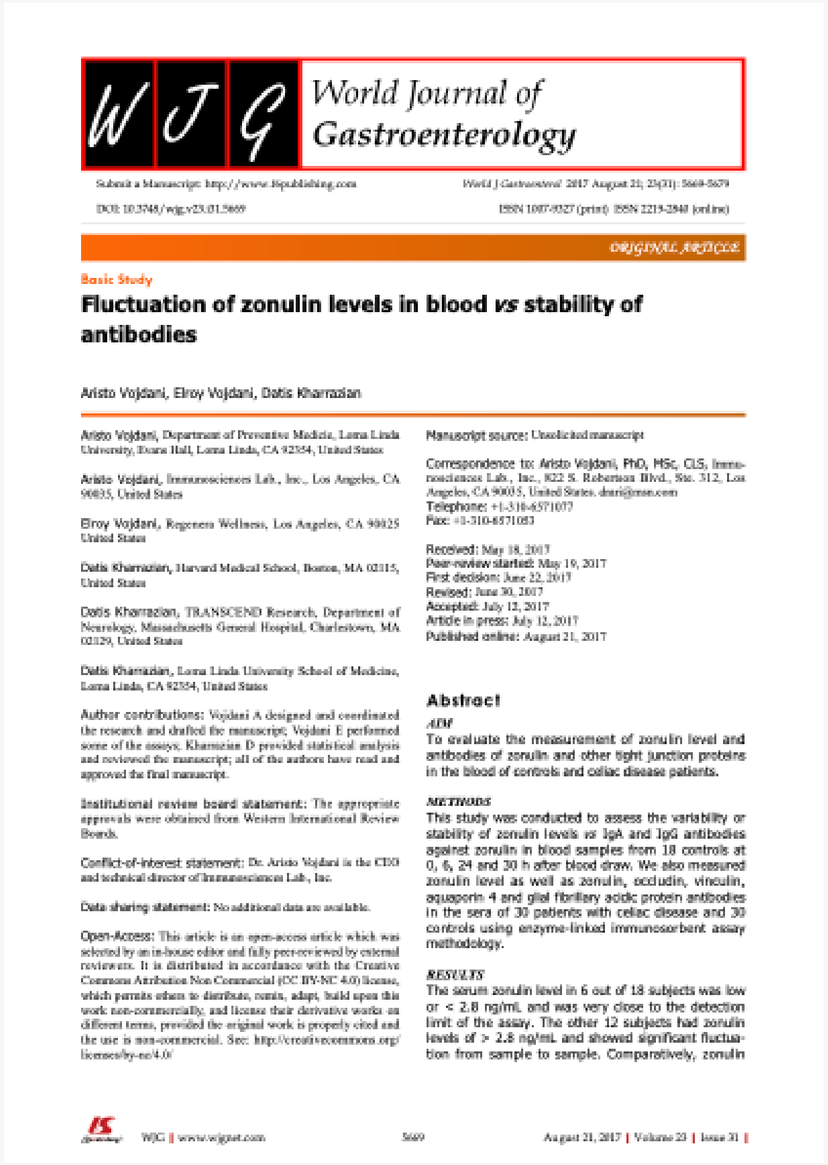

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The half-life of antibodies is about 21 days, the assessment of antibodies against zonulin provides a better clinical picture with one blood draw.

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REVIEW

All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases [version 1; peer review: 3 approved]

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Abstract

Improved hygiene leading to reduced exposure to microorganisms has been implicated as one possible cause for the recent "epidemic" of chronic inflammatory diseases (CIDs) in industrialized countries. That is the essence of the hygiene hypothesis that argues that rising incidence of CIDs may be, at least in part, the result of lifestyle and environmental changes that have made us too "clean" for our own good, so causing changes in our microbiota. Apart from genetic makeup and exposure to environmental triggers, inappropriate increase in intestinal permeability (which may be influenced by the composition of the gut microbiota), a "hyper-belligerent" immune system responsible for the tolerance–immune response balance, and the composition of gut microbiome and its epigenetic influence on the host genomic expression have been identified as three additional elements in causing CIDs. During the past decade, a growing number of publications have focused on human genetics, the gut microbiome, and proteomics, suggesting that loss of mucosal barrier function, particularly in the gastrointestinal tract, may substantially affect antigen trafficking, ultimately influencing the close bidirectional interaction between gut microbiome and our immune system. This cross-talk is highly influential in shaping the host gut immune system function and ultimately shifting genetic predisposition to clinical outcome. This observation led to a re-visitation of the possible causes of CIDs epidemics, suggesting a key pathogenic role of gut permeability. Pre-clinical and clinical studies have shown that the zonulin family, a group of proteins modulating gut permeability, is implicated in a variety of CIDs, including autoimmune, infective, metabolic, and tumoral diseases. These data offer novel therapeutic targets for a variety of CIDs in which the zonulin pathway is implicated in their pathogenesis.

Keywords

Chronic inflammatory diseases, Gut permeability, microbiome, zonulin

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Among the several potential intestinal luminal stimuli that can stimulate zonulin release (thus Intestinal Permeability), small exposure to large amounts of bacteria (and its shell LPS) and gluten, have been identified as the two most powerful triggers

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CLINICAL TAKEAWAY?

When zonulin antibodies are elevated in a stool analysis, consider the Wheat Zoomer to:

- gauge (and demonstrate progress in followups) of excessive intestinal permeability
- Scale the immune reaction to both wheat and LPS
- With leaky gut the ‘Gateway’ in the development of autoimmune disease, consider the Autoimmune Zoomer

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Actin Antibodies

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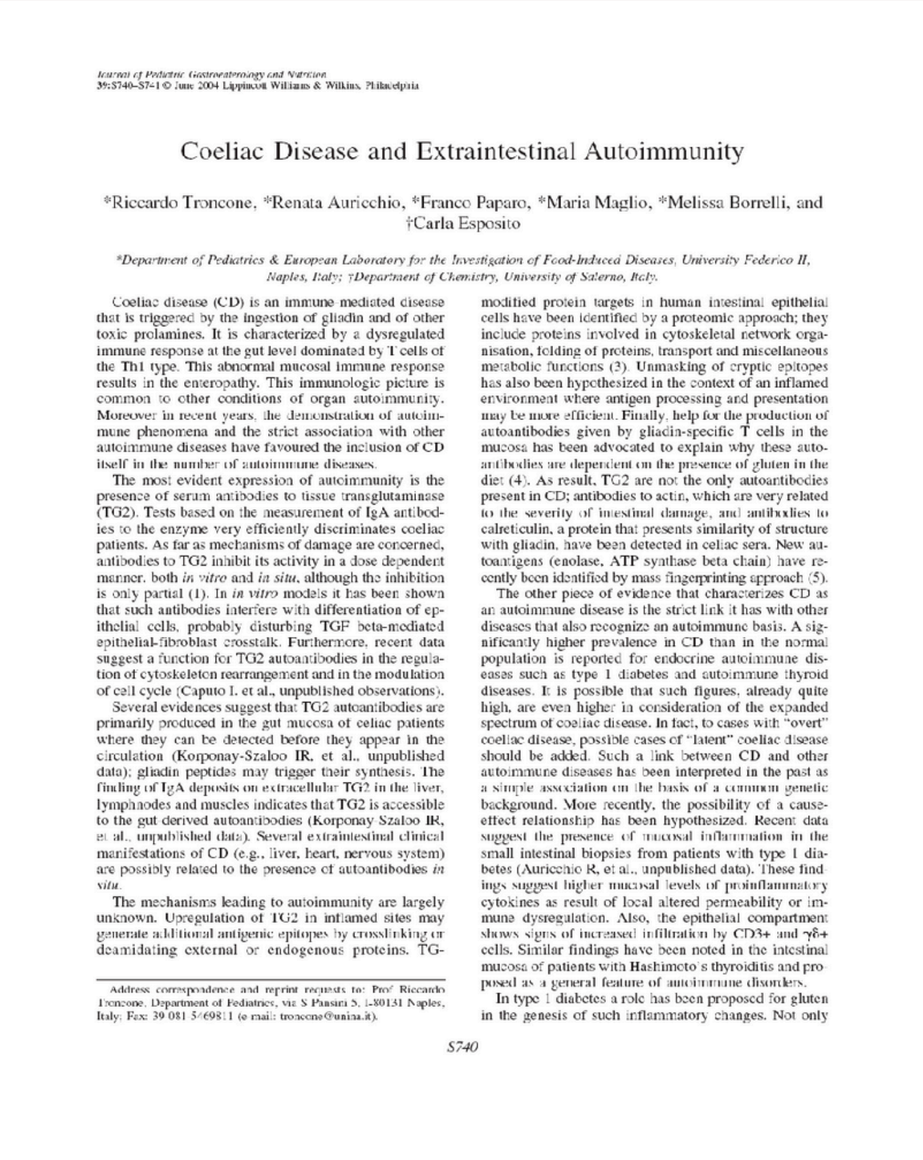

Journal of Pediatric Gastroenterology and Nutrition
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Coeliac Disease and Extraintestinal Autoimmunity

*Riccardo Troncone, *Renata Auricchio, *Franco Paparo, *Maria Maglio, *Melissa Borrelli, and †Carla Esposito

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Coeliac disease (CD) is an immune mediated disease that is triggered by the ingestion of gliadin and of other toxic prolamines. It is characterized by a dysregulated immune response at the gut level dominated by T cells of the Th1 type. This abnormal mucosal immune response results in the enteropathy. This immunologic picture is common to other conditions of organ autoimmunity. Moreover in recent years, the demonstration of autoimmune phenomena and the strict association with other autoimmune diseases have favoured the inclusion of CD itself in the number of autoimmune diseases.

The most evident expression of autoimmunity is the presence of serum antibodies to tissue transglutaminase (TG2). Tests based on the measurement of IgA antibodies to the enzyme very efficiently discriminates coeliac patients. As far as mechanisms of damage are concerned, antibodies to TG2 inhibit its activity in a dose dependent manner, both *in vitro* and *in situ*, although the inhibition is only partial (1). In *in vitro* models it has been shown that such antibodies interfere with differentiation of epithelial cells, probably disturbing TGF beta-mediated epithelial-fibroblast crosstalk. Furthermore, recent data suggest a function for TG2 autoantibodies in the regulation of cytoskeleton rearrangement and in the modulation of cell cycle (Caputo I, et al., unpublished observations).

Several evidences suggest that TG2 autoantibodies are primarily produced in the gut mucosa of celiac patients where they can be detected before they appear in the circulation (Korponay-Szaloo IR, et al., unpublished data); gliadin peptides may trigger their synthesis. The finding of IgA deposits on extracellular TG2 in the liver, lymphnodes and muscles indicates that TG2 is accessible to the gut derived autoantibodies (Korponay Szaloo IR, et al., unpublished data). Several extraintestinal clinical manifestations of CD (e.g., liver, heart, nervous system) are possibly related to the presence of autoantibodies *in situ*.

The mechanisms leading to autoimmunity are largely unknown. Upregulation of TG2 in inflamed sites may generate additional antigenic epitopes by crosslinking or deamidating external or endogenous proteins. TG-modified protein targets in human intestinal epithelial cells have been identified by a proteomic approach; they include proteins involved in cytoskeletal network organisation, folding of proteins, transport and miscellaneous metabolic functions (3). Unmasking of cryptic epitopes has also been hypothesized in the context of an inflamed environment where antigen processing and presentation may be more efficient. Finally, help for the production of autoantibodies given by gliadin-specific T cells in the mucosa has been advocated to explain why these autoantibodies are dependent on the presence of gluten in the diet (4). As result, TG2 are not the only autoantibodies present in CD; antibodies to actin, which are very related to the severity of intestinal damage, and antibodies to calreticulin, a protein that presents similarity of structure with gliadin, have been detected in celiac sera. New autoantigens (enolase, ATP synthase beta chain) have recently been identified by mass fingerprinting approach (5).

The other piece of evidence that characterizes CD as an autoimmune disease is the strict link it has with other diseases that also recognize an autoimmune basis. A significantly higher prevalence in CD than in the normal population is reported for endocrine autoimmune diseases such as type 1 diabetes and autoimmune thyroid diseases. It is possible that such figures, already quite high, are even higher in consideration of the expanded spectrum of coeliac disease. In fact, to cases with "overt" coeliac disease, possible cases of "latent" coeliac disease should be added. Such a link between CD and other autoimmune diseases has been interpreted in the past as a simple association on the basis of a common genetic background. More recently, the possibility of a cause-effect relationship has been hypothesized. Recent data suggest the presence of mucosal inflammation in the small intestinal biopsies from patients with type 1 diabetes (Auricchio R, et al., unpublished data). These findings suggest higher mucosal levels of proinflammatory cytokines as result of local altered permeability or immune dysregulation. Also, the epithelial compartment shows signs of increased infiltration by CD3+ and γδ+ cells. Similar findings have been noted in the intestinal mucosa of patients with Hashimoto's thyroiditis and proposed as a general feature of autoimmune disorders.

In type 1 diabetes a role has been proposed for gluten in the genesis of such inflammatory changes. Not only

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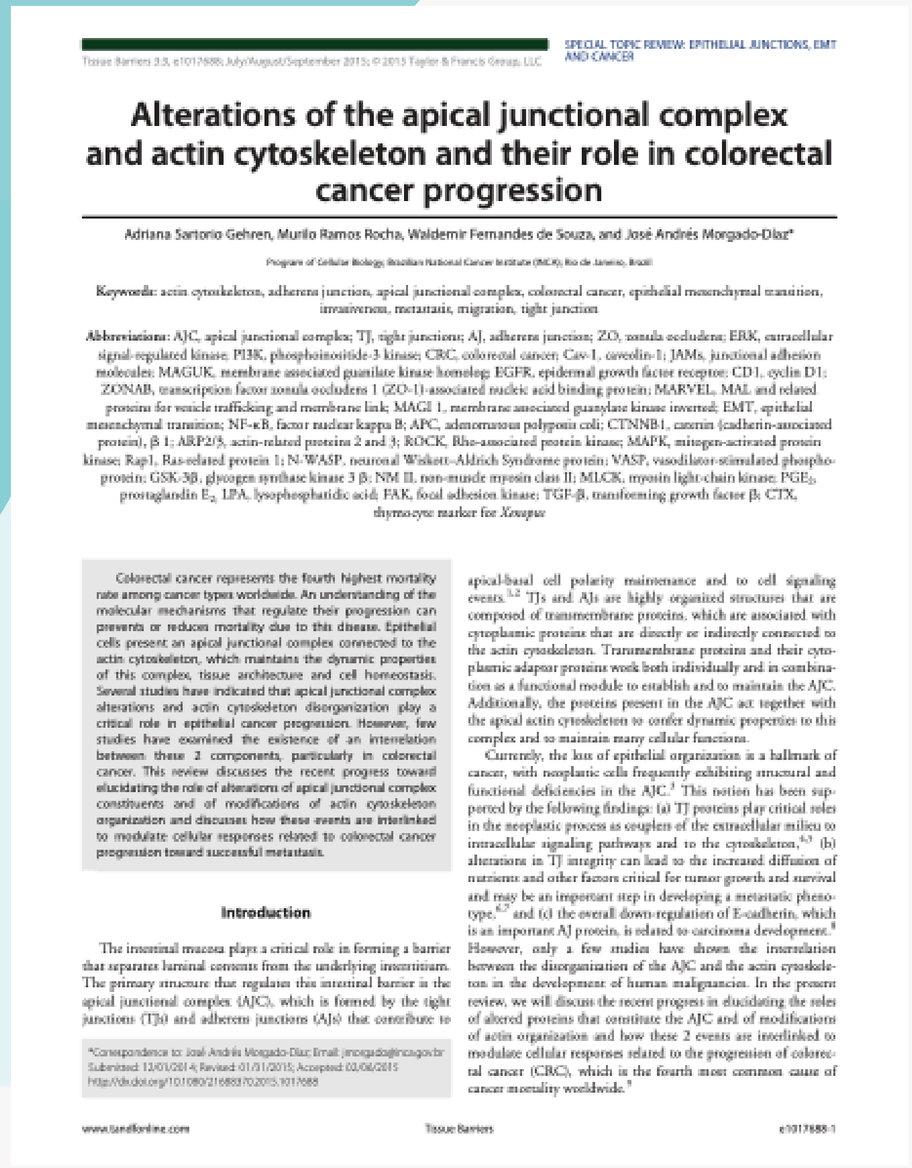

Tissue Barriers 3:3, e1017688; July/August/September 2015; © 2015 Taylor & Francis Group, LLC

Alterations of the apical junctional complex and actin cytoskeleton and their role in colorectal cancer progression

Adriana Sartorio Gehren, Murilo Ramos Rocha, Waldemir Fernandes de Souza, and José Andrés Morgado-Díaz*

Program of Cellular Biology, Brazilian National Cancer Institute (INCA); Rio de Janeiro, Brazil

Keywords: actin cytoskeleton, adherens junction, apical junctional complex, colorectal cancer, epithelial mesenchymal transition, invasiveness, metastasis, migration, tight junction

Abbreviations: AJC, apical junctional complex; TJ, tight junctions; AJ, adherens junction; ZO, zonula occludens; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide-3 kinase; CRC, colorectal cancer; Cav-1, caveolin-1; JAMs, junctional adhesion molecules; MAGUK, membrane associated guanilate kinase homolog; EGFR, epidermal growth factor receptor; CD1, cyclin D1; ZONAB, transcription factor zonula occludens 1 (ZO-1)-associated nucleic acid binding protein; MARVEL, MAL and related proteins for vesicle trafficking and membrane link; MAGI 1, membrane associated guanylate kinase inverted; EMT, epithelial mesenchymal transition; NF-κB, factor nuclear kappa B; APC, adenomatous polyposis coli; CTNNB1, catenin (cadherin-associated protein), β 1; ARP2/3, actin-related proteins 2 and 3; ROCK, Rho-associated protein kinase; MAPK, mitogen-activated protein kinase; Rap1, Ras-related protein 1; N-WASP, neuronal Wiskott–Aldrich Syndrome protein; VASP, vasodilator-stimulated phosphoprotein; GSK-3β, glycogen synthase kinase 3 β; NM II, non-muscle myosin class II; MLCK, myosin light-chain kinase; PGE_2, prostaglandin E_2; LPA, lysophosphatidic acid; FAK, focal adhesion kinase; TGF-β, transforming growth factor β; CTX, thymocyte marker for *Xenopus*

Colorectal cancer represents the fourth highest mortality rate among cancer types worldwide. An understanding of the molecular mechanisms that regulate their progression can prevents or reduces mortality due to this disease. Epithelial cells present an apical junctional complex connected to the actin cytoskeleton, which maintains the dynamic properties of this complex, tissue architecture and cell homeostasis. Several studies have indicated that apical junctional complex alterations and actin cytoskeleton disorganization play a critical role in epithelial cancer progression. However, few studies have examined the existence of an interrelation between these 2 components, particularly in colorectal cancer. This review discusses the recent progress toward elucidating the role of alterations of apical junctional complex constituents and of modifications of actin cytoskeleton organization and discusses how these events are interlinked to modulate cellular responses related to colorectal cancer progression toward successful metastasis.

Introduction

The intestinal mucosa plays a critical role in forming a barrier that separates luminal contents from the underlying interstitium. The primary structure that regulates this intestinal barrier is the apical junctional complex (AJC), which is formed by the tight junctions (TJs) and adherens junctions (AJs) that contribute to apical-basal cell polarity maintenance and to cell signaling events.[1,2] TJs and AJs are highly organized structures that are composed of transmembrane proteins, which are associated with cytoplasmic proteins that are directly or indirectly connected to the actin cytoskeleton. Transmembrane proteins and their cytoplasmic adaptor proteins work both individually and in combination as a functional module to establish and to maintain the AJC. Additionally, the proteins present in the AJC act together with the apical actin cytoskeleton to confer dynamic properties to this complex and to maintain many cellular functions.

Currently, the loss of epithelial organization is a hallmark of cancer, with neoplastic cells frequently exhibiting structural and functional deficiencies in the AJC.[3] This notion has been supported by the following findings: (a) TJ proteins play critical roles in the neoplastic process as couplers of the extracellular milieu to intracellular signaling pathways and to the cytoskeleton,[4,5] (b) alterations in TJ integrity can lead to the increased diffusion of nutrients and other factors critical for tumor growth and survival and may be an important step in developing a metastatic phenotype,[6,7] and (c) the overall down-regulation of E-cadherin, which is an important AJ protein, is related to carcinoma development.[8] However, only a few studies have shown the interrelation between the disorganization of the AJC and the actin cytoskeleton in the development of human malignancies. In the present review, we will discuss the recent progress in elucidating the roles of altered proteins that constitute the AJC and of modifications of actin organization and how these 2 events are interlinked to modulate cellular responses related to the progression of colorectal cancer (CRC), which is the fourth most common cause of cancer mortality worldwide.[9]

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Epithelial cells present an apical junctional complex connected to the actin cytoskeleton, which maintains the dynamic properties of this complex, tissue architecture and cell homeostasis.

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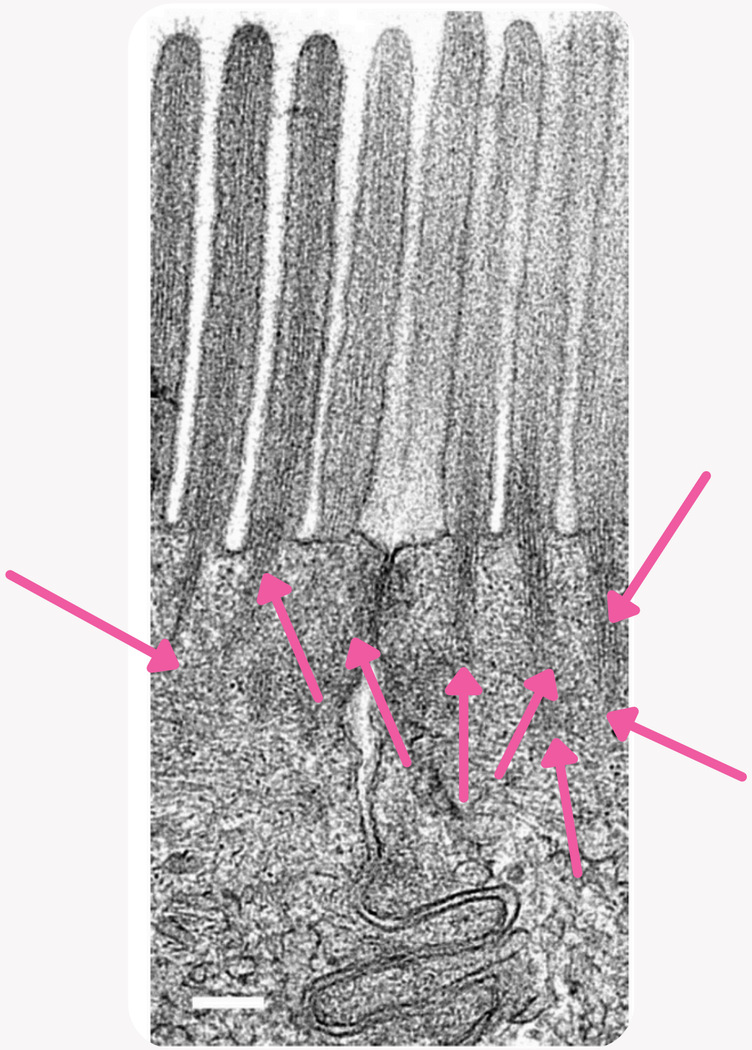
Actin-myosin network



Each microvillus contains a core bundle of ~20–30 parallel actin filaments.

Amer Jour of Path, Vol. 169, No. 6, Dec 2006

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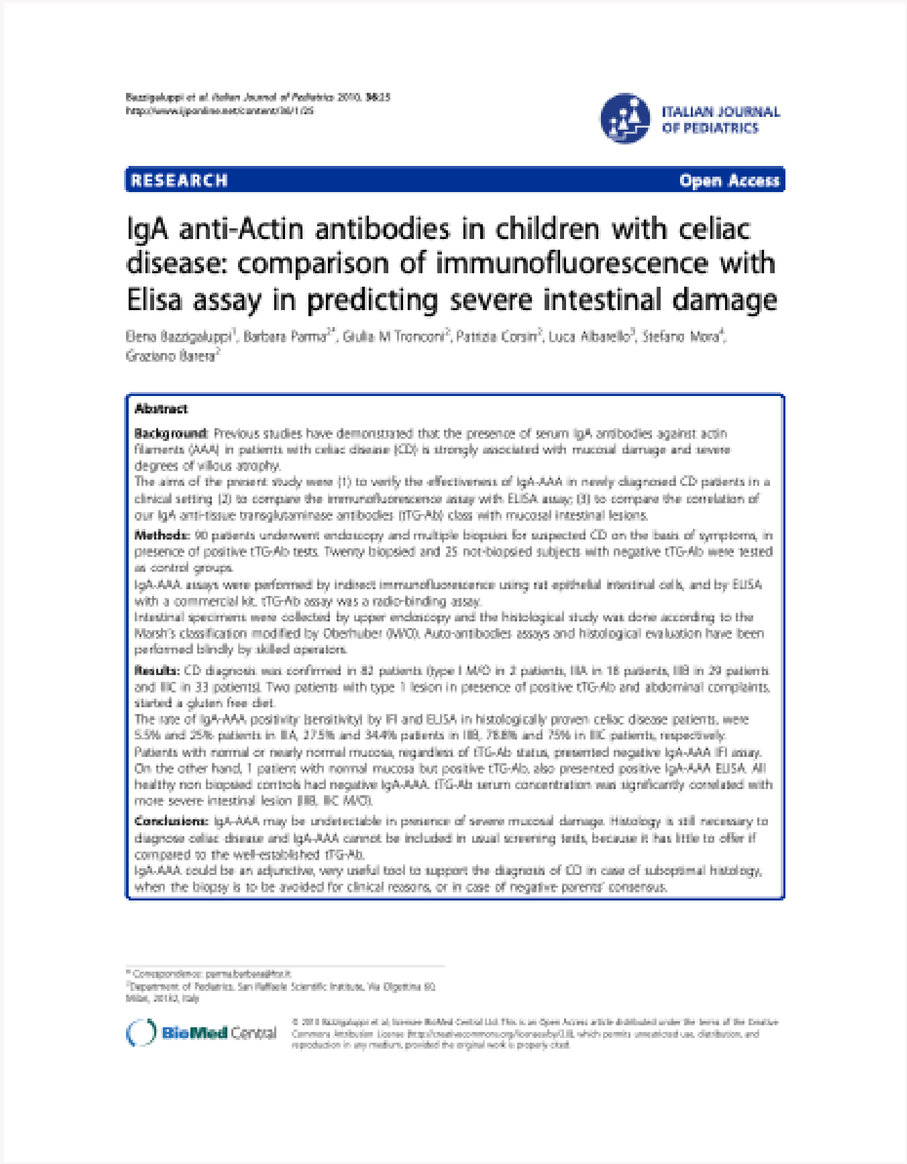

Bazzigaluppi et al. Italian Journal of Pediatrics 2010, 36:25
http://www.ijponline.net/content/36/1/25

ITALIAN JOURNAL OF PEDIATRICS

RESEARCH Open Access

IgA anti-Actin antibodies in children with celiac disease: comparison of immunofluorescence with Elisa assay in predicting severe intestinal damage

Elena Bazzigaluppi[1], Barbara Parma[2*], Giulia M Tronconi[2], Patrizia Corsin[2], Luca Albarello[3], Stefano Mora[4], Graziano Barera[2]

Abstract

Background: Previous studies have demonstrated that the presence of serum IgA antibodies against actin filaments (AAA) in patients with celiac disease (CD) is strongly associated with mucosal damage and severe degrees of villous atrophy.
The aims of the present study were (1) to verify the effectiveness of IgA-AAA in newly diagnosed CD patients in a clinical setting (2) to compare the immunofluorescence assay with ELISA assay; (3) to compare the correlation of our IgA anti-tissue transglutaminase antibodies (tTG-Ab) class with mucosal intestinal lesions.

Methods: 90 patients underwent endoscopy and multiple biopsies for suspected CD on the basis of symptoms, in presence of positive tTG-Ab tests. Twenty biopsied and 25 not-biopsied subjects with negative tTG-Ab were tested as control groups.
IgA-AAA assays were performed by indirect immunofluorescence using rat epithelial intestinal cells, and by ELISA with a commercial kit. tTG-Ab assay was a radio-binding assay.
Intestinal specimens were collected by upper endoscopy and the histological study was done according to the Marsh's classification modified by Oberhuber (MVO). Auto-antibodies assays and histological evaluation have been performed blindly by skilled operators.

Results: CD diagnosis was confirmed in 82 patients (type I MVO in 3 patients, IIIA in 18 patients, IIIB in 29 patients and IIIC in 33 patients). Two patients with type 1 lesion in presence of positive tTG-Ab and abdominal complaints, started a gluten free diet.
The rate of IgA-AAA positivity (sensitivity) by IFI and ELISA in histologically proven celiac disease patients, were 5.5% and 25% patients in IIIA, 27.5% and 34.4% patients in IIIB, 78.8% and 75% in IIIC patients, respectively.
Patients with normal or nearly normal mucosa, regardless of tTG-Ab status, presented negative IgA-AAA IFI assay. On the other hand, 1 patient with normal mucosa but positive tTG-Ab, also presented positive IgA-AAA ELISA. All healthy non biopsied controls had negative IgA-AAA. tTG-Ab serum concentration was significantly correlated with more severe intestinal lesion (IIIB, IIC MVO).

Conclusions: IgA-AAA may be undetectable in presence of severe mucosal damage. Histology is still necessary to diagnose celiac disease and IgA-AAA cannot be included in usual screening tests, because it has little to offer if compared to the well-established tTG-Ab.
IgA-AAA could be an adjunctive, very useful tool to support the diagnosis of CD in case of suboptimal histology, when the biopsy is to be avoided for clinical reasons, or in case of negative parents' consensus.

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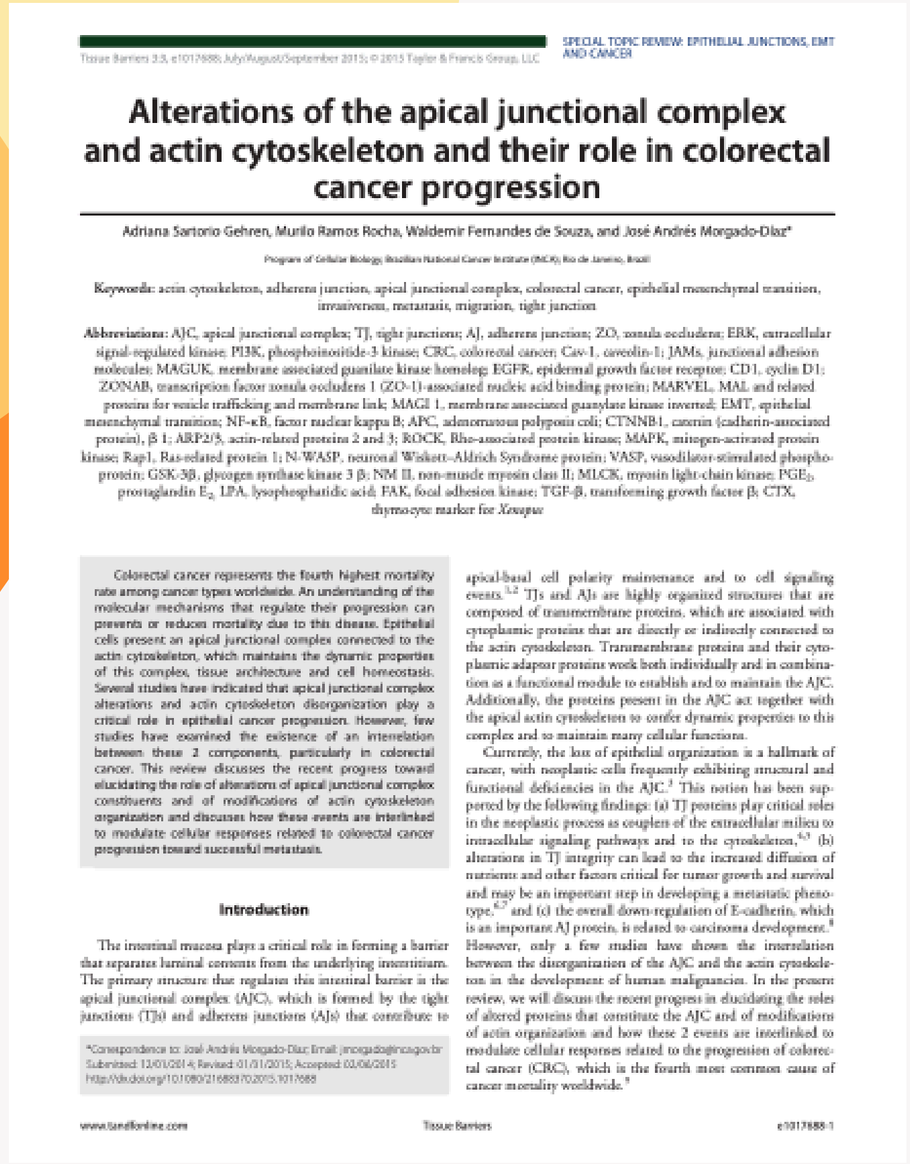
Serum IgA antibodies against actin filaments (AAA) in patients with celiac disease (CD) is strongly associated with mucosal damage and severe degrees of villous atrophy.

Italian Journal of Pediatrics 2010, 36:25



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Introduction

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Currently, the loss of epithelial organization is a hallmark of cancer, with neoplastic cells frequently exhibiting structural and functional deficiencies in the AJC.[3] This notion has been supported by the following findings: (a) TJ proteins play critical roles in the neoplastic process as couplers of the extracellular milieu to intracellular signaling pathways and to the cytoskeleton,[4,5] (b) alterations in TJ integrity can lead to the increased diffusion of nutrients and other factors critical for tumor growth and survival and may be an important step in developing a metastatic phenotype,[6,7] and (c) the overall down-regulation of E-cadherin, which is an important AJ protein, is related to carcinoma development.[8] However, only a few studies have shown the interrelation between the disorganization of the AJC and the actin cytoskeleton in the development of human malignancies. In the present review, we will discuss the recent progress in elucidating the roles of altered proteins that constitute the AJC and of modifications of actin organization and how these 2 events are interlinked to modulate cellular responses related to the progression of colorectal cancer (CRC), which is the fourth most common cause of cancer mortality worldwide.[9]

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Several studies have indicated that apical junctional complex alterations and actin cytoskeleton disorganization play a critical role in epithelial cancer progression.

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nutrients

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Review

Intestinal Barrier Function in Gluten-Related Disorders

Danielle Cardoso-Silva [1,†], Deborah Delbue [1,†], Alice Itzlinger [1], Renée Moerkens [2], Sebo Withoff [2], Federica Branchi [1] and Michael Schumann [1,*]

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Abstract: Gluten-related disorders include distinct disease entities, namely celiac disease, wheat-associated allergy and non-celiac gluten/wheat sensitivity. Despite having in common the contact of the gastrointestinal mucosa with components of wheat and other cereals as a causative factor, these clinical entities have distinct pathophysiological pathways. In celiac disease, a T-cell mediate immune reaction triggered by gluten ingestion is central in the pathogenesis of the enteropathy, while wheat allergy develops as a rapid immunoglobulin E- or non-immunoglobulin E-mediated immune response. In non-celiac wheat sensitivity, classical adaptive immune responses are not involved. Instead, recent research has revealed that an innate immune response to a yet-to-be-defined antigen, as well as the gut microbiota, are pivotal in the development in this disorder. Although impairment of the epithelial barrier has been described in all three clinical conditions, its role as a potential pathogenetic co-factor, specifically in celiac disease and non-celiac wheat sensitivity, is still a matter of investigation. This article gives a short overview of the mucosal barrier of the small intestine, summarizes the aspects of barrier dysfunction observed in all three gluten-related disorders and reviews literature data in favor of a primary involvement of the epithelial barrier in the development of celiac disease and non-celiac wheat sensitivity.

Keywords: epithelial barrier; permeability; celiac disease; non-celiac gluten sensitivity; non-celiac wheat sensitivity; wheat allergy

1. The Intestinal Barrier

The intestinal barrier has a crucial role in protecting the organism against pathogens and possible harmful substances derived from the external environment (Figure 1). It is formed by a mucus and epithelial layer and by the lamina propria underneath. Immune cells, components of the intestinal microbiota and anti-microbial peptides have crucial functions in maintaining the intestinal barrier function [1,2].

Gliadin exposure alone alters the barrier properties of intestinal epithelial cells.

Nutrients 2019, 11, 2325;

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218

COELIAC DISEASE

Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function

M G Clemente, S De Virgiliis, J S Kang, R Macatagney, M P Musu, M R Di Pierro, S Drago, M Congia, A Fasano

Gut 2003;52:218–223

See end of article for authors' affiliations

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Accepted for publication 9 September 2002

Background and aims: Despite the progress made in understanding the immunological aspects of the pathogenesis of coeliac disease (CD), the early steps that allow gliadin to cross the intestinal barrier are still largely unknown. The aim of this study was to establish whether gliadin activates a zonulin dependent enterocyte intracellular signalling pathway(s) leading to increased intestinal permeability.
Methods: The effect of gliadin on the enterocyte actin cytoskeleton was studied on rat intestinal epithelial (IEC-6) cell cultures by fluorescence microscopy and spectrofluorimetry. Zonulin concentration was measured on cell culture supernatants by enzyme linked immunosorbent assay. Transepithelial intestinal resistance (Rt) was measured on ex vivo intestinal tissues mounted in Ussing chambers.
Results: Incubation of cells with gliadin led to a reversible protein kinase C (PKC) mediated actin polymerisation temporarily coincident with zonulin release. A significant reduction in Rt was observed after gliadin addition on rabbit intestinal mucosa mounted in Ussing chambers. Pretreatment with the zonulin inhibitor FZI/0 abolished the gliadin induced actin polymerisation and Rt reduction but not zonulin release.
Conclusions: Gliadin induces zonulin release in intestinal epithelial cells in vitro. Activation of the zonulin pathway by PKC mediated cytoskeleton reorganisation and tight junction opening leads to a rapid increase in intestinal permeability.

Coeliac disease (CD) is an autoimmune enteropathy triggered by ingestion of gluten containing grains in genetically susceptible individuals. The gliadin fraction of wheat gluten represents the environmental factor responsible for the development of the intestinal damage typical of the disease.[1] While in recent years we have witnessed significant progress on the immunological aspects of CD pathogenesis,[2] no major achievements have been made in understanding the early steps that allow gliadin to cross the intestinal epithelial barrier to be recognised by the intestinal immune system.[3] Gliadin deamidation by tissue transglutaminase has been demonstrated to enhance the recognition of gliadin peptides by HLA DQ2/DQ8 T cells in genetically predisposed subjects and it might initiate the cascade of autoimmune reactions which are finally responsible for mucosal destruction through production of cytokines and matrix metalloproteinases.[4 5] These reactions imply that gliadin and/or its breakdown peptides in someway cross the intestinal epithelial barrier and reach the lamina propria of the intestinal mucosa where they are recognised by antigen presenting cells. Under physiological circumstances the intestinal epithelial barrier is described as being almost impermeable to macromolecules.[6] However, CD is characterised by enhanced paracellular permeability across intestinal epithelium— that is, "leaky gut", a condition that would allow passage of macromolecules through the paracellular space.[7 8] We have recently reported that zonulin, a modulator of tight junction (tj) permeability,[9] is upregulated during the acute phase of CD.[10] Following binding to its surface receptor, zonulin induces a protein kinase C (PKC) mediated polymerisation of intracellular actin filaments which are directly connected to structural proteins of the tj hence regulating epithelial permeability.[11 12] The complex actin cytoskeleton network of the enterocyte is known to be involved in the intracellular trafficking of molecules as well as in the regulation of paracellular permeability by its direct interaction with the tj structural proteins.[13 14] This study was aimed at establishing the interplay between gliadin and the enterocyte, with specific emphasis on the effect of gliadin on zonulin release and subsequent activation of intracellular signalling leading to the disassembly of intercellular tj.

METHODS

IEC-6 cell cultures

Rat intestinal epithelial cells (IEC-6 cells) were grown in cell culture flasks (Falcon Labware, Reston, Virginia, USA) at 37°C in an atmosphere of 95% air and 5% CO_2. The medium consisted of Dulbecco's modified Eagle's medium (Gibco, Rockville, Maryland, USA) containing 4500 mg/l D-glucose, pyridoxine hydrochloride, 5% heat inactivated (56°C, 30 minute) fetal bovine serum, 0.1 U/ml bovine insulin, 4 mM L-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin.

Gliadin peptides

Gliadin (Sigma, St Louis, Missouri, USA) was freshly prepared in a 70% ethanol solution (20 mg/ml) and used at serial dilutions in the cell culture medium, ranging from the 1:20 dilution (final concentration: gliadin 1 mg/ml; ethanol 3.5%) to the 1:200 dilution (final concentration: gliadin 0.1 mg/ml; ethanol 0.35%). The pH was adjusted to 7.4 when necessary by 1 M NaOH buffer. Similar ethanol concentrations were added to the final concentration of bovine serum albumin (BSA) and zein from maize (Sigma) used as negative controls. Ethanol concentration was never more than 3.5% in the final solution in order to avoid any direct effect of ethanol on cultured cells. Synthetic peptides 31–55 and 22–59 (Biopolymer Laboratories, University of Maryland, Baltimore, Maryland, USA) were

Abbreviations: CD, coeliac disease; Rt, transepithelial electrical resistance; Zot, zonula occludens toxin; tj, tight junctions; PKC, protein kinase C; BSA, bovine serum albumin; PBS, phosphate buffered saline; CV, coefficient of variation.

Gut 2003;52:218–223

The cellular response observed only a few minutes after gliadin (exposure) was characterized by significant cytoskeleton reorganization with a redistribution of actin filaments mainly in the intracellular subcortical compartment.

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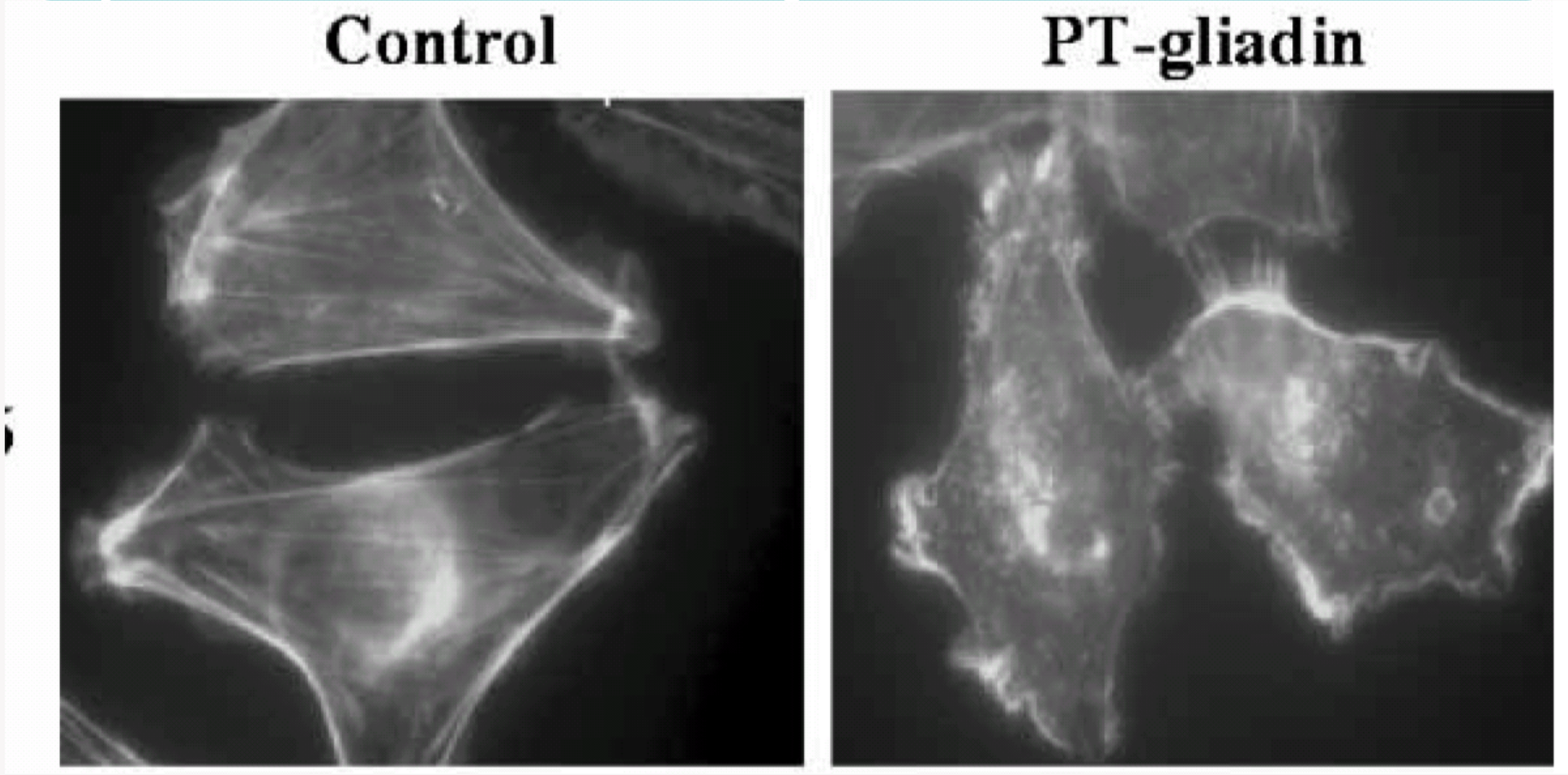
Figure 2. Effect of gliadin on intestinal epithelial cells cytoskeleton leads to a reorganization of actin filaments



Scand J of Gastro, 2006; 41: 408/419

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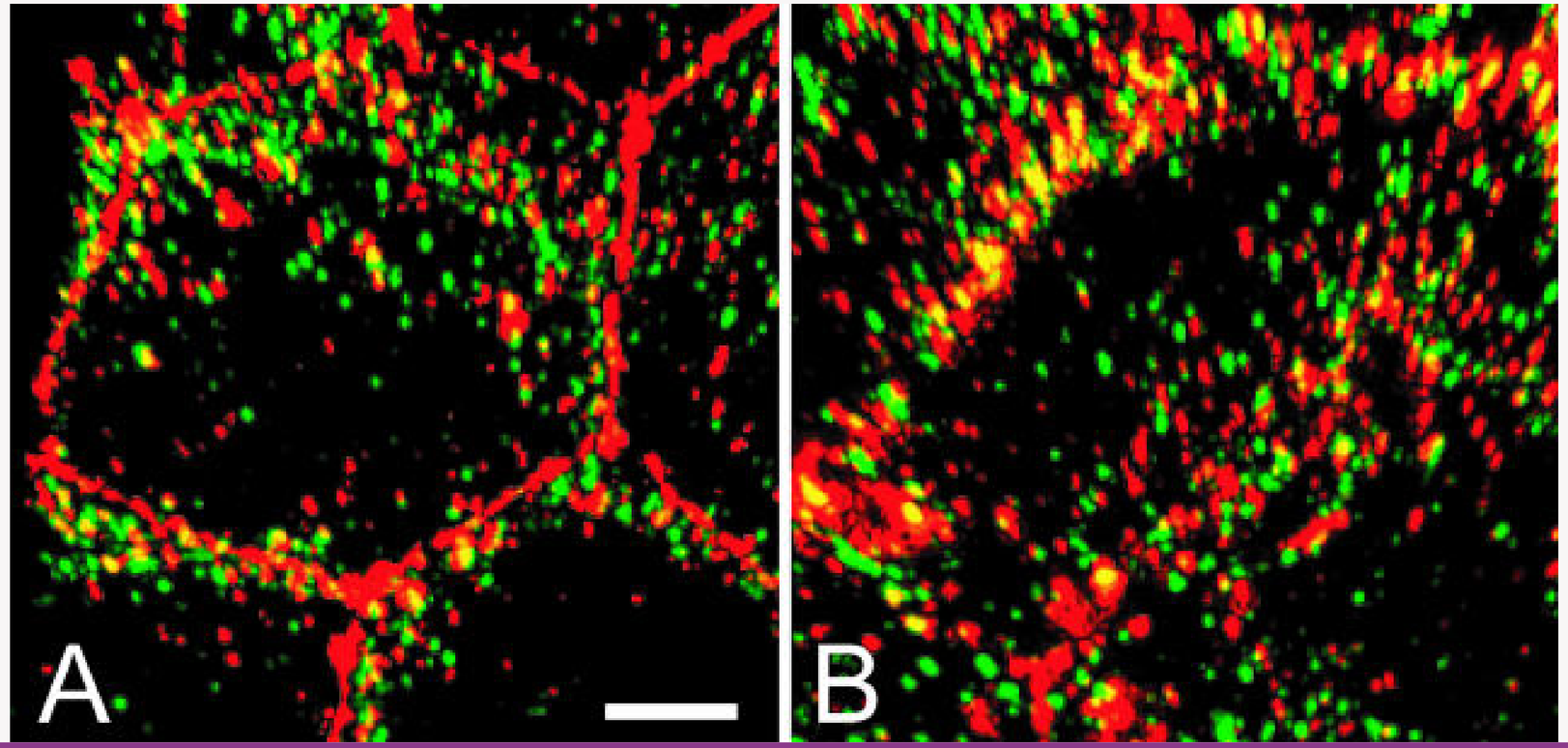

Actin depolymerization induces caveolae-mediated occludin endocytosis. Three-dimensional projections of monolayers labeled for occludin (red) and caveolin-1 (green), a marker of caveolae, are shown. Rather than the ordered appearance of occludin encircling the apical portion of the cell with few intracellular occludin-continuing vesicles in control epithelia (A), abundant intracellular occludin-continuing vesicles are evident after actin depolymerization (B). Many of these newly formed vesicles also contain caveolin-1, as apparent from the yellow colocalization signal. Bar = 5 μm.

Am J Pathol. 2006 Dec;169(6):1901-9

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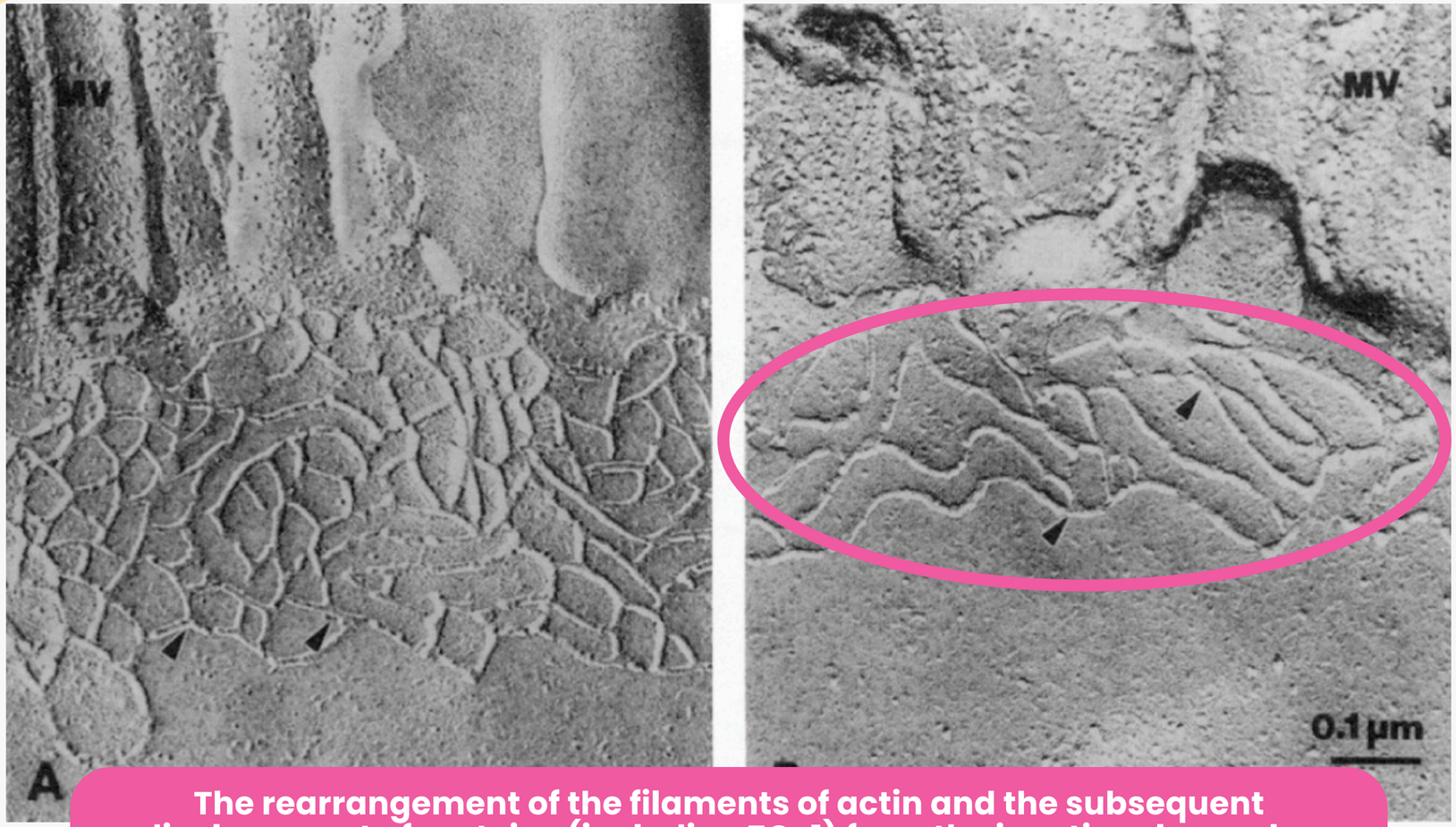

The rearrangement of the filaments of actin and the subsequent displacement of proteins (including ZO-1) from the junctional complex

Ann. N.Y. Acad. Sci. 1165: 195–205 (2009).

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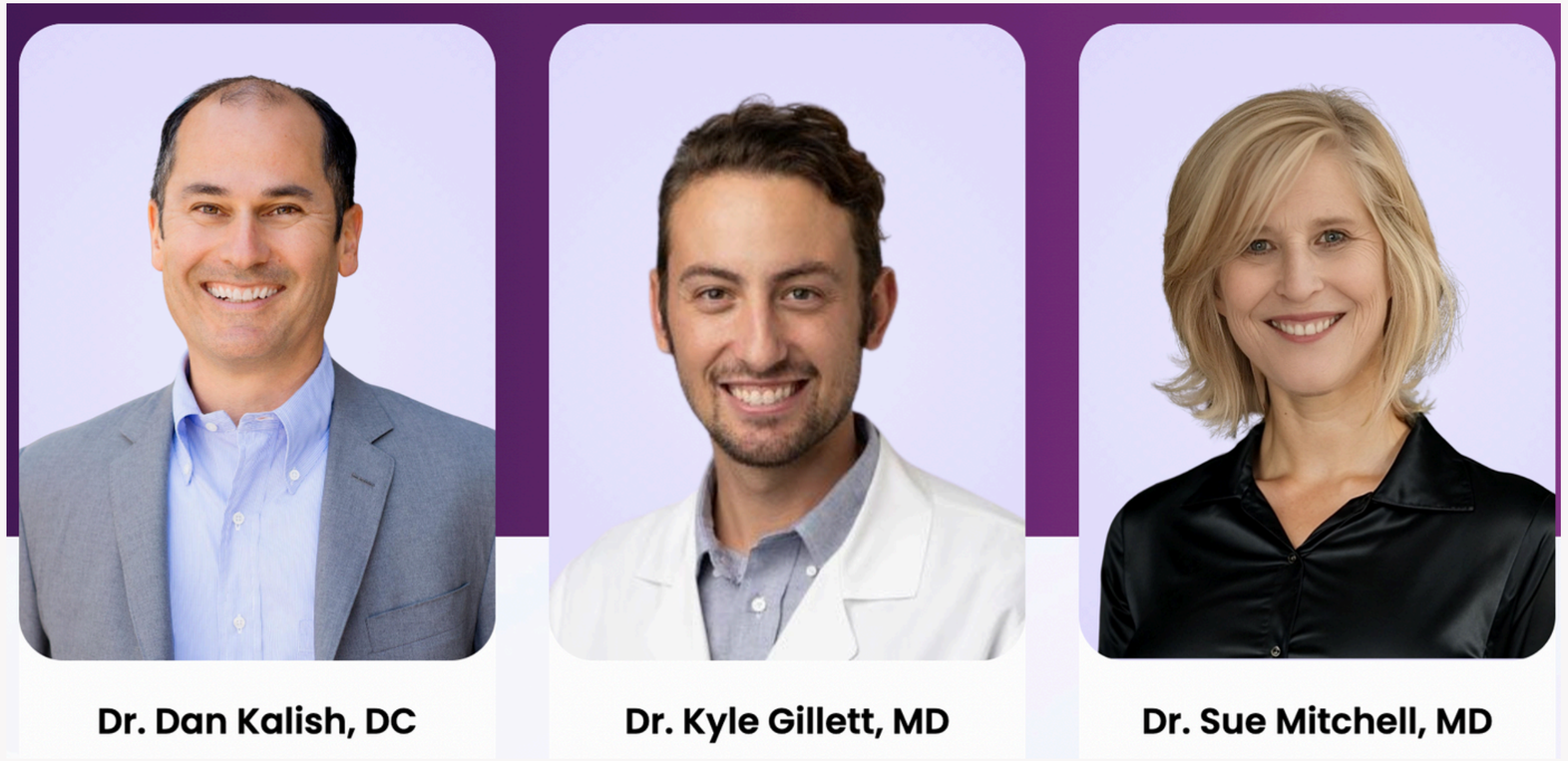
3 Excellent Presentations on Bringing Balance and Achieving Excellent Results with Protocols Designed to Heal the Gut



Dr. Dan Kalish, DC

Dr. Kyle Gillett, MD

Dr. Sue Mitchell, MD

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Sue Mitchell, MD
Board-Certified Gastroenterologist

My Gastroparesis Diet

Nourishing the Microbiome

Berries (blueberries, raspberries, etc.)
Prebiotic & Phytonutrients

Green banana - optional
Prebiotic

Inflammacore Powder
Protein/Glutamine/Antioxidants

Goat milk
Prebiotic (HMO)

Goat milk kefir
Probiotic

Add Protein
Add SBI/Sacc B, etc.

*Developed with Dr. Margaret Harris, PhD

15-19

Goals:

Rest stomach - Optimize MMC's - Nourish proximal small intestine

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Sue Mitchell, MD
Board-Certified Gastroenterologist

Two Week Detox Protocol

Breakfast
Healing
Smoothie
30-35 g protein

Lunch
Soft, easy to digest
Ground meats/ steamed
veggies
30-35 g protein

Dinner
Healing Smoothie
30-35 g protein
Optimize nighttime
MMC's

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Dr. Dan Kalish DC, *IFMCP*

Founder, Kalish Institute of Functional Medicine

Case Study #1: Gut + Hormones + Detox + Oxidative Stress
Female patient, age 34, PMS, fatigue, and occasional GI symptoms – big picture, impaired estrogen clearance, oxidative stress, estrogen metabolites, cancer risk

Case Study #2: Gut + Immune + Absorption + Cardiovascular Male age 59, Diagnosis of MALT lymphoma

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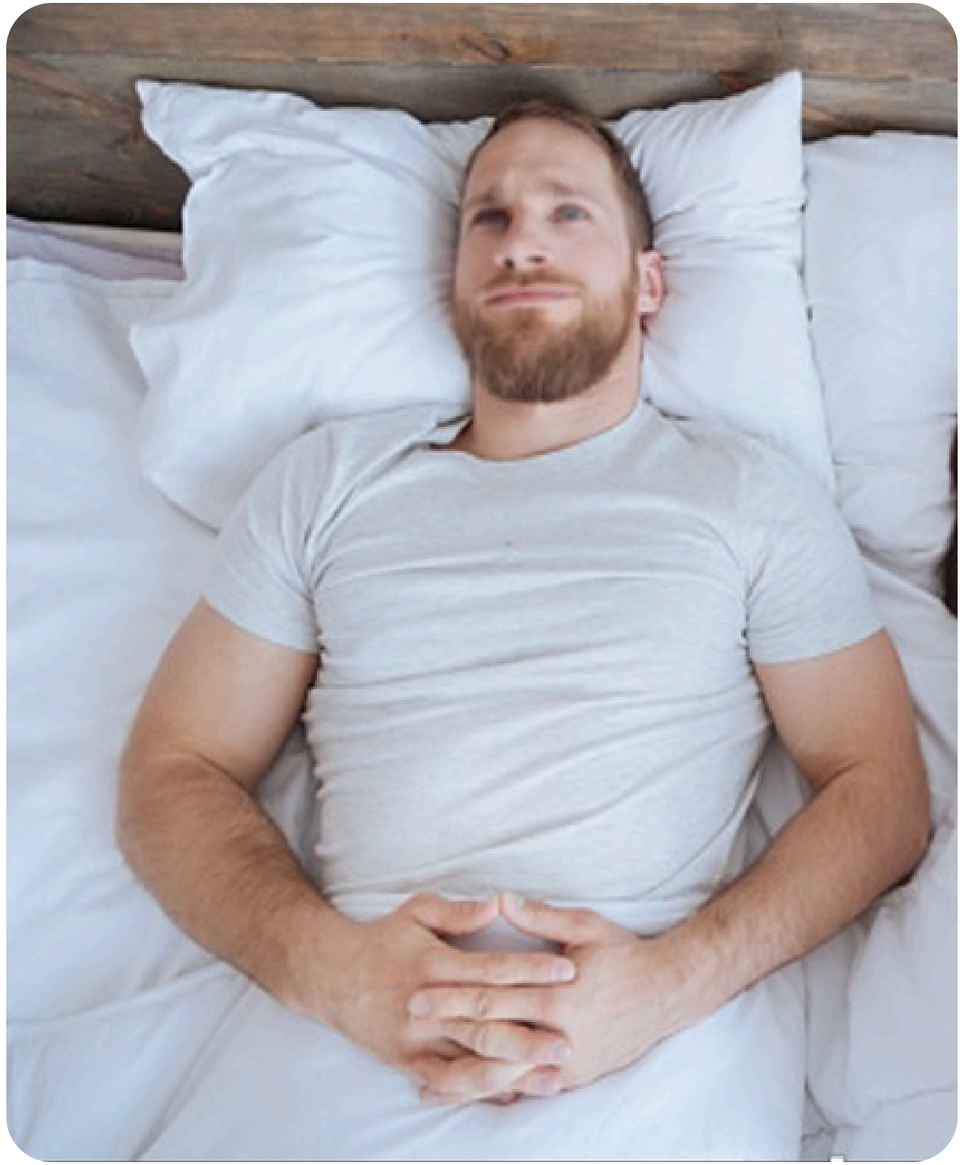

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A Gift For You

The presentation, plus ALL of 51 are available to you for free at www.theDr.com/longevity

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Take Care of Yourself

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Make Sure to Tell those Important to You How Much You Love them

The Vibrant Longevity Summit


Thank You for Your Kind Attention
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Wishing you Sunrises of Beauty
throughout your life
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Summit

The Gut Microbiome Connection

Advancing Systemic Health Protocols

Session 2

Dr. Dan Kalish, DC

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The Gut Microbiome Connection

Testing Solutions

Meet Your Speaker

Dr. Dan Kalish

***DC**, IFMCP*

Founder, Kalish Institute
of Functional Medicine

The Gut’s Impact on Key Body Systems

- Hormone System
- Detoxification System
- Immune System
- Cardiovascular System
- Oxidative Stress/Nutrient Absorption

30-minute systems-level overview of Gut Zoomer test report using case studies – systems, not symptoms

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Testing Packages

While we are focusing on GI testing today, I use a foundational longevity work up that includes:

- Gut Zoomer
- Hormone Zoomer
- Total Tox Burden and Oxidative Stress Profile

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Case Study #1: Gut + Hormones + Detox + Oxidative Stress

- Female patient, age 34, PMS, fatigue, and occasional GI symptoms – big picture, impaired estrogen clearance, oxidative stress, estrogen metabolites, cancer risk
- Key findings:
  - High beta-glucuronidase > poor glucuronidation > recirculation of estrogens
  - Low glutathione + high toxin markers on Total Tox > poor clearance of both hormones and environmental chemicals
  - Estrogen metabolite issues on Hormone Zoomer

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Case Study #2: Gut + Immune + Absorption + Cardiovascular

- Male age 59, long history of heartburn, no major GI symptoms
- Key findings:
  - H. pylori identified via RT-PCR despite prior negative workup at top hospital
  - Diagnosis of MALT lymphoma links gut-immune dysfunction
  - Evidence of malabsorption of fats > decreased absorption of fat-soluble antioxidants
  - High oxidative stress despite taking antioxidants and clean diet

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GI Inflammation Impacts on Longevity

Inflammation from GI issues can lead to systemic inflammation and oxidative stress, in turn impacting impact longevity:

- DNA damage (8-OHDG)
- Lipid damage (lipid peroxides)
- Mitochondrial dysfunction (organic acids)

Brook, Wellness Program for Healthy Pregnancy

Referred by her mother, a former patient

- Social worker, high stress but rewarding job
- PMS symptoms, debilitating fatigue 2-3 days a month, seemed like obvious PMS issue
- Emotionally healthy, ready to start a family and wants to spend a year preparing

Pathways, Root Causes

1. Beta glucuronidation (pathway)
2. GI pathogens (root cause)

(3) Estrogen metabolites/estrogen dominance (pathway)

(4)Toxin burden (root cause)

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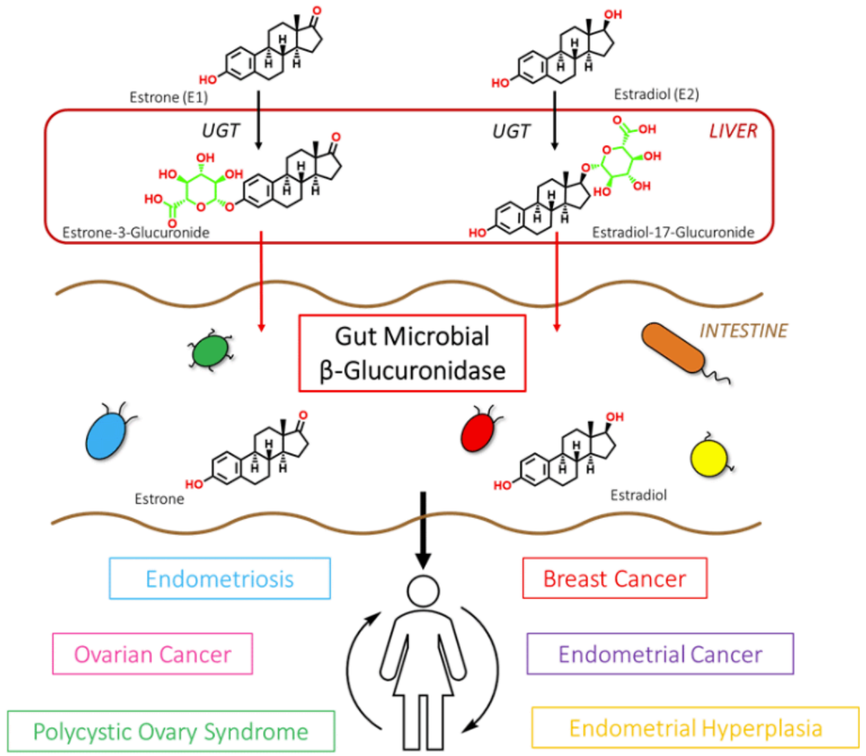
Essential Longevity Tests Reveal

High beta glucuronidase and parasitic infection,

- Gut Zoomer - Cryptosporidium, e. histo
- Hormones Zoomer – Estrogen metabolite issues, low progesterone
- Total Tox Burden and Oxidative Stress Profile - high levels oxidative stress low glutathione



Gut microbiome–derived β-glucuronidases are components of the estrobolome that reactivate estrogens

October 2019 · Journal of Biological Chemistry
294(49):jbc.RA119.010950

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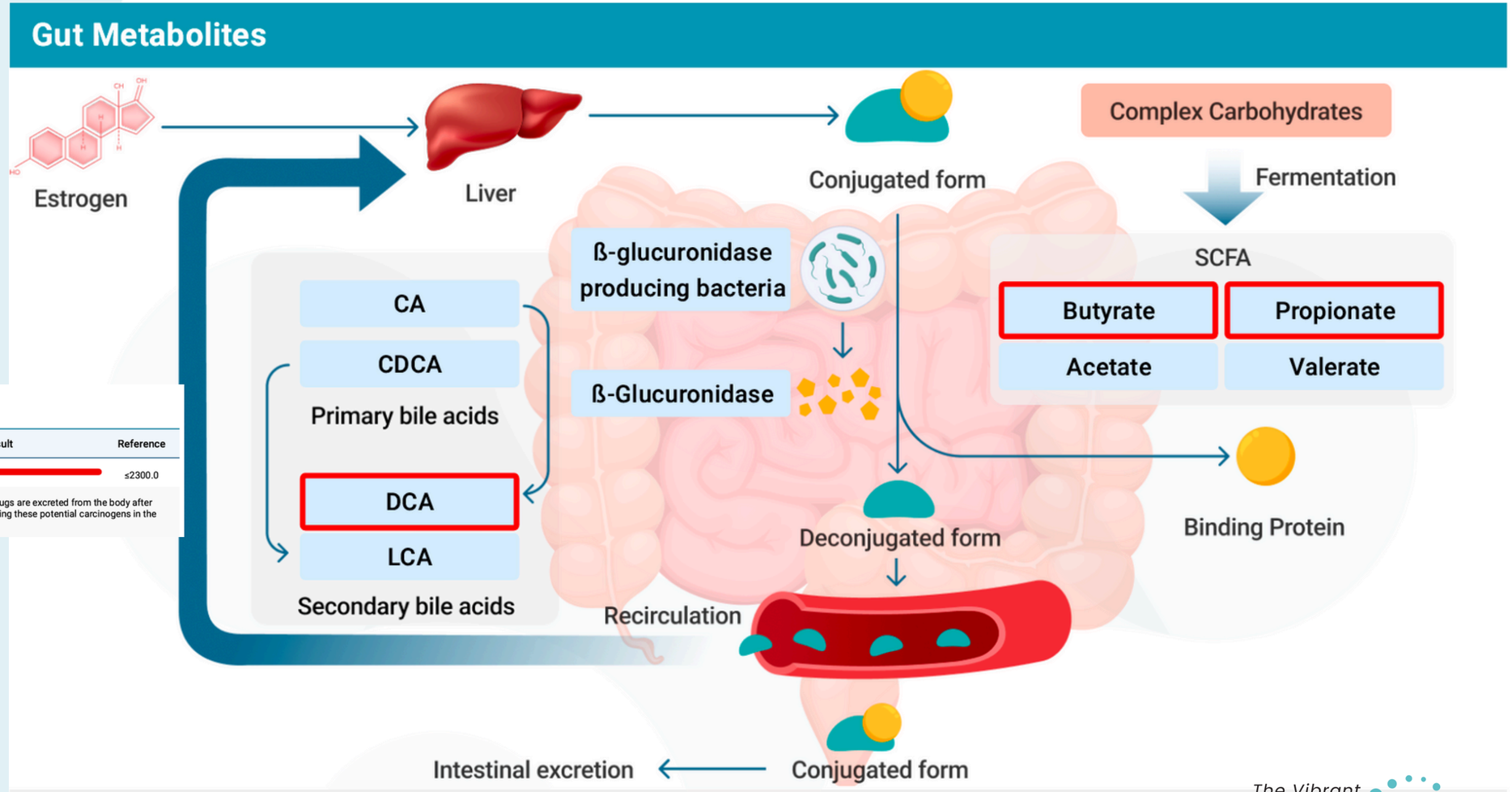
Beta-Glucuronidase: A Hormone Recycling Enzyme



Test Name	Current	Previous	Result	Reference
ß-glucuronidase (U/mL)	2569		0 2300	≤2300.0

Beta-glucuronidase is an enzyme induced by anaerobic bacteria. Many toxins, hormones, and drugs are excreted from the body after conjugation to a glucuronide molecule. Beta-glucuronidase can uncouple these conjugates, freeing these potential carcinogens in the bowel and increase cancer risk.

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GI Pathogens

Parasitic infections

- *Led to monthly crash*
- *Immediately relieved by treating parasites with Alinia*

GUT PATHOGENS

Parasites - Protozoans	Current	Previous	Reference	Parasites - Protozoans	Current	Previous	Reference
Cryptosporidium	3.2e4		≤1e2	Entamoeba histolytica	1.9e3		≤1e2

Cryptosporidium: Consider anti-parasitic herbal treatments containing ingredients such as black walnut, garlic oil, oil of oregano, Artemisia, berberine, goldenseal, gentian root extract, quassia bark extract, citrus seed extract.

Entamoeba histolytica: Consider anti-parasitic herbal treatments containing ingredients such as black walnut, garlic oil, oil of oregano, Artemisia, berberine, goldenseal, gentian root extract, quassia bark extract, citrus seed extract.

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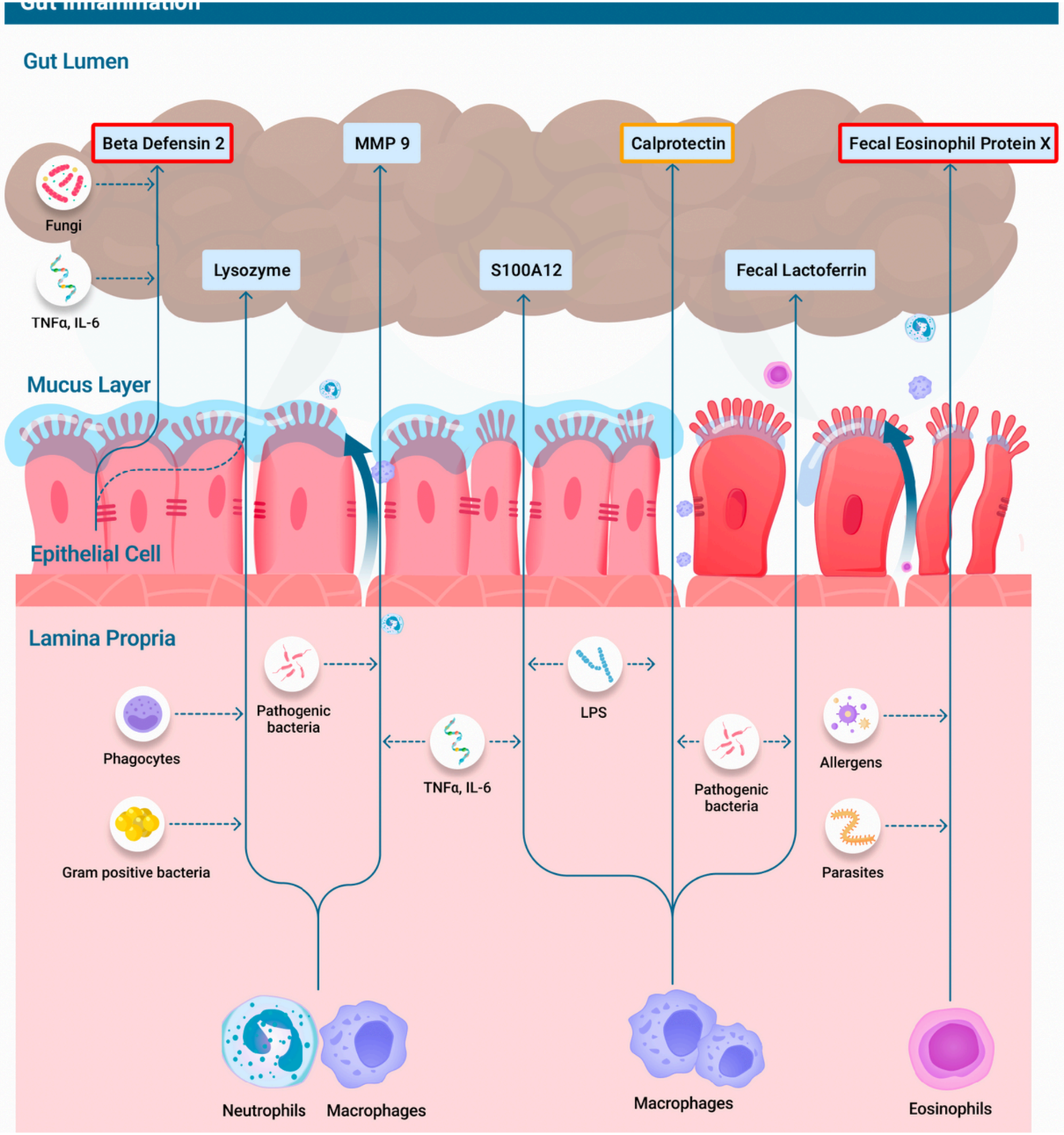
Gut Inflammation

GUT INFLAMMATORY MARKERS	
Test Name	Current
Beta Defensin 2 (ng/mL)	51.0
Lysozyme (ng/mL)	463.1
MMP 9 (ng/mL)	0.2
S100A12 (mcg/ml)	30.0
Calprotectin (mcg/g)	81.7
Fecal Lactoferrin (mcg/ml)	2.1
Fecal Eosinophil Protein X (mcg/g)	9.4



Estrogen Metabolites

- Elevated 4-OH-E1
- Low 2-OH-E1
- High oxidative stress, low glutathione, low cysteine, low glycine

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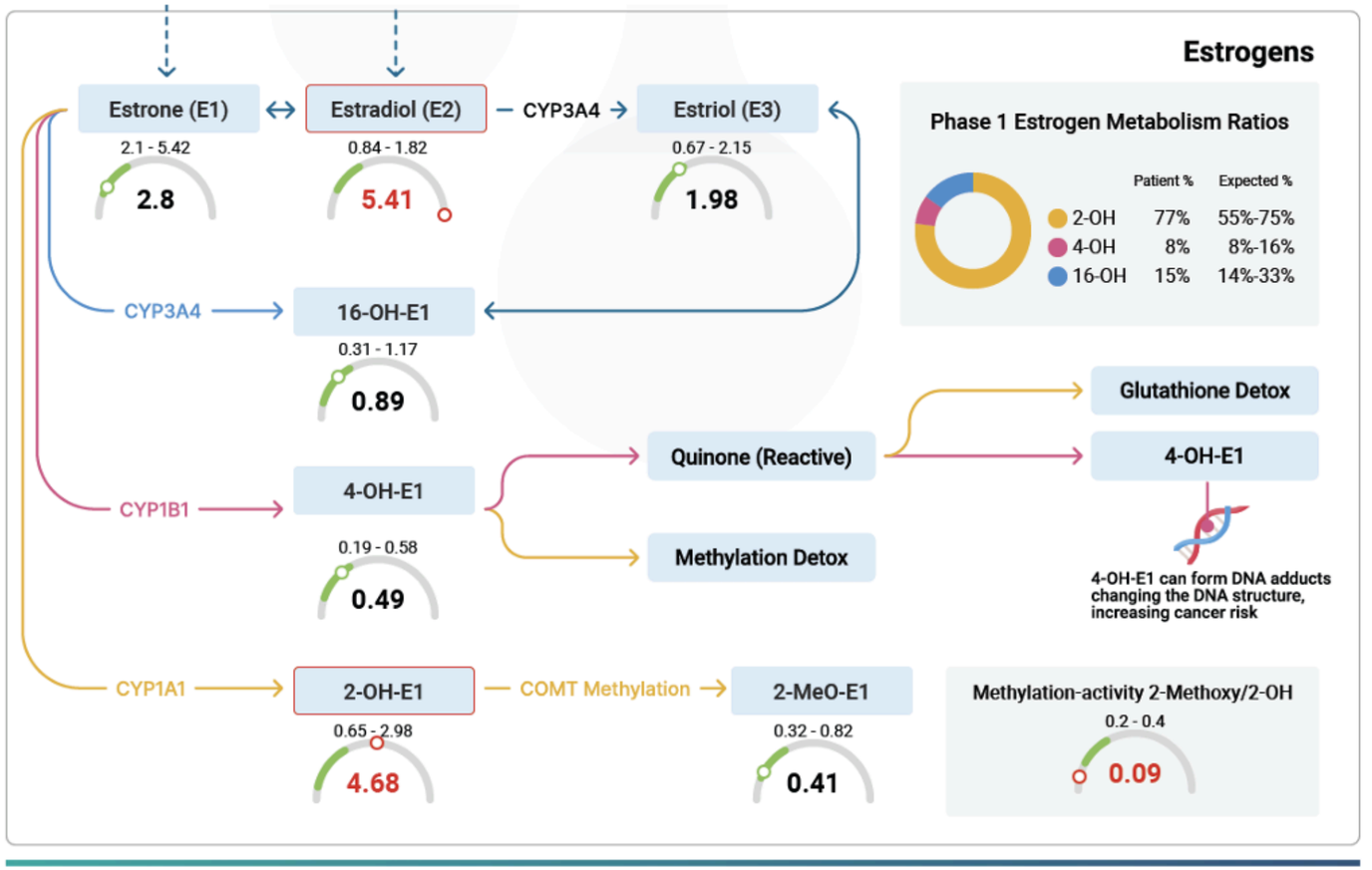
Estrogen Metabolites



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Treatment Plan

- Bioidentical progesterone, cyclical augmentation
- Calcium D-glucarate
- NAC/Glycine
- Multi Pack
- Alinia used for parasites
- Retest at 6 month mark, cleared parasites

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Results

- Parasite treatment eliminated what seemed like PMS problem
- Progesterone and estrogen balance improved energy and mood

Ted, Longevity Work up with Surprising Discovery

Referred by his personal trainer for weight loss resistance despite perfect exercise and diet routines (personal chef, personal trainer)

- High stress 39-year career in business
- High cholesterol, wanting to avoid statins, interest in longevity, overweight, mild GI issues
- Best medical care possible in United States
- Early stage MALT lymphoma

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Interpreting Gut Zoomer

Case study #2 covers these key topics

- Role of GI infections in immune health, antioxidant deficiencies from malabsorption
- The importance of using cutting edge tech for lab analysis, what can happen with use of old tech
- The role of microbial metabolites in the efficacy of commonly used longevity supplements like resveratrol and curcumin

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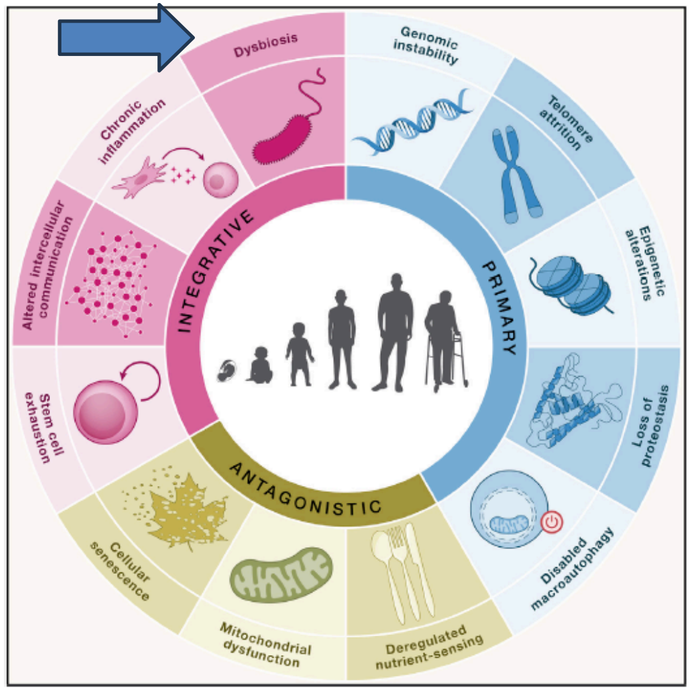
Hallmarks of Aging

Cell
Leading Edge
CellPress
Review

Hallmarks of aging: An expanding universe

Carlos López-Otín,[1,2,3,*] María A. Blasco,[4] Linda Partridge,[5,6] Manuel Serrano,[7,8,9] and Guido Kroemer[10,11,12,*]
[1]Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, Oviedo, Spain



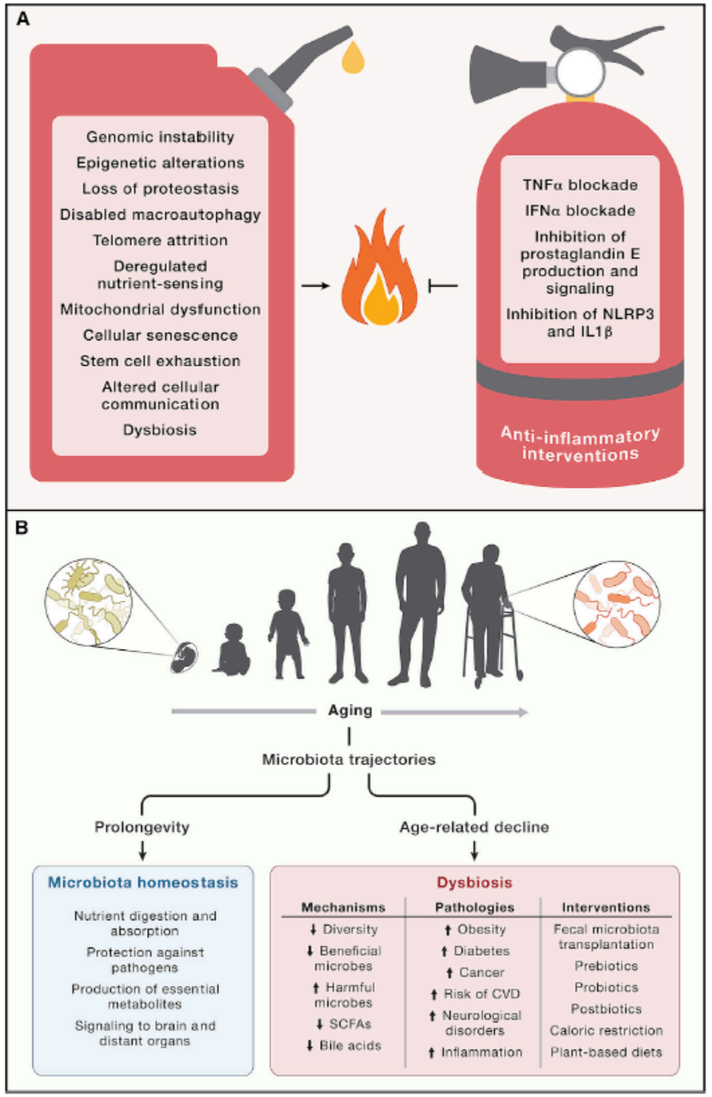


Figure 6. Derangement of supracellular functions

Altered intercellular communication bridges the cell-intrinsic hallmarks to meta-cellular hallmarks including the chronic inflammation, and the alterations in the crosstalk between human genome and microbiome, which finally result in dysbiosis. (A) Chronic inflammation during aging occurs as a consequence of multiple derangements that stem from all the other hallmarks. Several representative examples of anti-inflammatory interventions with positive effects on healthspan and lifespan are shown in the right part of the figure. (B) Dysbiosis contributes to multiple pathological conditions associated with aging. The human gut microbiota significantly changes during aging, finally leading to a general decrease in ecological diversity. The main features of the mechanisms underlying these microbiota changes and some examples of interventions on the gut microbiota composition which can promote healthy aging are shown in the lower part of the right panel. CVDs, cardiovascular diseases; SCFAs, short-chain fatty acids.

CHRONIC INFLAMMATION

Inflammation increases during aging ("inflammaging") with systemic manifestations, as well as with pathological local phenotypes including arteriosclerosis, neuroinflammation, osteoarthritis, and intervertebral discal degeneration. Accordingly, the circulating concentrations of inflammatory cytokines and biomarkers (such as CRP) increase with aging. Elevated IL-6 levels in plasma constitute a predictive biomarker of all-cause mortality in aging human populations.[271] In association with enhanced inflammation, immune function declines, a phenomenon that can be captured by high-dimensional monitoring of myeloid and lymphoid cells in the blood from patients and from mouse tissues.[272] For example, a population of age-associated T cells—termed Taa cells—is composed of exhausted memory cells that mediate pro-inflammatory effects via granzyme

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Case Study #2: Polyphenol Connection

- Patient already on longevity protocol (e.g., curcumin and resveratrol, green drinks and many anti-oxidant supplements
- Testing demonstrated:
  - Altered short-chain fatty acids, bile acids and organic acids
  - Microbial metabolism was insufficient for full polyphenol utilization
  - Research backed insight: Gut-derived microbial metabolites are required for full efficacy of curcumin, resveratrol, and for absorption of anti-oxidant supplements

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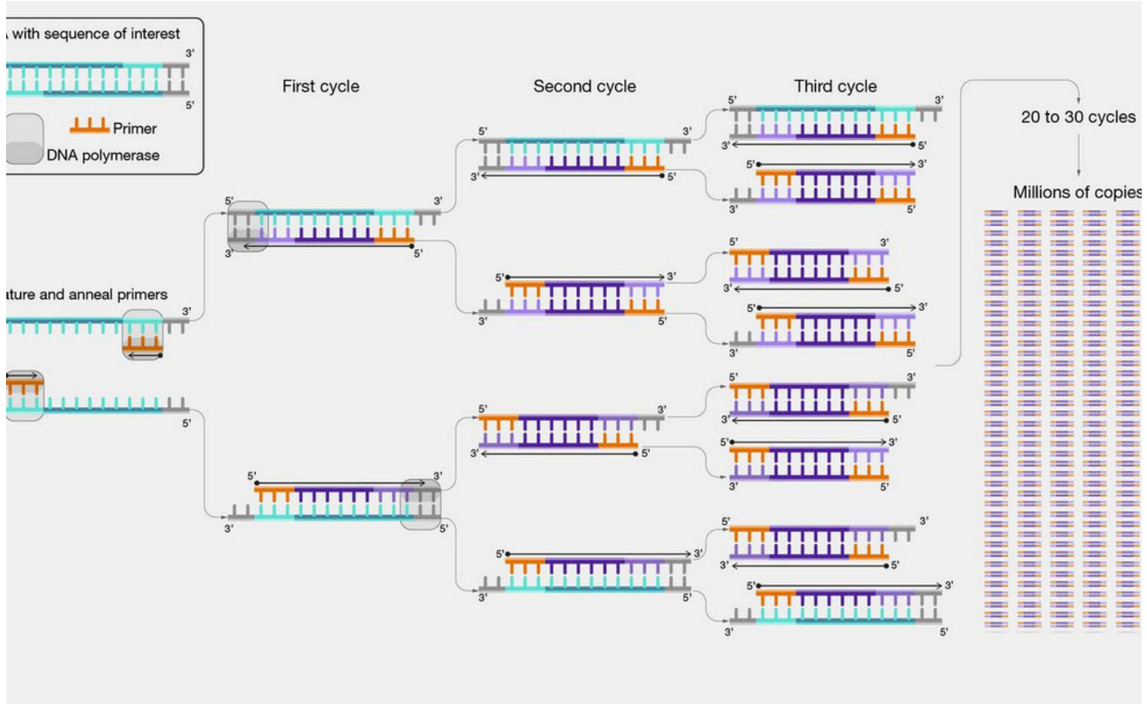
Essential Longevity Tests Reveal

H. Pylori detected on RT PCR, missed by his previous work ups

- Gut Zoomer - H. pylori, poor GB function
- Hormones Zoomer – HPA axis dysregulation
- Total Tox Burden and Oxidative Stress Profile - high levels oxidative stress



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Start With Pathways and Mechanisms

(1) Impact of GI infections on immune system
(2) Fiber to fat –production of SCFA (butyrate)
(3) Bile acid issues leading to fat soluble antioxidant malabsorption
(4)Microbial metabolites and longevity supplement utilization – why GI always matters with longevity programs

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GI Infections and Immune Dysregulation

GI infections disrupt mucosal immunity (SIgA), alter microbial diversity, contribute to system inflammation, and can trigger autoimmunity, and drive long-term immune activation.

- Dysbiosis and h. pylori overgrowth can trigger pro-inflammatory cytokine release
- Chronic GI infections correlate with increased risk of immune-related disorders (e.g. IBD, arthritis, allergies)
- Persistent H. pylori infection linked to MALT lymphoma, a form of B-cell lymphoma in gastric mucosa, due to chronic antigenic stimulation

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SCFAs: Immune-modulating Metabolites from Dietary Fiber

- Gut bacteria ferment dietary fiber and as a by-product generate SCFAs, acetate, propionate, and butyrate
- Butyrate promotes regulatory T cell (Treg) differentiation and suppresses presses pro-inflammatory cytokines
- SFCAs modulate innate immune cells (e.g., macrophages, dendritic cells), reducing inflammation

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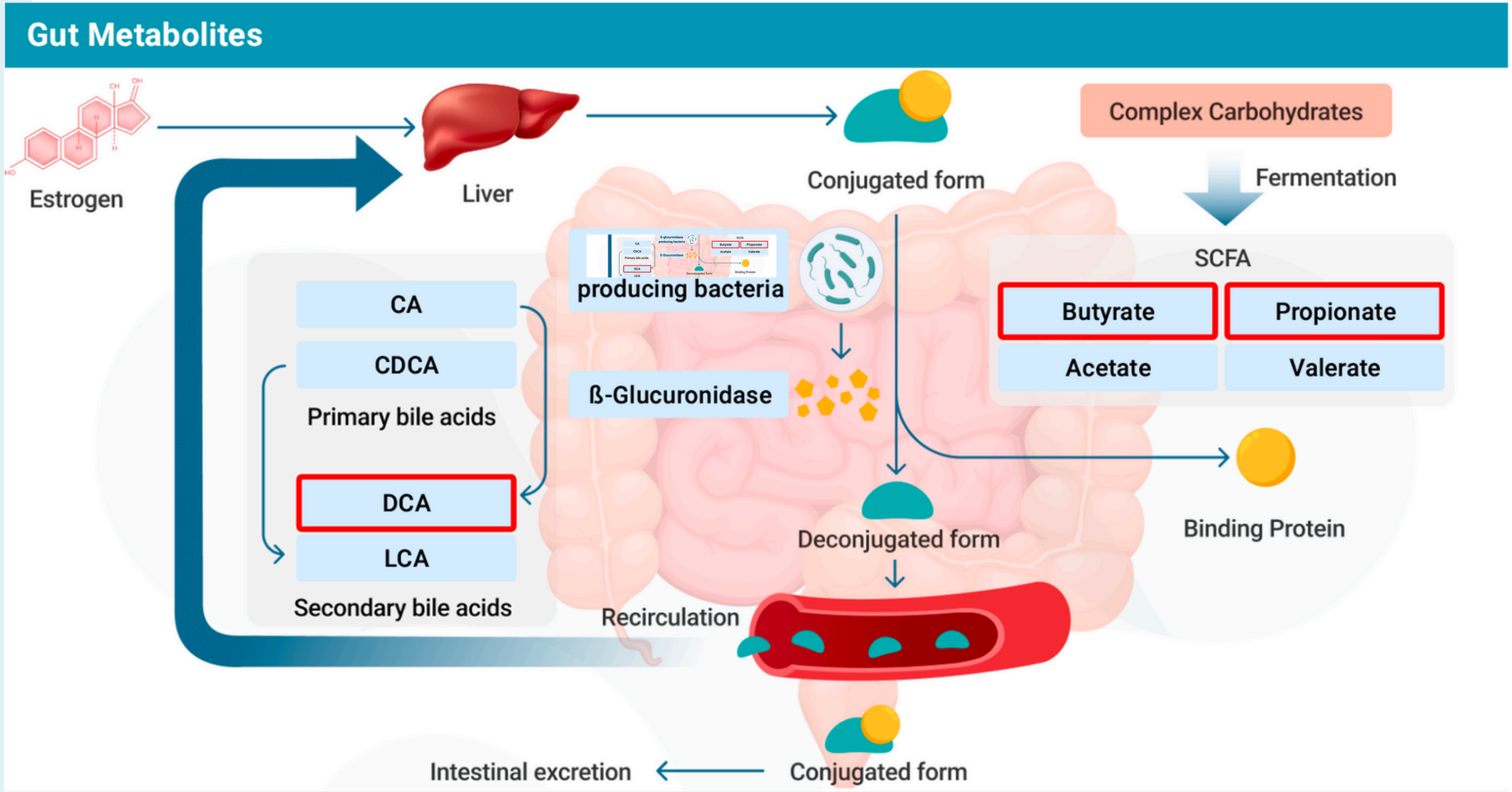
Gut Metabolites

GUT METABOLITES	
BILE ACID METABOLITES	Current
Cholic Acid (CA) (%)	0.17
Chenodeoxycholic Acid (CDCA) (%)	1.14
Deoxycholic Acid (DCA) (%)	16.75
Lithocholic Acid (LCA) (%)	73.23
LCA/DCA Ratio	4.37
SHORT CHAIN FATTY ACIDS	Current
Acetate (%)	62.1
Propionate (%)	30.5
Butyrate (%)	1.5
Valerate (%)	2.0
Total Short Chain Fatty Acids (micromol/g)	176.4
ESTROGEN METABOLISM	Current
ß-Glucuronidase (U/mL)	1299



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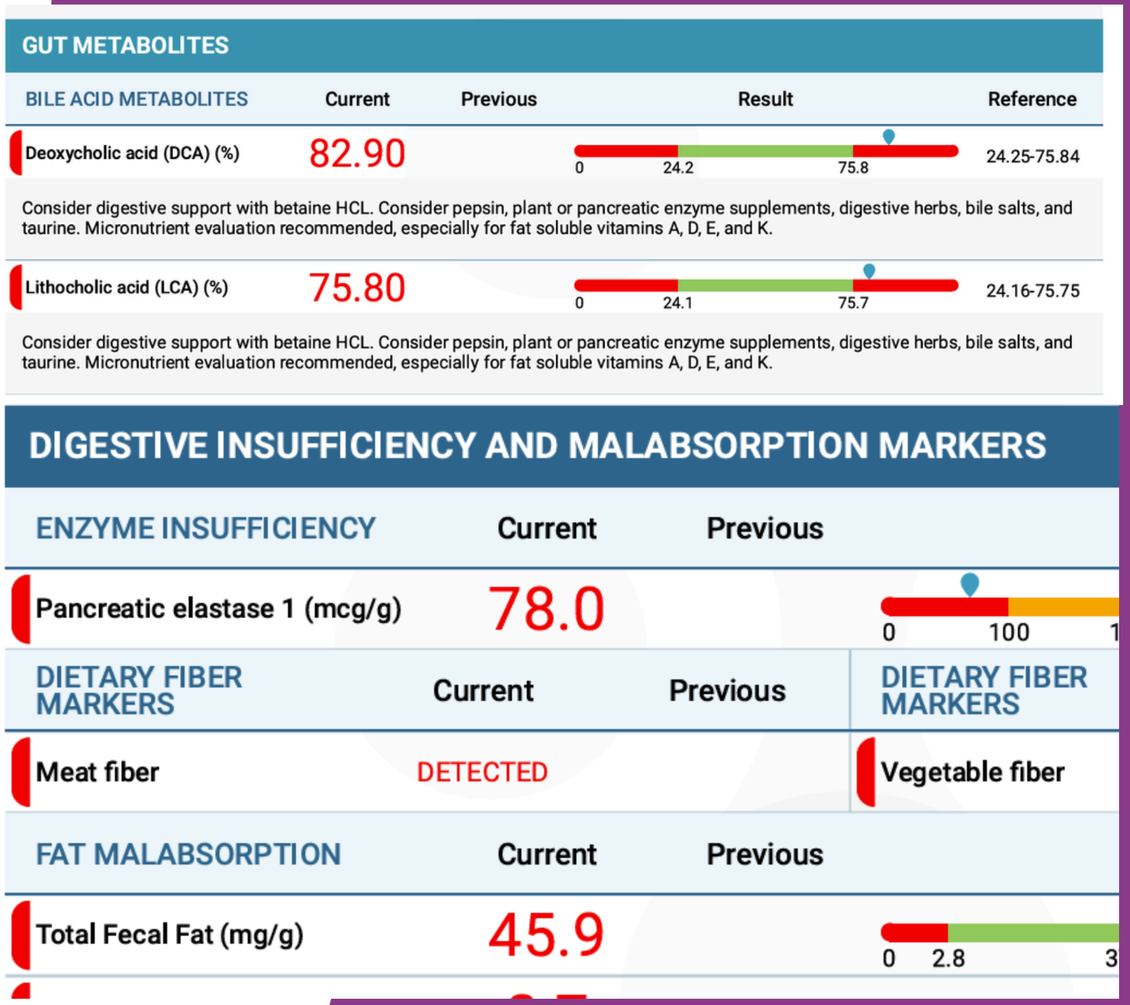
Fat Malabsorption

Bile acid metabolite issues reflect fat absorption issues, lead to fat soluble antioxidant malabsorption, increasing oxidative stress and impacting longevity

- *Fecal fat reveals lipid digestion and pancreatic enzyme sufficiency*
- *Fat soluble antioxidants like vitamin E and vitamin A are key to projection of lipid membranes and low antioxidant capacity triggers many of the Hallmarks of Aging*



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Gut Zoomer Test Reporting Features

CHOLIC ACID (CA)

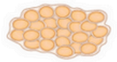
- Fat malabsorption (greasy stools) from dysregulated bile synthesis and affected cholesterol metabolism.
- Digestive discomfort due to gut dysbiosis.

Gallbladder, Liver, Gut

CHENODEOXYCHOLIC ACID (CDCA)

- Affected bowel movements from gut inflammation and impaired motility.
- Insulin resistance and poor blood sugar regulation due to disrupted GLP-1 sensitivity.

Brown adipose tissue, Gut



DEOXYCHOLIC ACID (DCA)

- Elevated gut inflammation via NF-κB.
- Bowel discomfort due to low stool water content affecting gut motility and bowel movement.

Immune cells, Colon

LITHOCHOLIC ACID (LCA)

- Toxin build-up due to poor detoxification
- Frequent gut infections from reduced immunity via VDR.
- Bloating and irregular stools from gut dysbiosis.

Liver, Gut

β-GLUCORONIDASE

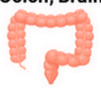
- Increased toxin reabsorption due to impaired glucuronidation.
- Hormonal imbalances leading to estrogen dominance.
- Elevated risk of inflammation.

Liver, Gut

ACETATE

- Dysregulated cholesterol levels due to altered lipid metabolism.
- Mood swings from affected neuronal signaling.
- Increased inflammation.

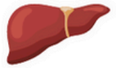
Colon, Brain



PROPIONATE

- Potential weight regulation issues due to altered energy homeostasis.
- Impaired satiety leading to overeating due to affected GLP-1 secretion.

Liver, Pancreas



BUTYRATE

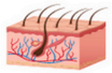
- Gastric discomfort from weakened intestinal lining.
- Poor blood sugar control due to disrupted glucose regulation via GLP-1.
- Brain fog from impaired neurogenesis.

Gut, Brain

VALERATE

- Affected skin barrier function leading to dry, irritated, and itchy skin

Skin



β-GLUCORONIDASE PRODUCING BACTERIA

- Increased toxin reabsorption due to glucuronide cleavage (release of toxins or hormones).
- Hormonal disruptions, including estrogen dominance.
- Gut microbiota imbalance leading to inflammation.

Liver, Gut

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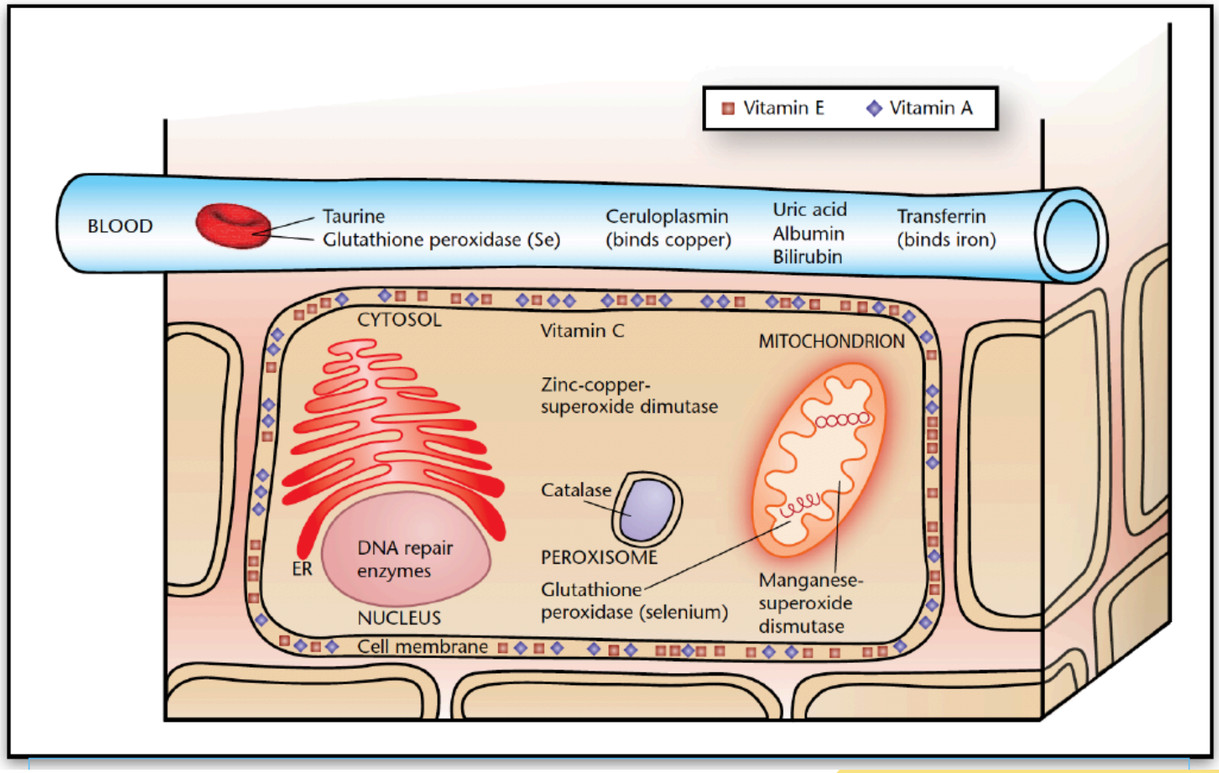
Bile Acids and Fat Soluble Antioxidants

Antioxidants we must eat and digest vs. antioxidants we produce endogenously

- **Only from diet**, vitamin E, vitamin C, polyphenols
- **90% endogenous production**: CoQ10, glutathione (require precursors, minerals, amino acids)
- Why this matters: aging of cells accelerates with mitochondrial damage and increased oxidative stress
- We must absorb antioxidants via healthy GI tract and produce the endogenous ones to keep our cells healthy and long-lived

Figure 10.2 — Distribution of Antioxidants



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Gut Metabolites

GUT METABOLITES				
BILE ACID METABOLITES	Current	Previous	Result	Reference
Cholic Acid (CA) (%)	0.17		0 0.36	≤0.36
Chenodeoxycholic Acid (CDCA) (%)	1.14		0 1.25	≤1.25
Deoxycholic Acid (DCA) (%)	16.75		0 24.2 75.8	24.25-75.84
Lithocholic Acid (LCA) (%)	73.23		0 24.1 75.7	24.16-75.75
LCA/DCA Ratio	4.37		0 0.31 3.38	0.32-3.38
SHORT CHAIN FATTY ACIDS	Current	Previous	Result	Reference
Acetate (%)	62.1		0 60.1 72.7	60.2-72.7
Propionate (%)	30.5		0 15.3 30.3	15.4-30.3
Butyrate (%)	1.5		0 5 12.4	5.1-12.4
Valerate (%)	2.0		0 0.7 3.5	0.8-3.5
Total Short Chain Fatty Acids (micromol/g)	176.4		0 45.3 210	45.4-210.1
ESTROGEN METABOLISM	Current	Previous	Result	Reference
ß-Glucuronidase (U/mL)	1299		0 2300	≤2300.0

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Microbial Metabolites Impact on Longevity Supplements

Abstract

(Poly)phenols (PPs) constitute a large family of phytochemicals with high chemical diversity that are known to be active principles of plant-derived nutraceuticals and herbal medicinal products. Their pharmacological activity, however, is difficult to demonstrate due to their mild physiological effects, and to the large inter-individual variability observed. Many PPs have little bioavailability and reach the colon almost unaltered. There they encounter the gut microbes resulting in a two-way interaction in which PPs modulate the gut microbiota composition, and the intestinal microbes catabolize the ingested PPs to release metabolites that are often more active and better absorbed than the native phenolic compounds. The type and quantity of the PP metabolites produced in humans depend on the gut microbiota composition and function, and different metabotypes have been identified. However, not all the metabolites have the same biological activity, and therefore the final health effects of dietary PPs depend on the gut microbiota composition. Stratification in clinical trials according to individuals' metabotypes is necessary to fully understand the health effects of PPs. In this review, we present and discuss the most significant and updated knowledge regarding the reciprocal interrelation of the gut microbiota with dietary PPs as a key factor that modulates the health effects of these compounds. The review will focus in those PPs that are known to be metabolized by gut microbiota resulting in bioactive metabolites.

ELSEVIER

Biochemical Pharmacology

Volume 139, 1 September 2017, Pages 82-93

Review

The gut microbiota: A key factor in the therapeutic effects of (poly)phenols

Juan Carlos Espín, Antonio González-Sarrías, Francisco A. Tomás-Barberán

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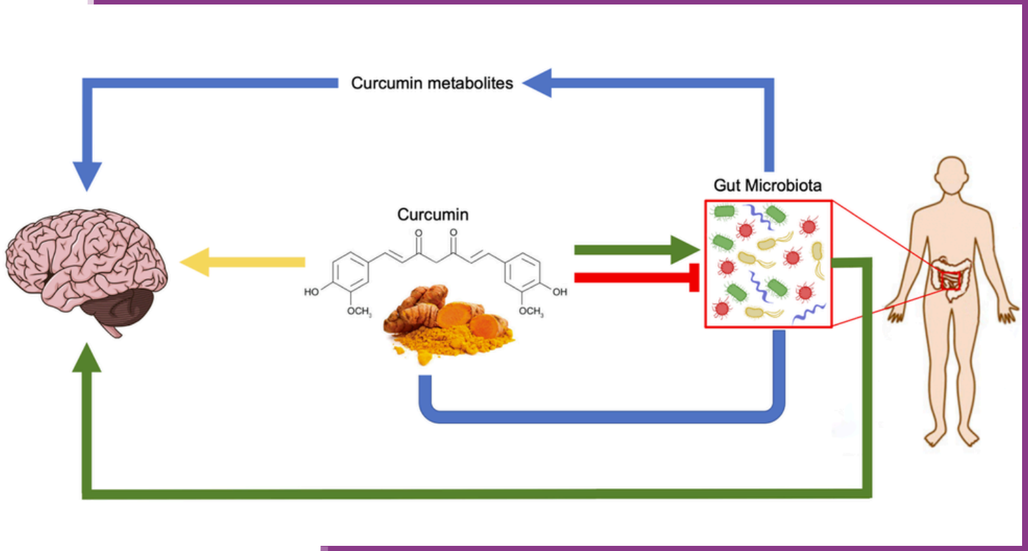
Enhancing Effects of Curcumin and Resveratrol



Focusing on curcumin's systemic absorption may miss the point.

- *Curcumin extensively metabolized by the gut bacteria*
- *Local actions in gut and its impact on microbial metabolites may be key to its benefits*

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Treatment Plan

- Treatment h. pylori with Prev-Pak and follow up herbal program, addressed dysbiosis with antimicrobial herbs and follow-up with probiotics and GI repair program
- HPA axis program
- Antioxidant support
- Retest at 6 month mark showed elimination of h. pylori and dysbiosis, improvement of fat absorption

Results

- Elimination of early-stage MALT lymphoma with h. pylori treatment
- Improvement of oxidative stress, normalization of absorption and microbial metabolites
- Reinvigored longevity program

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Closing Thoughts

- The true power of testing lies in root-cause discovery, not just patient symptom relief. Treating GI is about much more than relieving GI symptoms
- Fixing the gut can reduce oxidative stress, reduce inflammation and improve mitochondrial function, this impacting longevity via many paths.

  Key takeaways:

  - Always run GI testing, even in asymptomatic patients

  - Use a multi-panel approach to uncover connections

  - Longevity programs depend on nuances including metabolites and nutrient absorption, not just intake of anti-aging products

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Thank You!

The Gut Microbiome Connection

Advancing Systemic Health Protocols

Session 3

Dr. Kyle Gillett, MD

The Vibrant Longevity Summit

The Gut Microbiome Connection: Protocols

Kyle Gillett MD

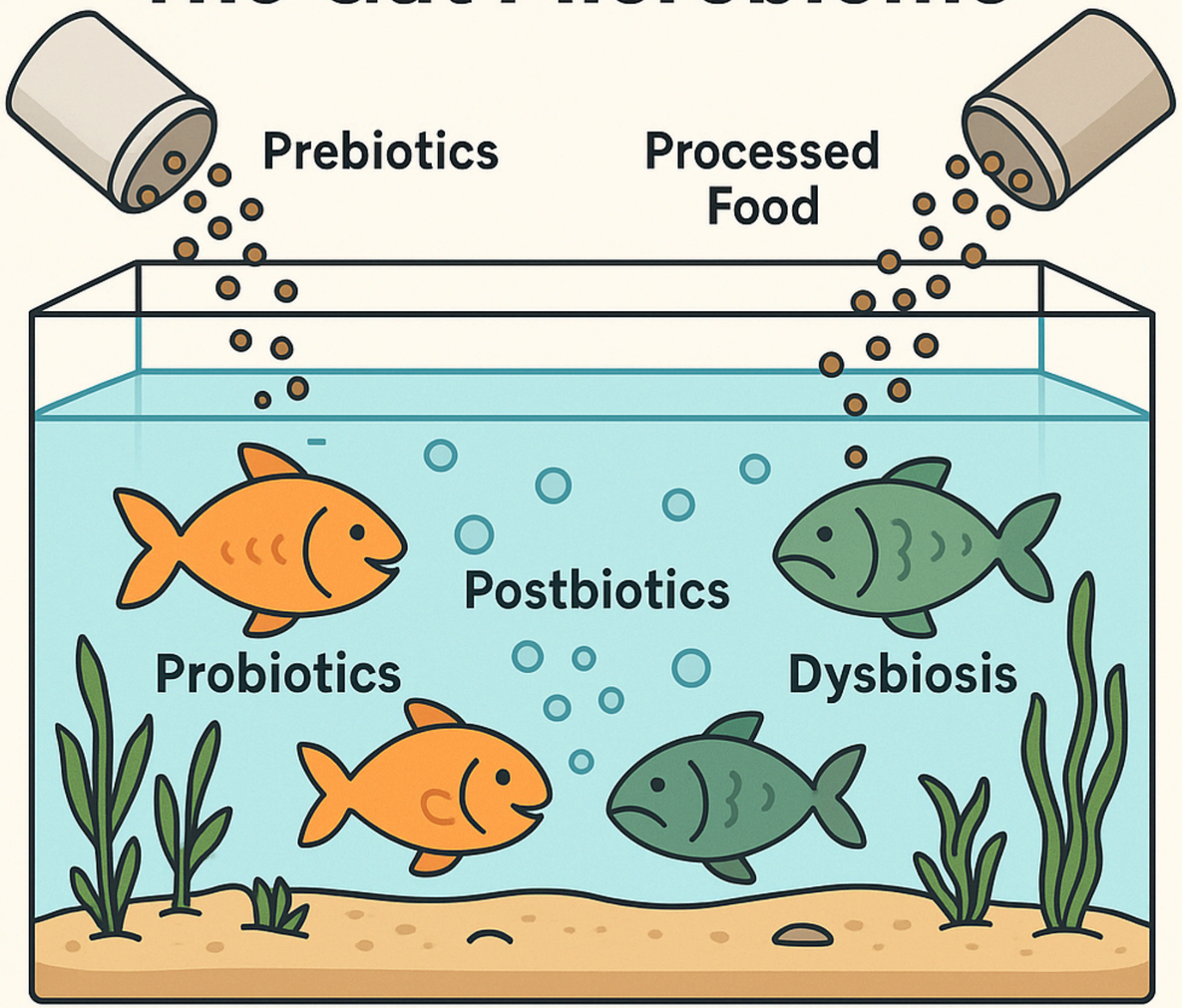
Meet Your Speaker

Kyle Gillett MD

Board Certified:
Family Medicine
Obesity Medicine

Gillett Health
Gillett Health Podcast
SageBio Visualizer

The Gut Microbiome



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Cookie cutter
gut protocol bad

Cookie cutter
gut protocol
good

34% 34%

68%

14% 14%

95%

0.1% 2% 2% 0.1%

IQ score 55 70 85 100 115 130 145

Cookie cutter
gut protocol
good

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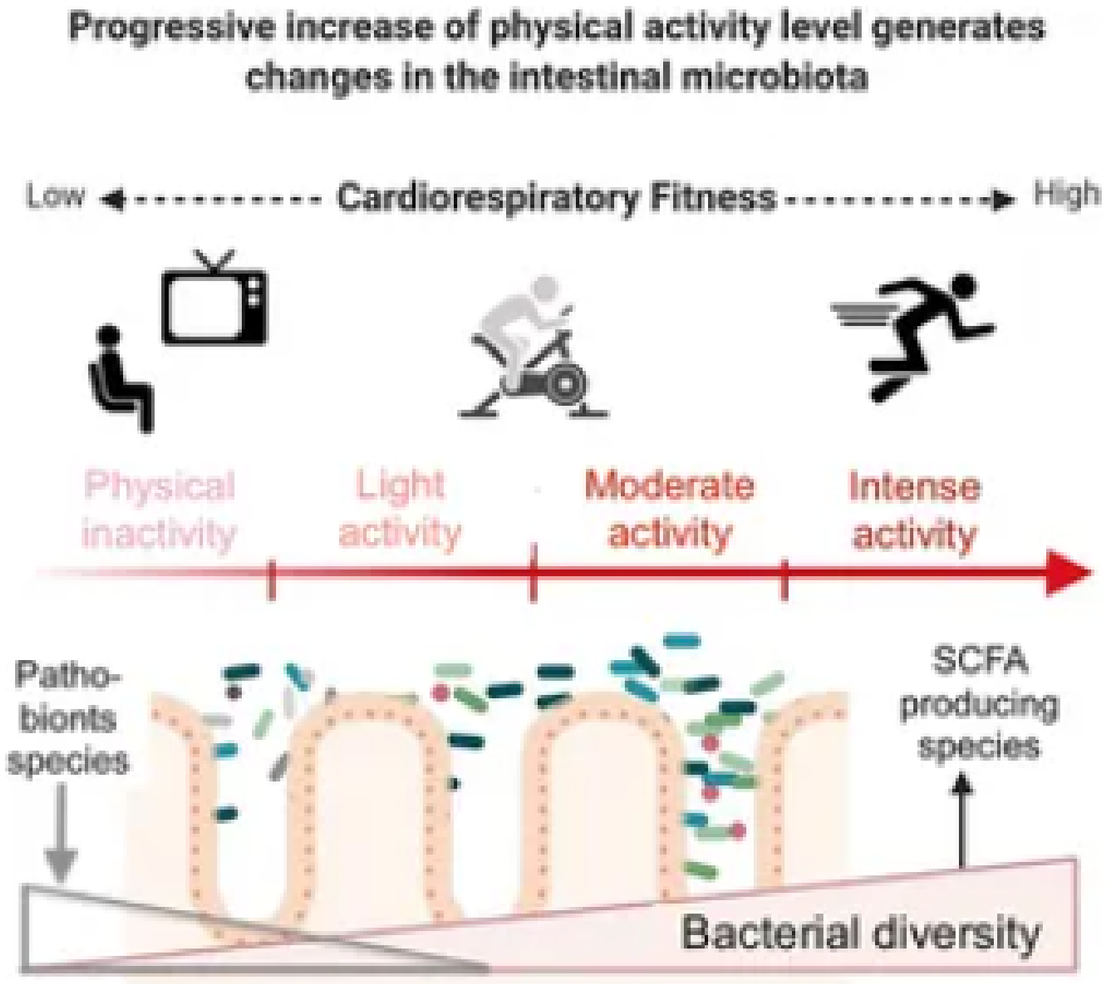
General Gut Health Protocols

Category	Protocol	Description	Supporting Evidence
Exercise	Mix of low, moderate, and high intensity exercise	Promotes gut microbiome diversity and improves intestinal barrier integrity	Maillet et al., 2021
Diet	Whole food diet (e.g., Mediterranean)	Enhances beneficial microbes and reduces inflammatory species	Sanchez-Garcia et al., 2024
Environmental Exposure	Outdoor and natural microbial environment exposure	Contact with biodiverse nature improves immune system via microbiome modulation	Roslund et al., 2021
Fiber Intake	Broad spectrum fiber supplement to meet daily fiber target	Increases microbial diversity and metabolite production like SCFAs	Lewis et al., 2021
Bitters	Bitters before meals	May enhance natural digestive enzyme secretion and bile flow (limited clinical evidence)	— *(Traditional/clinical support; not yet well-studied in RCTs)*

🧪 Optional Additions

Optional Supplement	Purpose
Emulsified oregano oil	Potential antimicrobial against pathogenic overgrowth
TUDCA	May support bile flow and intestinal barrier integrity
Avoid binders, biofilm disruptors, anti-nutrients	Minimizes microbiome disruption and gut lining damage

Figure 1



GUT METABOLITES

SHORT CHAIN FATTY ACIDS	Current	Previous	Result	Reference
Butyrate (%)	1.2		0 5 12.4	5.1-12.4

Butyrate is a short-chain fatty acid (SCFA) primarily produced through the bacterial fermentation of resistant starch and dietary fibers. This process involves the microbial hydrolysis of dietary polysaccharides into monosaccharides, which are then fermented to form butyrate. Butyrate serves as a vital energy source for colonocytes and supports gut barrier function by enhancing tight junction integrity. It also reduces intestinal inflammation and oxidative stress, promoting a healthy gut environment. Butyrate exerts its effects through G-protein-coupled receptors 41 and 43 (GPR41 and GPR43), contributing to insulin sensitivity via glucagon-like peptide-1 (GLP-1), which aids in glucose metabolism and enhances insulin secretion. Recent studies have shown that butyrate can support neurogenesis (the formation of new neurons) in the brain via the 'gut-brain axis.' Low fecal butyrate levels can cause gastrointestinal issues due to a compromised intestinal lining, impaired blood sugar regulation from disrupted GLP-1 activity, and cognitive symptoms like brain fog due to affected neurogenesis.

Total Short Chain Fatty Acids (micromol/g)	36.4		0 45.3 210	45.4-210.1

Total short-chain fatty acids (SCFAs) refer to the combined concentration of acetate, butyrate, propionate, valerate, iso-butyrate, and other SCFAs in the gut. They are produced through the anaerobic fermentation of indigestible dietary fibers, such as resistant starch and polysaccharides, by gut microbiota. SCFAs play essential roles in maintaining gut health by serving as energy sources for intestinal epithelial cells, strengthening the gut barrier, and regulating microbial diversity. They help suppress intestinal inflammation, support gut homeostasis, and influence systemic metabolic and immune responses. SCFAs interact with G-protein-coupled receptors 41 and 43 (GPR41 and GPR43), affecting gut motility, energy metabolism, and inflammatory pathways. Their benefits extend beyond the gut, impacting insulin sensitivity, lipid metabolism, and neuroimmune interactions. Low fecal SCFA levels indicate dysbiosis and are linked to various health conditions, including irritable bowel syndrome, inflammatory bowel disease, obesity, and metabolic disorders. Symptoms of reduced SCFAs may include bloating, abdominal discomfort, fatigue, and irregular bowel movements.

Supplement Suggestions

SUPPLEMENTS

Butyrate: Fructans, Inulin, Vitamin B2

Total Short Chain Fatty Acids: Fructans, Inulin

Consider these supplements in relation to medical history and symptoms. Not all recommended supplements are appropriate in all individual cases. Consult a knowledgeable healthcare provider before taking any supplemental nutrients or probiotics.

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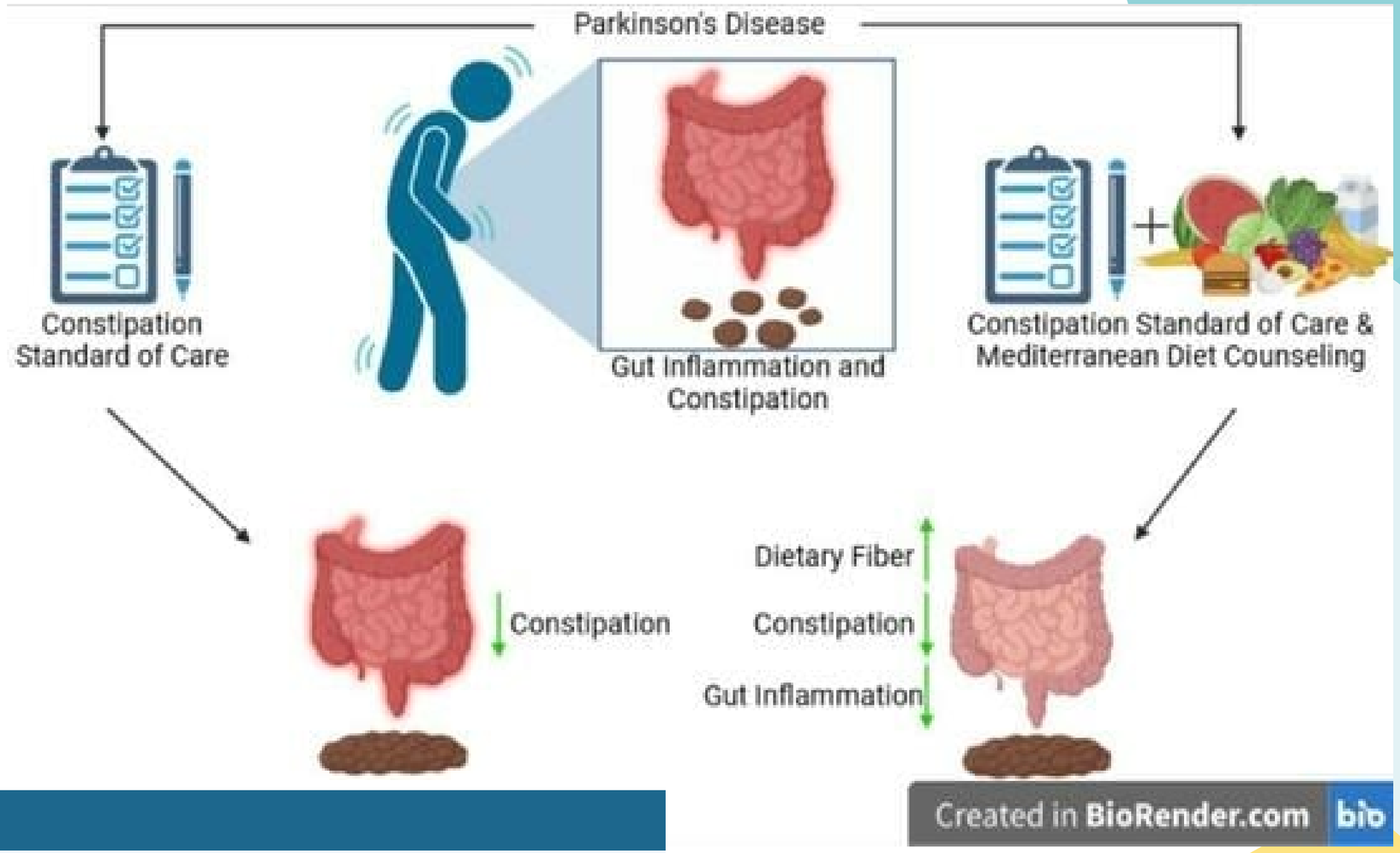
First step for high calprotectin?



GUT INFLAMMATORY MARKERS

Test Name	Current	Previous	Result	Reference
Calprotectin (mcg/g)	59.2		0 50 119	≤50.0

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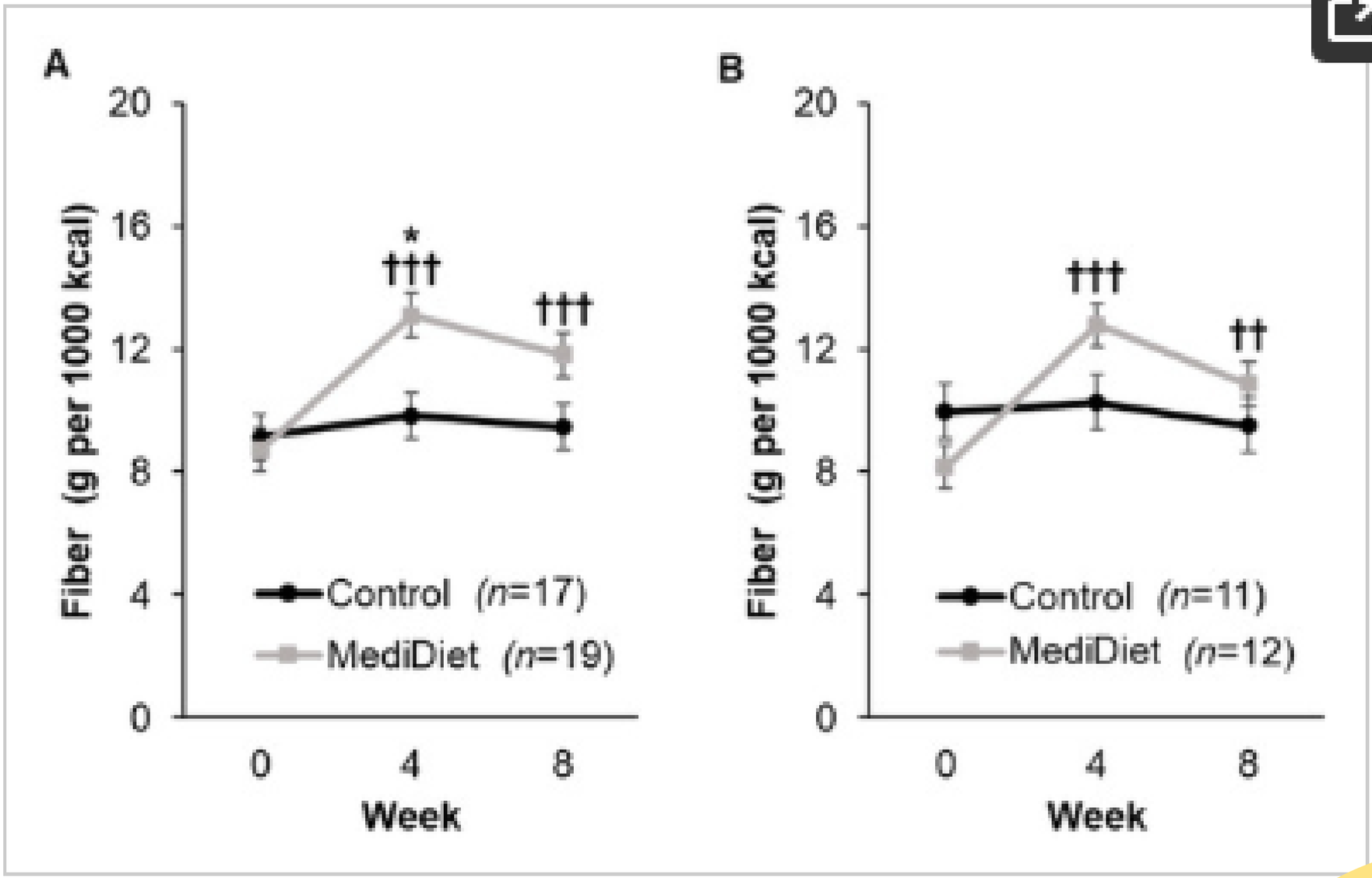

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Specific protocols: Beta-glucuronidase, intrahepatic recirculation, and gut metabolism

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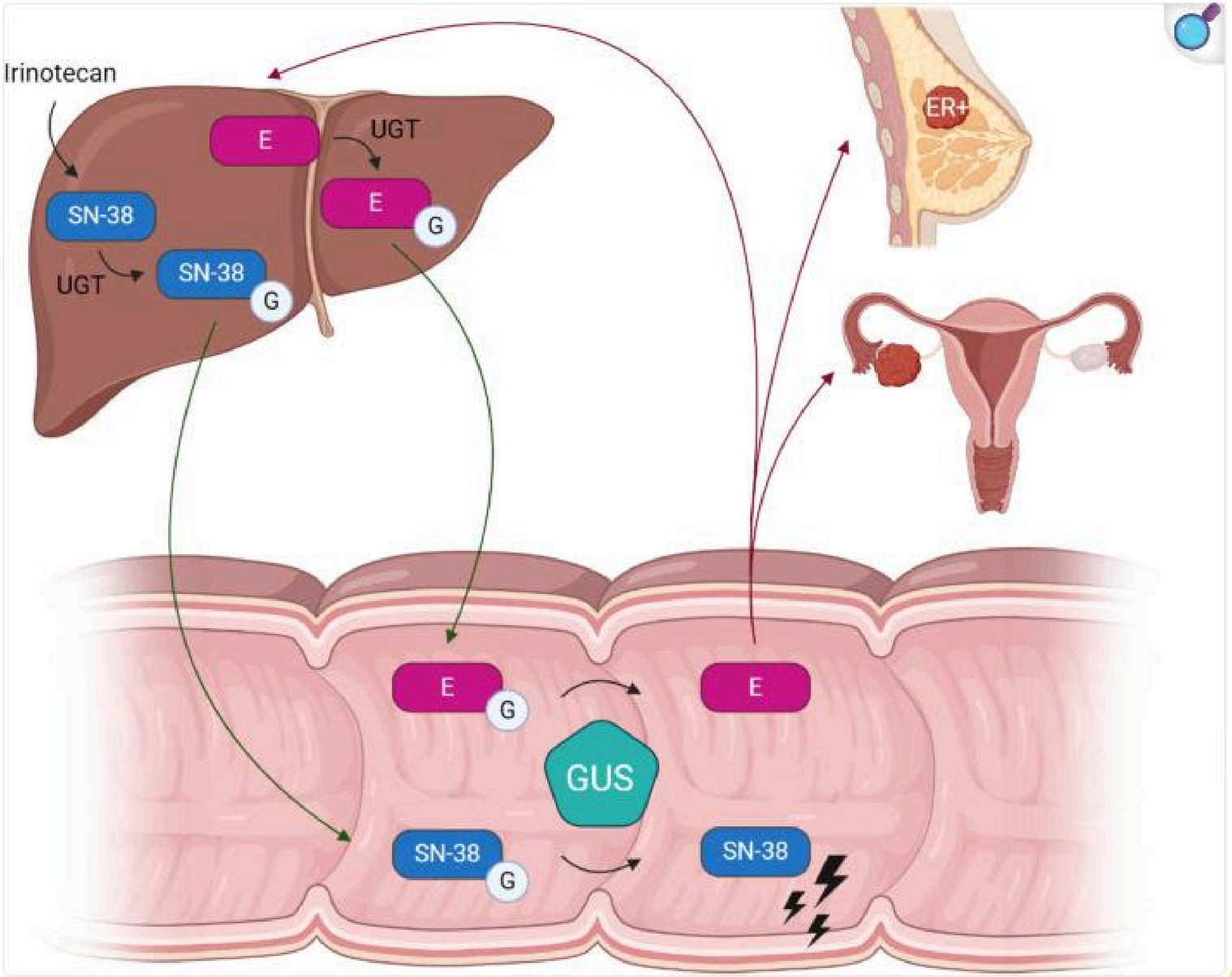

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Consider stool frequency in making protocol

Parameter		Correlatic activity
		β-GLN
Faecal frequency (times/week)	6·2 ± 1·3 (4–8)	−0·74†
Faecal wet weight (g)*	158 ± 48 (97–211)	0·12
	186 ± 88 (55–308)	
Transit time (h)	53·9 ± 13·5 (43·3–81·4)	0·43
Intake of fluid (g d^{-1})	2527 ± 476 (2080–3743)	−0·02
Intake of fibre (g d^{-1})	18·9 ± 2·5 (15·8–22·6)	−0·09

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🔬 Control of Gut β-Glucuronidase (GUS) Activity

Strategy	Mechanism	Examples / Notes
1. Inhibit the Enzyme	Directly block GUS enzyme activity	- **Calcium D-Glucarate** (converted to glucaric acid in gut) - GUS inhibitors from polyphenols, flavonoids (e.g., EGCG, curcumin) - Pharmaceuticals in trials targeting microbial GUS
2. Reduce GUS-Producing Bacteria (esp. *E. coli*)	Antimicrobial suppression of high GUS-expressing strains	- **Rifaximin** (non-systemic antibiotic) - Herbal antimicrobials: **berberine**, **oregano oil**, neem - Reduces abundance of *E. coli* and *Bacteroides* spp.
3. Outcompete with Beneficial Microbes	Probiotic or prebiotic use to crowd out or displace high-GUS producers	- **Probiotics:** *L. acidophilus*, *B. longum*, *Saccharomyces boulardii* - **Prebiotics:** D-mannose, inulin, GOS - Boosts *Alistipes*, *Akkermansia* (potentially lower GUS activity)

*Plus effects of bile acids, and estrogens

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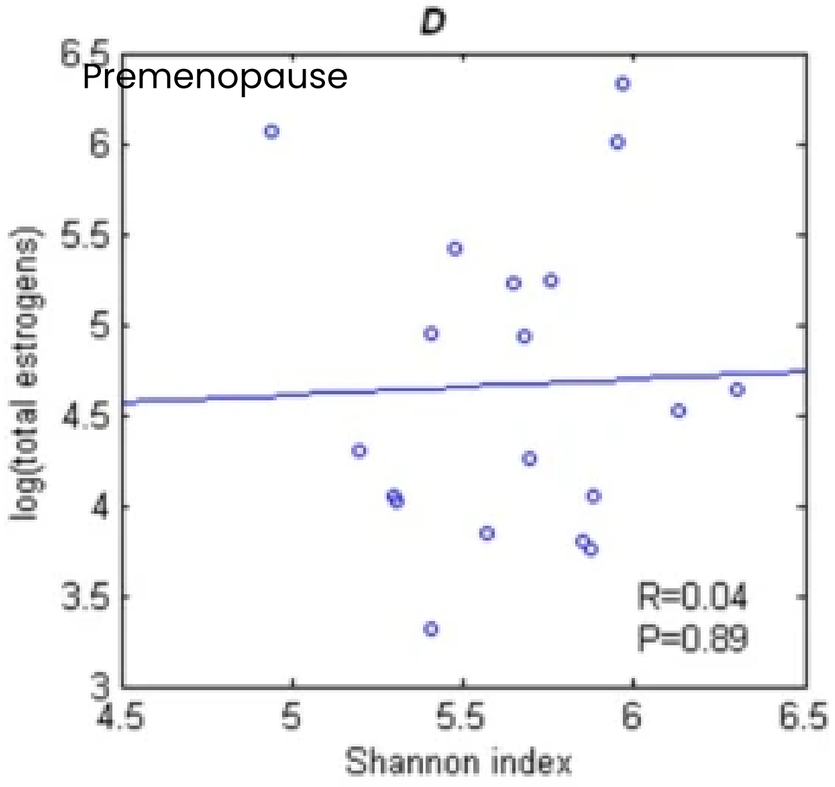
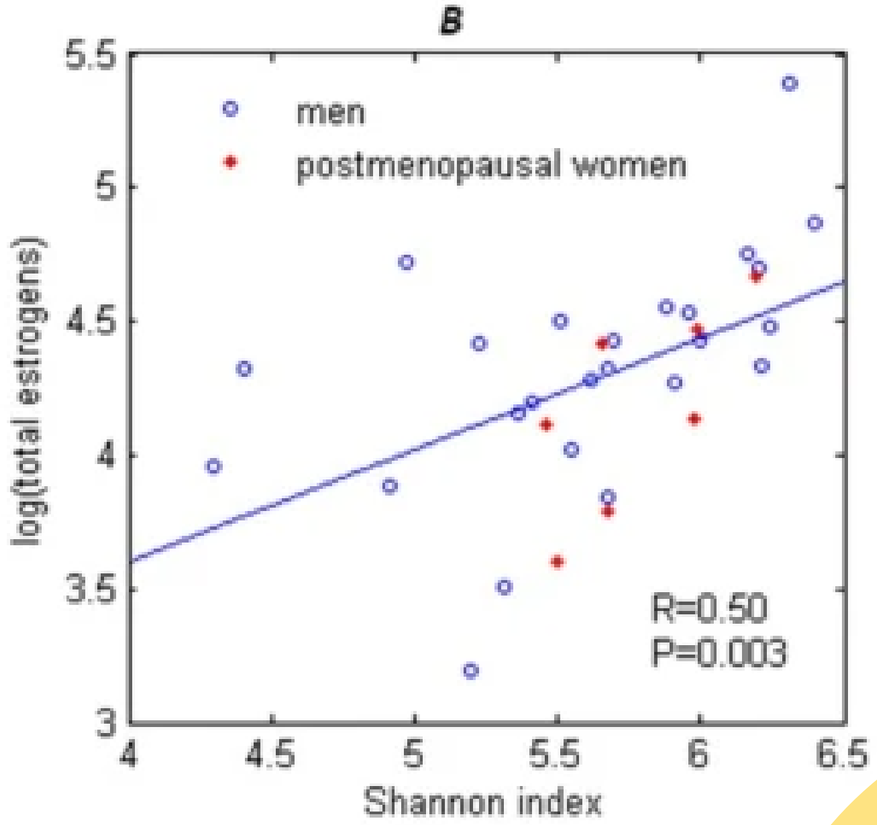
Estrone, mean (SE)	15.4 (2.0)		12.1 (1.6)		39.9 (13.8)	
- β-glucuronidase correlation	R=0.45	*P*=0.03	R=0.27	*P*=0.56	R=0.03	*P*=0.89
- β-glucosidase correlation	R=0.32	*P*=0.12	R=-0.13	*P*=0.79	R=0.06	*P*=0.81
- Shannon index	R=0.35	*P*=0.08	R=0.74	*P*=0.06	R=0.005	*P*=0.98
- Observed species	R=0.45	*P*=0.02	R=0.80	*P*=0.03	R=0.09	*P*=0.71





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Specific protocols: Bile Acids, sequestrants, intrahepatic recirculation, and hormones

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Common interaction: Ezetimibe

Taken together, these results suggest that colestyramine has the potential to bind ezetimibe and reduce its systemic bioavailability when administered concomitantly (table III). Therefore, administration of colestyramine and ezetimibe should be spaced several hours apart (2 hours before or 4 hours after administration of a bile acid binding agent) to avoid impeding the absorption of ezetimibe and potentially minimising its therapeutic effect.[14] The

Bile acids, bile acid sequestrants, and any GUS inhibitor will affect:
Any med that is mostly absorbed with help of glucuronidation

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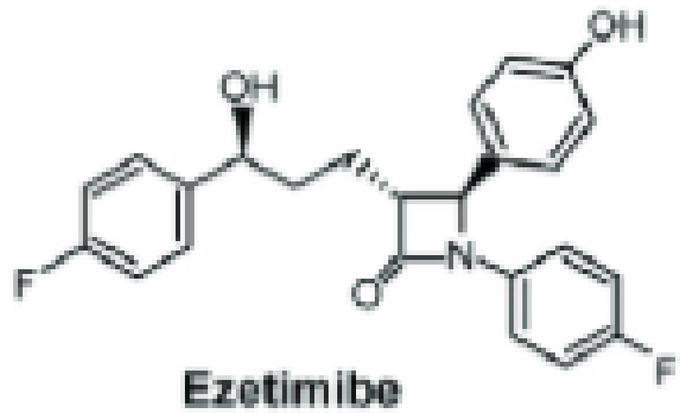
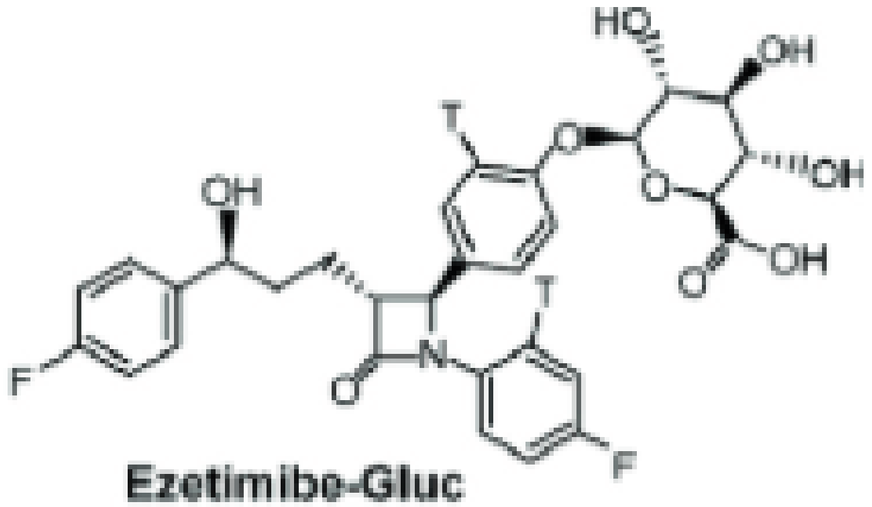
A



Ezetimibe



Ezetimibe-Gluc

Both are efficacious

Ezetimibe-Gluc is stronger

Long duration of action due to intrahepatic recirculation

Consider twice daily dosing for those on GUT inhibitors, low bile acid, or less potent glucuronidation genetics

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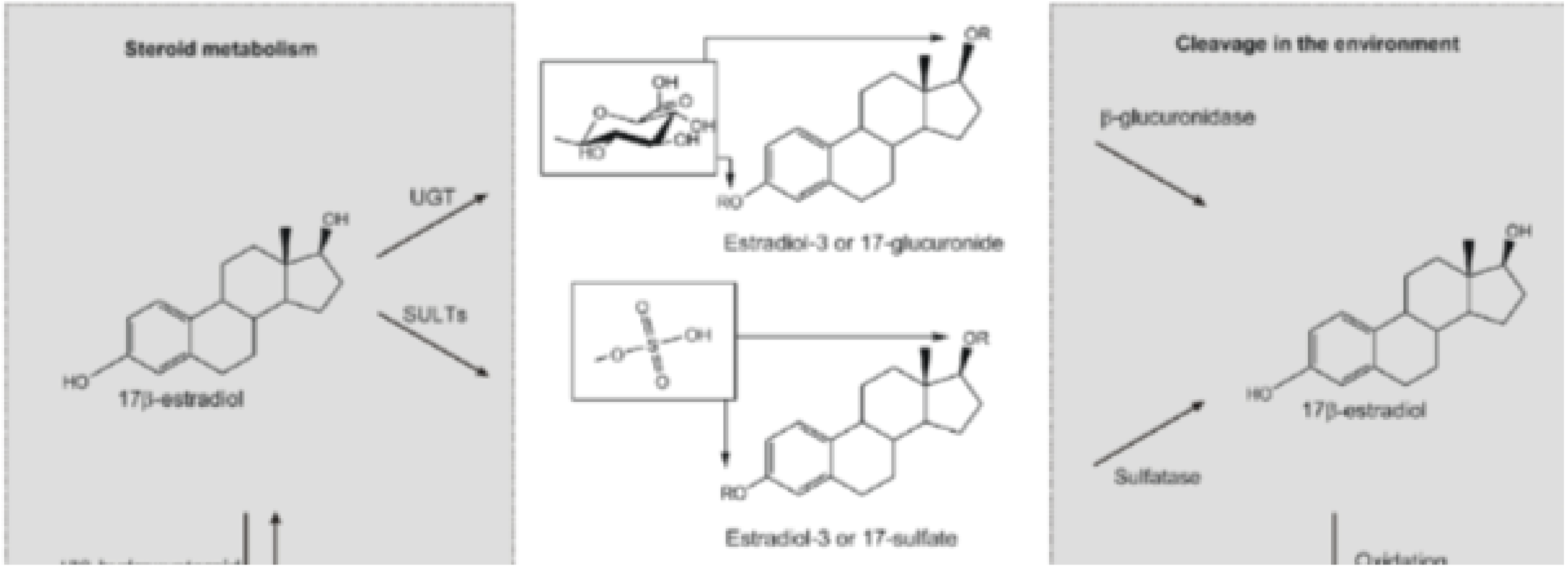

Opposite effect for many drugs like (oral) estradiol.

More absorbed with less glucuronidation

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Thank You!

Contact me: Kyle@gillettehealth.com

The Gut Microbiome Connection

Advancing Systemic Health Protocols

Session 4

Dr. Sue Mitchell, MD

The Vibrant Longevity Summit

The Gut-Microbiome Connection

How Systemic Health Starts in the Gut

Sue Mitchell, MD

Meet Your Speaker

Sue Mitchell, MD

Board-Certified Gastroenterologist

Founder of Gutwell Medical

www.gutwellmedical.com

T.719-238-6664

Objectives

- My journey from traditional to functional
- Importance of detailed GI symptom history
- Abnormal gut motility's link to systemic inflammation
- Vibrant Panels in my clinical practice
- My approach and GI pearls using Vibrant 

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Full Transparency
10 years ago

"Oh, you're on a PPI?"
"No problem-stay on it indefinitely"

"Vitamin D deficiency and gut health?"
"Talk to your PCP"

"Candida overgrowth?"
"No such thing - Candida is normal in the gut"

"Fiber?"
"Just take Metamucil every day"

Traditional Gastroenterology

- **Traditional** private practice 1996 -2021
- Stong referral base of **“IBS” type patients**
- Referral center for **motility issues**
- 2018 - hired NP provider certified in **Functional Medicine**
- 2019- Gastroenterology Advanced Practice Module (IFM)

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Functional Gastroenterology

- The microbiome is **REVOLUTIONARY!** 
- **2019 –2025:** Margaret Harris, PhD, Professor of Nutrition at UCCS
  - Expert in microbiome, supplements, and nutrigenomics
- **Vibrant Wellness Panels**
- **Functional & Traditional GI –** 2 years to develop protocols
- **2021 until now – Gutwell Medical**

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THE IMPORTANCE OF A GOOD HISTORY

Functional Dyspepsia

Bloating
Epigastric pain
Gas
Early satiety

Belching
Nausea

Gastroparesis

Bloating
Epigastric pain
Gas
Early satiety

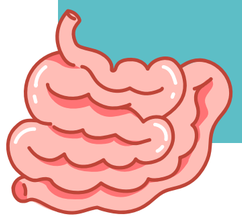
Belching
Nausea/Vomiting
Constipation

STOMACH

SIBO/SIFO/IMO

Bloating
Abdominal pain
Gas/Flatulence
Early satiety

Diarrhea
Constipation



SMALL INTESTINE

Clinical GI Questions

Stomach vs Small Intestine

- Bloating?
- Abdominal pain?
- Early satiety?
- What type of foods make you feel worse?
- Do you feel full when you go to bed?

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Gastroparesis

Making the Diagnosis

- **Gold Standard = 4-hour Gastric Emptying Scintigraphy (GES)**
  - Diet = radiolabeled egg whites/toast/jam
  - Diagnostic Criteria:
    - 2 hours: > 60 % of meal remains **OR** 4 hours: >10% of meal remains

- On EGD: see food in stomach from night before → then probable gastroparesis but patients likely to be dx with Functional Dyspepsia

Pitfalls with GES:

- **Circadian rhythms affect GES**
- **Test meal does not contain fat or fiber**

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The Same Spectrum of Disease

Functional Dyspepsia & Gastroparesis

- 2025 - Paper suggesting part of same spectrum of disease[1]
- **SXS overlap** and **FD** 25 -37% have delayed gastric emptying[2]
- 2022 studies link **FD to bx proven leaky gut[3]**
  - Dx Celiac disease - duodenal biopsies
- Idiopathic gastroparesis - associated with increased hsCRP/HgA1C[4] which is linked to systemic inflammation

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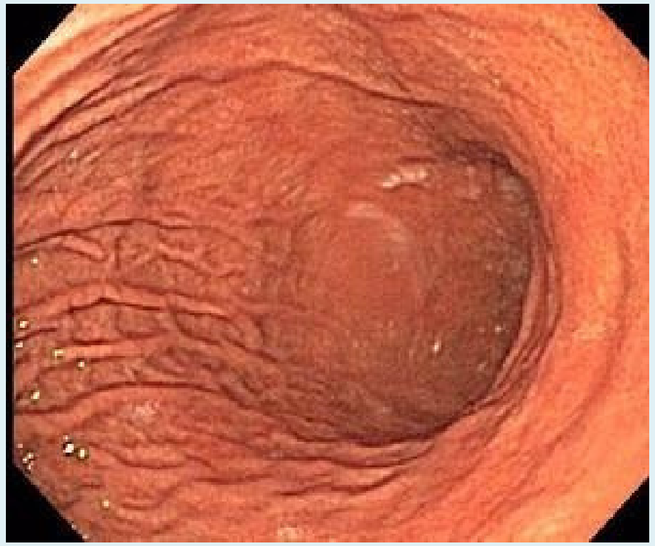
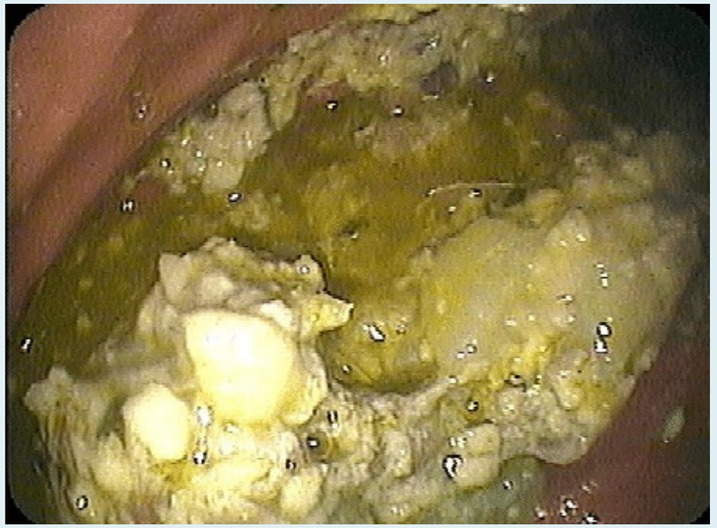
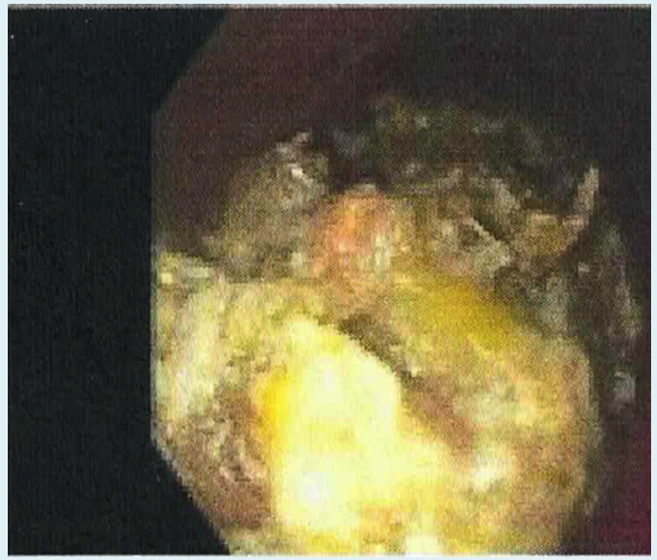
Optimizing GI Microbiome Begins in the Stomach



Normal Stomach EGD





Retained Plant Fibers

Both patients had normal GES

Plant fiber requires mechanical grinding by stomach[5]

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Traditional Treatments

Functional Dyspepsia	Gastroparesis	SIBO/IMO/SIFO
Proton Pump Inhibitors SSRI's Carafate	Promotility drugs Gastroparesis Diet *Standard of Care*	Antibiotics Promotility drugs

What do all these entities have in common?
All three are associated with **MMC impairment[6-8].**

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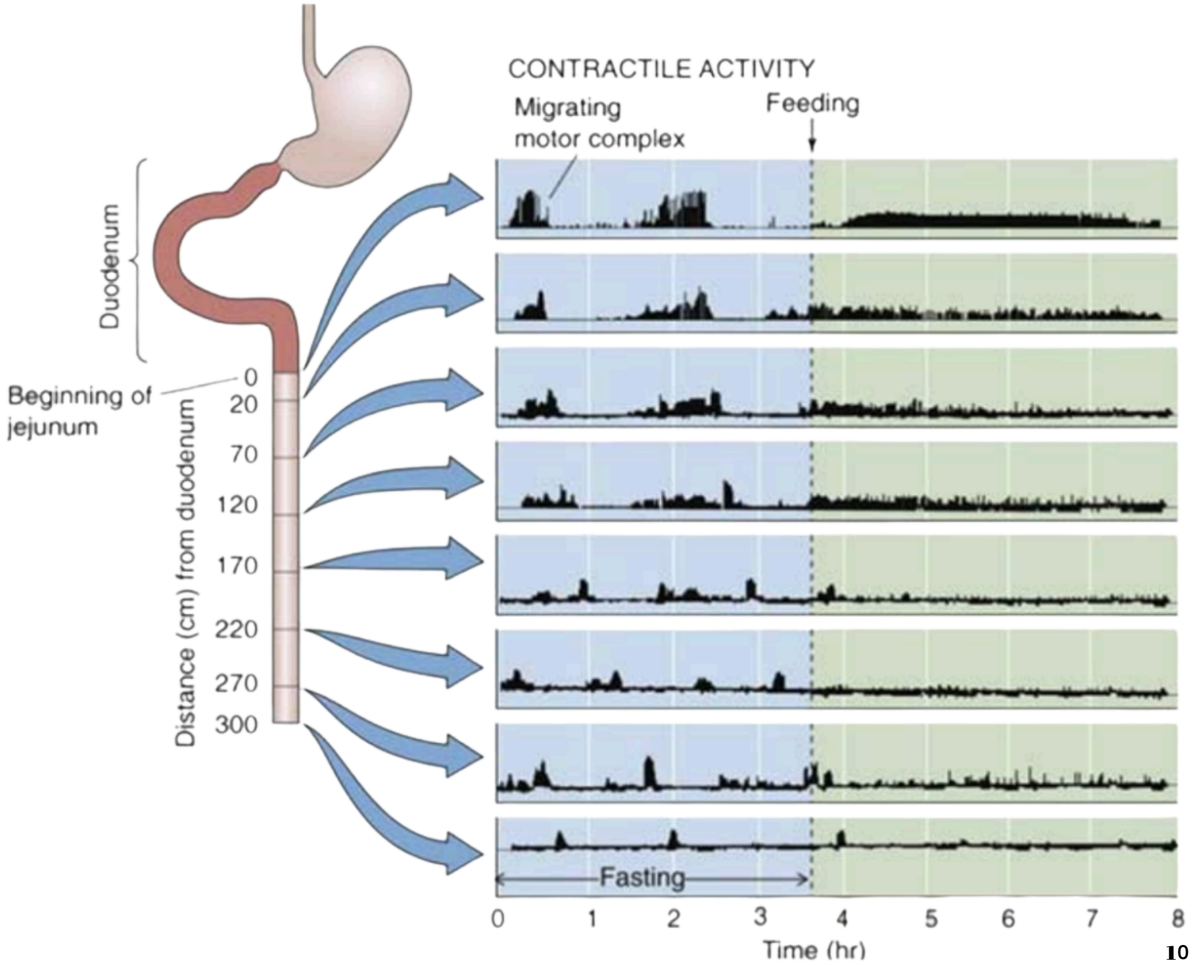
Migrating Motor Complex

Normal MMCs are vital for a healthy microbiome

- Push food, debris, toxins, pathogens through SI (90 mins)
- Pacemaker cells initiate MMC
  - Antrum ~ 72%
  - Duodenum ~28%
- Antrum origin = stronger amplitude and longer duration[9]



10

Image from: Doctorlib

Overview of Motility

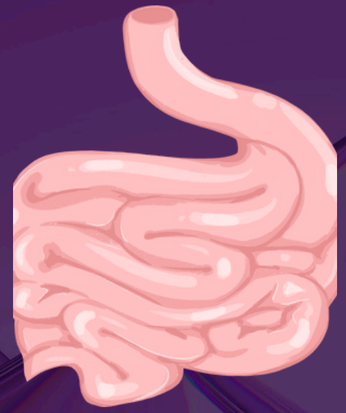
Functional Dyspepsia Gastroparesis

SIBO/IMO/SIFO

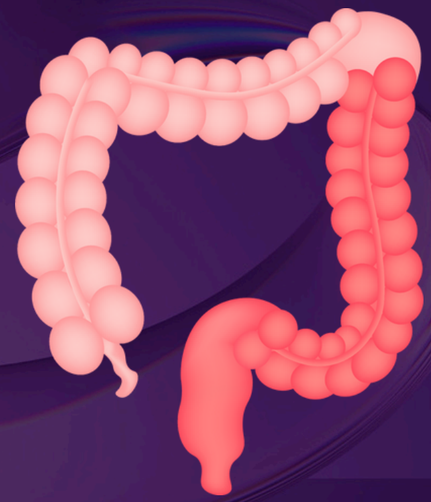
Microbiome Dysbiosis Systemic Inflammation

Originate MMC 72%

Originate MMC 28%



Abnormal MMCs



MMCs begin when stomach is empty[11]
Fat delays MMCs the longest and protein the shortest[12]

My Protocol
Initial Appointment

90-minute initial appointment includes:

- **Detailed history**
- **Review initial labs - Health Span Assessment**
- **Education on the gut**
- **Two-week detox protocol**
- **Symptom tracker**
- **Additional testing (Vibrant panels, SIBO etc.)**

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Health Span Assessment Panel

- Nutritional Status: iron panel, **folate**, **vitamin D, vitamin B12, ferritin, omega index**
- Blood sugar regulation: **glucose, HgA1c, insulin**, glycated serum protein, adiponectin, leptin
- Inflammation: **hsCRP, ox-LDL, homocysteine, MPO, PLAC**
- Autoimmunity: rheumatoid factor, ANA
- Adrenal and Sex Hormones: cortisol, estradiol, testosterone, progesterone
- Thyroid: **T3, T4, free T3, free T4, TSH, anti-TPO, rT3, anti-TG**
- Immune: IgG, IgM **(add on IgA when you order)**
- Hematologic: **CBC with differential**
- Chemistries: **CMP (includes GGT)**
- Cardiovascular: **LDL, sdLDL, HDL, Apo-A-1, ApoB**

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My Gut-Healing Protocol

- Liquid/**Smoothie** meals - **to ensure optimal conditions for antral MMC**
- **Nourish small intestine**
- **Remove inflammatory foods**
  - **Vibrant Food Sensitivities and Food Zoomers**
- Ensure **empty stomach** when going to **bed**
  - Increase in jejunal phase 3 MMCs while sleeping[13]
- Quiet the brain!

Standard of Care – Gastroparesis

Cleveland Clinic's Gastroparesis Diet

Stage 1	Stage 2	Stage 3
• PowerAde • Gatorade • Soft drinks • Bouillon	• Skim milk • Fat free broth • Cream of wheat • Scrambled eggs • Vegetable and fruit juices • Cooked vegetables without skins	• Blended foods • Lean meats (ground) • Poultry • Peanut butter

My Gastroparesis Diet

Nourishing the Microbiome

Berries (blueberries, raspberries, etc.)
Prebiotic & Phytonutrients

Green banana - optional
Prebiotic

Inflammacore Powder
Protein/Glutamine/Antioxidants

Goat milk
Prebiotic (HMO)

Goat milk kefir
Probiotic

Add Protein
Add SBI/Sacc B, etc.

*Developed with Dr. Margaret Harris, PhD

15-19

Goals:

Rest stomach - Optimize MMC's - Nourish proximal small intestine

Favorite Vibrant Panels

- **Vibrant Gut Zoomer**
  - Good for *all GI sxs*
  - Inflammatory markers, SIFO, leaky gut, etc.
- **Vibrant Wheat Zoomer**
  - Helpful for confirming *leaky gut dx* and *wheat sensitivity*
- **Food Sensitivity Panel (Food Zoomers)**
  - Identify *inflammatory foods/leaky gut triggers*
- **Vibrant Candida/IBS Panel**
  - Helpful in *SIFO dx* and *root cause for IBS* (Ab to CdtB and vinculin)
- **Vibrant Micronutrient Panel**
  - Great to *personalize supplements*

Two Week Detox Protocol

Breakfast

Healing Smoothie

30-35 g protein

Lunch

Soft, easy to digest

Ground meats/ steamed veggies

30-35 g protein

Dinner

Healing Smoothie

30-35 g protein

Optimize nighttime MMC's

Symptom Tracker

Real Time

"Please rate your symptoms every day on a scale from 0-10, 10 being the worst it gets, and 0 being completely resolved. Some may be yes/no or other numerical ratings."

WEEK 1									
	Symptom	**Baseline**	**Mon**	**Tues**	**Wed**	**Thur**	**Fri**	**Sat**	**Sun**
Symptom 1	**Bloating**	7/10	7	7	6	5	5	4	4
Symptom 2	**Feeling Full**	8/10	8	8	8	6	6	4	3
Symptom 3	**Flatulence**	6/10	6	6	5	5	5	3	3
Symptom 4	**Fatigue**	8/10	8	8	7	7	6	6	6
Symptom 5	**Lack of Hunger**	9/10	9	9	5	5	4	4	4
Symptom 6	**Stomach Empty at bedtime**	No	No	No	Yes	Yes	Yes	Yes	Yes

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Two Week Follow Up

- Follow up in two weeks
- Collaborate on patient's next steps
- Our 6-week program - **Dr. Mitchell** + **Functional Nutritionist**
- Conditions that *always use* smoothie protocol:
  - Diverticulitis
  - C. *difficile*/Infectious/Microscopic colitis
  - SIBO/SIFO
  - IBD - Crohn's with narrowing of intestine
  - FD/Gastroparesis

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Case 1

Crohn's & Leaky Gut

HPI:
40 yo female with **hx of Crohn's, s/p subtotal colectomy/high ostomy output**. Mayo evaluation and **negative SIBO**

SXS:
Early satiety, bloating, abd distention followed by **explosive ostomy output**

Vibrant Testing:
Cardiac CRP - 2.5, severe vitamin D deficiency, **Food Sensitivity Panel (corn, wheat, eggs)**

TX:
Corrected nutrient deficiencies
Two-week protocol (SBI and Sacc. B)
3rd Week: ostomy output reduced by 50% /Solid lunch using **Vibrant Panels**
Added inulin (prebiotic fiber) to smoothie

6 weeks - solid stool from ostomy and cardiac CRP at 1.3

Protocol optimized feeding the small bowel

Case 2

SIFO Dx via Vibrant

HPI:
45 yo male came from Puerto Rico convinced he had SIFO, but GI wouldn't treat. **Recurrent UTI past 4 months,** tx with various Abx, no improvement. Diflucan for 4 days for yeast in urine and felt better.

SXS:
Severe **belching**, mild **bloating,** abdominal discomfort

Labs:
Cardiac CRP - 2.23

Testing:
Candida/IBS Panel
Gut Zoomer
Total Toxic Burden- normal

Traditonal GI does not tx SIFO

***Vibrant Gut Zoomer and IBS/Candida panel**

Case 2

SIFO Insight via Vibrant

Gut Zoomer Results

GUT PATHOGENS			
Fungi	Current	Previous	Reference
Candida spp.	8.9e3		≤1.1e2

Candida + IBS Panel Results

Candida	Current (IgG + IgA)	IgM
Candida albicans	6.8	4.6
Candida tropicalis	>30	4.2
Candida parapsilosis	22.4	4.9
Candida glabrata	11.1	4.3
Candida krusei	5.9	3.8
Candida lusitaniae	27.1	4.3
Candida dubliniensis	19.7	3.4
Candida guilliermondii	>30	3.1

Case 2

SIFO Dx via Vibrant

Treatment

- **Diflucan 100 mg po daily for 14 days**
- **Nystatin 500,000 unit tabs po QID for 2 months**
- **Discussed dietary protocols**
- **Consider adding SBI to smoothie protocols**

Flagyl/Metronidazole is good for methane producers

Case 3

Methanogens & Constipation

HPI:
43 yo female hx of **methane/hydrogen SIBO** - two rounds Abx
Hospitalized for food poisoning prior to symptoms beginning

SXS:
Bloating (abd distention), fatigue, diet fatigue, constipation (cannot evacuate rectum)

Labs:
HgA1C: 5.4, glucose: 93, Insulin: 10.7, **hsCRP: 2.89**, **IgA: 448** , vitamin D: 29

Vibrant Panels:
Gut Zoomer
Candida IBS panel

Case 3

Vibrant Panels

IBS Candida Panel

Anti-Vinculin IgG	3.4	0 10 20	≤10.0
Anti-Cytolethal Distending Toxin B IgG	14.4	0 10 20	≤10.0

***Candida Antibodies were also moderately positive**

Gut Zoomer

Methanobrevibacter smithii	26.3	0 20	≤20.0
Fecal Zonulin (ng/mL)	391.9	0 25 160	25.1-160.8
pH	6.2	0 6 7.8	6.1-7.8
sIgA (mcg/g)	1900.8	0 425 1450	426.0-1450.0

Case 3

Symptom Trackers

WEEK 1

	Symptom	Baseline	Mon	Tues	Wed	Thur	Fri	Sat	Sun
Symptom 1	Bloating	7/10	6	6	5	5	5	5	4
Symptom 2	Flatulence	7/10	6	6	6	5	6	5	4
Symptom 3	Fatigue	5/10	5	5	5	4	4	5	5
Symptom 4	Brain Fog	5/10	5	5	5	4	4	4	4
Symptom 5	Puffiness	6/10	6	5	5	5	5	5	5
Symptom 6	Crazy Brain	6/10	5	5	5	5	4	4	4

WEEK 5

	Symptom	Baseline	Mon	Tues	Wed	Thur	Fri	Sat	Sun
Symptom 1	Bloating	7/10	2	2	2	2	2	2	2
Symptom 2	Flatulence	7/10	3	3	3	2	2	2	2
Symptom 3	Fatigue	5/10	2	2	2	2	2	2	2
Symptom 4	Brain Fog	5/10	2	2	2	2	2	2	2
Symptom 5	Puffiness	6/10	2	2	2	2	2	2	2
Symptom 6	Crazy Brain	6/10	2	2	2	2	2	2	2

Case 4

Root Cause & IBS Panel

HPI:
37 yo WF (Functional Nutritionist) - 20 years of GI issues - started after severe **food poisoning event**, subsequent food poisoning resulted in flare of symptoms and slowing of motility.

SXS:
Early satiety, nausea, **visceral hypersensitivity**, abd distention and bloating, abd pain - worse as the day goes on

Previous testing:
GI workup:
EGD - normal, GES - normal, Colonoscopy - normal

Could not find root cause of symptoms

Case 4

Root Cause & IBS Panel

IBS testing:
Showed positive Anti-Vinculin antibodies

Antibody Detected	Patient Value (OD)	Antibody Levels
Anti-CdtB Ab	1.50	Not Elevated
Anti-Vinculin Ab	1.97	Elevated

Correlation with IBS:

- Anti-CdtB antibodies can mistakenly target and damage gut neurons, **disrupting gut motility** and leading to **post-infectious IBS**

- Anti-vinculin antibodies are correlated with decreased pacemaker cells in stomach and associated with **altered MMCs** in SIBO[20]

Vibrant IBS/Candida panel can help find root cause of IBS symptoms

Final Case

C.diff Colitis vs C. diff Carrier

GUT PATHOGENS

Bacteria	Current	Previous	Reference	Bacteria	Current	Previous	Reference
Clostridium difficile	<1e1		≤5e2	Clostridium difficile Toxin A	5.5e4		≤5.8e2
Clostridium difficile Toxin B	<1e2		≤5.8e2	Clostridium perfringens	<1e2		≤1e2

Pt. can be a carrier of C. diff, but to have C. diff colitis, you must prove that the toxin is present (further testing).

For carriers, use caution with antibiotics and work to optimize microbiome.

C. *diff* Carrier vs. C. *diff* Colitis

Bacteria

Campylobacter *(C. jejuni/ C. coli)*	Not Detected
Clostridium difficile *Toxin A/B gene only * Correlate with C. diffcile Toxin A/B, EIA*	Detected
Escherichia coli O157 *(E. coli O157)*	Not Detected
Enteroaggregative E.coli *(EAEC)*	Not Detected
Enterotoxigenic E.coli *(ETEC) lt/st*	Not Detected
Salmonella spp.	Not Detected
Shiga-like toxin producing E.coli *(STEC) stx1/stx2*	Not Detected
Shigella spp. / Enteroinvasive E.coli *(EIEC)*	Not Detected
Vibrio spp. *(V. vulnificus/ V. cholerae)*	Not Detected
Vibrio parahaemolyticus	Not Detected
Yersinia enterocolitica	Not Detected

Viruses

Adenovirus F 40/41	Not Detected
Norovirus GI/GII	Not Detected
Rotavirus A	Not Detected

Parasites

Cryptosporidium	Not Detected
Entamoeba histolytica	Not Detected

Result: No ova and parasites seen

Comments: *One negative specimen does not rule out the possibility of a parasitic infection.*

WHITE BLOOD CELLS, STOOL

Result: No white blood cells seen. Normal

Reference Range: Normal: No white blood cells seen.

FECAL FAT, QUALITATIVE

Neutral Fat: < 60 fat globules / HPF Normal

Reference Range: Normal: < 60 fat globules/HPF
Increased: ≥ 60 fat globules/HPF

CALPROTECTIN, STOOL

Result: 80.8 mg/kg Borderline

Reference Range: Normal: < 50 mg/kg
Borderline: ≥ 50 – < 120 mg/kg
Abnormal: ≥ 120 mg/kg

PANCREATIC-ELASTASE ELISA, STOOL

Result: 450.19 µg/mL Normal

Reference Range: Normal: > 200 µg/mL
Slight to moderate pancreatic insufficiency: 100-200 µg/mL
Severe pancreatic insufficiency: < 100 µg/mL

C. difficile TOXIN A/B, STOOL (EIA) *

Result: Positive Abnormal

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Clinical Pearl Takeaways

- Gastroparesis is underdiagnosed in traditional GI
- Focus on optimizing MMCs as treatable root cause to systemic inflammation.
- Consider SIFO as a root cause patients with SIBO, gastroparesis and leaky gut
- **Vibrant IBS/Candida Panel and Gut Zoomers can help identify root causes**

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Thank You!

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Scan the QR code to access GI Restoration Protocol.

Password "**Longevity**"

This link will be active for the next 30 days!

www.gutwellmedical.com

Citations

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