The 111th Abbott Nutrition Research Conference

June 29–July 1, 2010
Columbus, Ohio

Subject: Inflammation and Nutrition in Chronic Disease
Welcome

We are pleased to provide you with the Proceedings of the 111th Abbott Nutrition Research Conference, entitled “Inflammation and Nutrition in Chronic Disease.”

The 111th Abbott Nutrition Research Conference is one of a series of conferences designed to connect the latest in science and research with the practice of clinical nutrition. We strongly believe that advancing therapeutic nutrition into clinical practice will play a vital role in the future of health care.

Inflammation is an intricate process that can present blatantly (eg, asthma, inflammatory bowel disease, and chronic obstructive pulmonary disease), work in concert with other chronic pathologies (eg, cardiovascular disease, diabetes, and kidney disease), or hide inconspicuously (obesity). Inflammation also plays a significant role in malnutrition and wasting in the clinical setting. Many of the conference participants research some aspects of inflammation pathways as they relate to these pathologies. T-cell activity, certain cytokines, inflammation-related mediators, and microbes all can play a role in the process that occurs in the course of acute upset or chronic disease.

A variety of foods, nutritional components, and diet patterns can affect the inflammation milieu and thus affect the outcomes in disease and malnourished states. Many practical issues in addressing chronic inflammation from a nutrition perspective were discussed in the 111th Abbott Nutrition Research Conference.

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Inflammation and Nutrition in Chronic Disease

The 111th Abbott Nutrition Research Conference was held in Columbus, Ohio, on June 29–July 1, 2010. This Report contains summaries of presentations given by the following contributors:

Keynote Address

Bioactive Molecules That Target Inflammation
Charles N. Serhan, PhD
Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA
Director, Center for Experimental Therapeutics and Reperfusion Injury

The identification of endogenous specialized proresolving mediators biosynthesized during inflammation resolution indicates that the resolution of acute inflammation is an active programmed process at the tissue level. This changes the century-old concept that resolution of acute inflammation is a passive process. Dr Serhan describes findings indicating that natural resolution pathways may underlie many prevalent diseases associated with uncontrolled inflammation and open the potential for resolution-based pharmacology.

Dietary Factors That Influence Inflammation

Inflammation and Nutrition in Chronic Disease
Gordon L. Jensen, MD, PhD
Pennsylvania State University, University Park and Hershey, PA, USA
Head, Department of Nutritional Sciences
Professor of Medicine, Hershey Medical Center

Over the last decade, the central role of inflammation in the burden of chronic disease that afflicts mankind has become increasingly appreciated. Growing evidence also shows that the pathophysiology of malnutrition that often accompanies disease invariably consists of a combination of varying degrees of undernutrition or overnutrition and inflammation leading to altered body composition and impairment of biological function. Dr Jensen describes the categories and characteristics of malnutrition syndromes, as well as nutrition interventions to address these syndromes.

Western Diet as a Trigger for Inflammatory Bowel Disease
Karen Madsen, PhD
University of Alberta, Edmonton, Alberta, Canada
Co-Director, Center of Excellence for Gastrointestinal Inflammation and Immunity Research
Professor of Medicine, Gastroenterology

Inflammatory bowel diseases, consisting of Crohn’s disease and ulcerative colitis, are chronic inflammatory conditions of the gut believed to occur in genetically predisposed individuals who are exposed to unknown environmental and microbial triggers. Dr Madsen explores the role of such triggers as gut microflora and dietary components and argues that with the advent of new molecular techniques, future studies examining a role for diet in triggering inflammatory bowel diseases also should include an assessment of this microflora, as well as the patient’s genetic makeup.

Rationale and Efficacy of Manipulating Intestinal Bacteria in Chronic Intestinal Inflammation by Probiotics, Prebiotics, and Diet
R. Balfour Sartor, MD
University of North Carolina, Chapel Hill, NC, USA
Midirectt Distinguished Professor of Medicine
Director, Microbiology and Immunology, Multidisciplinary IBD Center

Humans coexist with an incredibly complex diversity of bacteria, fungi, and viruses that increases in both complexity and number in the distal relative to the proximal GI tract. Some of these organisms are integrally involved in the pathogenesis of inflammatory bowel diseases. Dr Sartor argues that antibiotics, probiotics, and prebiotics, as well as the combination of all three approaches, have great potential to treat these diseases, but what maybe is needed is individualized treatment for each patient. To date, manipulating the intestinal microbiota in inflammatory bowel diseases has not substantially altered the underlying disease process or changed the natural history of these disorders, but dietary manipulation no doubt offers the most physiologic and least toxic approach to treating inflammatory bowel disease and has tremendous potential for long-term use.

Targeting Inflammation in the Metabolic Syndrome
Ishwarlal Jialal, MD, PhD
University of California, Davis, CA, USA
Professor of Internal Medicine
Stowell Endowed Chair, Experimental Pathology
Director, Laboratory for Atherosclerosis and Metabolic Research
The metabolic syndrome comprises a constellation of features, including abdominal obesity, high levels of triglycerides, high blood pressure, and fasting glucose ≥100 mg/dL. Patients with the syndrome are in a proinflammatory state, as evidenced by numerous biomarkers of inflammation. Dr. Jialal explains that therapeutic lifestyle changes, including weight loss and aerobic exercise, significantly reduce biomarkers of inflammation. The best studied is high-sensitivity C-reactive protein (hsCRP) levels. Developing evidence supports use of statin therapy in patients with the syndrome.

Obesity-Associated Inflammation and Its Role in Insulin Resistance

Andrew S. Greenberg, MD (with Martin S. Obin, PhD)
Tufts University, Boston, MA, USA
Atkins Professorship in Metabolism and Nutrition
Director, Laboratory of Obesity and Metabolism

A body of data has suggested that obesity promotes inflammation, which has become increasingly implicated as an important etiologic protagonist in the development of insulin resistance. Dr. Greenberg summarizes research showing that adipocytes may act as an initiating agent in adipose tissue inflammation and/or in promoting a feed-forward cycle of macrophage recruitment to adipose tissue. The increased level of adipocytes and adipose tissue inflammation appears to have an important role in the development of obesity-associated inflammation and insulin resistance.

Obesity-Induced Inflammation and Insulin Resistance

Jongsoon Lee, PhD
Harvard University, Boston, MA, USA
Assistant Investigator, Cellular and Molecular Physiology, Joslin Diabetes Center
Assistant Professor of Medicine (pending)

Obesity is universally accepted as the culprit of insulin resistance and type 2 diabetes but the underlying molecular mechanisms are not yet fully established. Recently, inflammation was recognized as the pathogenic mediator of not only these diseases but also metabolic syndromes such as cardiovascular disease. Dr. Lee argues that circulating monocytes may systemically regulate adipose tissue macrophage inflammation by regulating macrophage numbers and their fate in adipose tissue, and that salicylate treatment may reverse these processes by specifically suppressing monocyte activation, thereby improving obesity-induced inflammation and insulin resistance.

Inflammation and Wasting in Chronic Kidney Disease: Partners in Crime

Peter Stenvinkel, MD, PhD
Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden
Professor of Renal Medicine

Nearly 4000 articles were published on inflammation in chronic kidney disease since the first reports in the late 1990s connecting uremic inflammation with a wasted, fatigued, and calcified phenotype that leads to premature death. What was described as a “novel” risk factor 10 years ago has by now evolved into an established finding in end-stage renal disease patients. Dr. Stenvinkel examines the interrelationships of inflammation and wasting, describes biomarkers of inflammation, and offers recommendations for clinical intervention, as well as future research.

Current Asthma Management: Opportunities for a Nutrition-Based Intervention

Stanley J. Szefler, MD
National Jewish Health, Denver, CO, USA
Director, Weinberg Clinical Research Unit/Pediatrics Section
Head, Pediatric Clinical Pharmacology

Several potential methods can improve asthma outcomes by directing a management concept to a personalized medicine approach, including early recognition and treatment of asthma, application of genetics and epigenetics to predict risk for developing persistent asthma, utilization of biomarkers to monitor disease activity, and the development of new approaches to manage inflammation, including immunomodulator therapy. Dr. Szefler describes recent accomplishments in asthma management, some of the unmet needs in light of current management principles, and the potential role of nutrition in future asthma management.

COPD: Inflammation, Phenotypes, and Nutrition

Barry J. Make, MD
National Jewish Health, Denver, CO, USA
Co-Director, Chronic Obstructive Pulmonary Disease (COPD) Program
Director, Pulmonary Rehab and Respiratory Care

COPD phenotypes are defined by using the presence and severity of airflow limitation, with COPD clinical practice guidelines defining COPD severity by the presence of forced expiratory volume in 1 second (FEV1). However, the strength of the correlation of FEV1 with clinical features is modest, making other phenotypes
Perhaps more important than lung function alone, Dr. Make looks at these clinically meaningful phenotypes and how COPD patients with these features may be the best candidates for adequate macronutrient intake, as well as novel nutritional interventions, such as use of docosahexaenoic acid, eicosapentaenoic acid, gamma-linolenic acid, vitamin D, antioxidants, and omega-3 and omega-6 fatty acids.

**Therapies for Inflammation: Gaps for Nutrition To Fill**

**Nutritional Effects on Inflammation and Infectious Disease: Is There a Role for Specific Nutrients?**

Melinda Beck, PhD  
University of North Carolina, Chapel Hill, NC, USA  
Professor and Director, Department of Nutrition,  
Division of Nutritional Biochemistry  
Associate Chair, Gillings School of Global Public Health

Specific nutrients can influence host inflammation, either in a beneficial or deleterious manner, depending on individual nutrients and circumstances. Dr. Beck discusses her work with fish oil, antioxidants associated with providing cellular protection against inflammation, the importance of nutrients such as vitamin E and selenium in a functioning immune system, and the effect of nutrition on the immune system to reduce inflammation in order to treat chronic disease. By dampening down the inflammation in order to treat a chronic illness, the host may have less ability to fight infection and therefore become more susceptible to infections. Dr. Beck tests this hypothesis using a well-established mouse model of influenza virus infection.

**A Nutrigenomic Approach to Prevention of Inflammation**

Henk F. J. Hendriks, PhD  
TNO Quality of Life, Zeist, Netherlands  
Senior Scientist and Project Leader, Human Physiology Group  
Product Manager, Human Volunteer Studies in Nutrition

Reduction of the inflammatory status may prevent the occurrence of disorders and diseases related to overweight. In contrast to accepted biomarkers, the application of nutrigenomics techniques for large-scale profiling of genes, proteins, and metabolites shows that an anti-inflammatory dietary mix is able to influence the processes of inflammation, oxidative stress, and metabolism in humans. Dr. Hendriks concludes that the use of comprehensive techniques, such as metabolic, protein, and gene profiling, facilitates the accurate and detailed quantification and description of the molecular processes involved.

**A Dietary Inflammatory Index to Predict Changes in Inflammatory Markers**

James R. Hébert, MSPH, ScD  
University of South Carolina, Columbia, SC, USA  
Director, South Carolina Statewide Cancer Prevention and Control Program  
Professor of Epidemiology and Biostatistics

Diet plays a central role in the regulation of chronic inflammation. After searching and not finding a diet-based inflammatory index, Dr. Hébert started developing one. Qualifying articles were indexed, read, and scored, with the overall goal of defining and validating an index that assesses the inflammatory potential of the diet on a continuum from maximally anti-inflammatory to maximally pro-inflammatory. Results were consistent with the effect of a diet-derived estimate of inflammation in interval changes in hsCRP. Dr. Hébert also shares the issues that still need to be addressed as part of the ongoing commitment to refining and improving the Dietary Inflammatory Index.
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<tbody>
<tr>
<td>AA</td>
<td>arachidonic acid</td>
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<tr>
<td>AGE</td>
<td>advanced glycation end product</td>
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<tr>
<td>AIDM</td>
<td>anti-inflammatory dietary mix</td>
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<tr>
<td>ATBC</td>
<td>Alpha-Tocopherol and Beta-Carotene (study)</td>
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<td>ATM</td>
<td>adipose tissue macrophage</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>BAC</td>
<td>bacterial artificial chromosome</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CARET</td>
<td>Beta-Carotene and Retinol Efficacy Trial</td>
</tr>
<tr>
<td>CARS</td>
<td>compensatory anti-inflammatory response syndrome</td>
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<tr>
<td>CCFA</td>
<td>Crohn’s &amp; Colitis Foundation of America</td>
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<tr>
<td>CD</td>
<td>Crohn’s disease</td>
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<tr>
<td>CKD</td>
<td>chronic kidney disease</td>
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<tr>
<td>CLS</td>
<td>crown-like structures</td>
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<tr>
<td>COPD</td>
<td>chronic obstructive pulmonary disease</td>
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<tr>
<td>COX-2</td>
<td>cyclooxygenase 2</td>
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<tr>
<td>cPLA2</td>
<td>cytosolic phospholipase A2</td>
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<tr>
<td>CRH</td>
<td>corticotrophin-releasing hormone</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DGLA</td>
<td>dihomo-gamma-linolenic acid</td>
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<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
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<tr>
<td>DIO</td>
<td>diet-induced obesity (animal model)</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
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<td>EPR-3</td>
<td>Expert Panel Report-3</td>
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<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>FDA</td>
<td>US Food and Drug Administration</td>
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<tr>
<td>FEV</td>
<td>forced expiratory volume</td>
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<td>FFQ</td>
<td>food-frequency questionnaire</td>
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<tr>
<td>FGF</td>
<td>fibroblast growth factor</td>
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<tr>
<td>GC-MS</td>
<td>gas chromatography-mass spectrometry</td>
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<tr>
<td>GFP</td>
<td>green fluorescent protein</td>
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<tr>
<td>GI</td>
<td>gastrointestinal</td>
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<td>GLA</td>
<td>gamma-linolenic acid</td>
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<td>GOLD</td>
<td>Global Initiative for Chronic Obstructive Lung Disease</td>
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<td>GPX</td>
<td>glutathione peroxidase</td>
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<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
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<tr>
<td>HPLC</td>
<td>high-pressure liquid chromatography or high-performance liquid chromatography</td>
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<tr>
<td>hscRP</td>
<td>high-sensitivity C-reactive protein</td>
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<tr>
<td>HSL</td>
<td>hormone-sensitive lipase</td>
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<tr>
<td>IKK</td>
<td>I kappaB kinase</td>
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<tr>
<td>IBD</td>
<td>inflammatory bowel disease</td>
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<tr>
<td>IFN-γ</td>
<td>interferon-gamma</td>
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<tr>
<td>IGF-1</td>
<td>insulin-like growth factor 1</td>
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<tr>
<td>IL</td>
<td>interleukin (eg, IL-6, IL-8, IL-10, IL-18)</td>
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<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
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<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
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<tr>
<td>LO</td>
<td>lipoxigenase (eg, 5-LO, 12-LO, 15-LO)</td>
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<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>LVRS</td>
<td>lung volume reduction surgery</td>
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<tr>
<td>MARS</td>
<td>mixed anti-inflammatory response or mixed antagonist response syndrome</td>
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<tr>
<td>MCP-1</td>
<td>monocyte chemotactic protein-1 or monocyte-chemoattractant protein-1</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic Syndrome</td>
</tr>
<tr>
<td>MMP-9</td>
<td>matrix metalloproteinase 9</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NASH</td>
<td>National Asthma Education and Prevention Program</td>
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<tr>
<td>NDS</td>
<td>Nutrition Data System</td>
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<tr>
<td>NFkB</td>
<td>nuclear factor kappa B</td>
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<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
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<tr>
<td>NNI</td>
<td>novel nutritional formula</td>
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<tr>
<td>NPO</td>
<td>nothing by mouth</td>
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<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor-1</td>
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<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cell</td>
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<tr>
<td>PEW</td>
<td>protein-energy wasting</td>
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<tr>
<td>PIO</td>
<td>pioglitazone</td>
</tr>
<tr>
<td>PMN</td>
<td>polymorphonuclear (leukocytes)</td>
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<tr>
<td>PGE2</td>
<td>prostaglandin E2</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acid (omega 3, omega 6 [n-3, n-6])</td>
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<tr>
<td>RBP4</td>
<td>retinol-binding protein 4</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxidant species</td>
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<tr>
<td>SAA</td>
<td>serum amyloid A protein</td>
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<tr>
<td>Se</td>
<td>selenium</td>
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<tr>
<td>SEASONS</td>
<td>Seasonal Variation of Cholesterol Levels Study</td>
</tr>
<tr>
<td>SIRS</td>
<td>systemic inflammatory response syndrome</td>
</tr>
<tr>
<td>SPM</td>
<td>specialized pro-resolving mediator</td>
</tr>
<tr>
<td>TB</td>
<td>tuberculosis</td>
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<tr>
<td>TC</td>
<td>total cholesterol</td>
</tr>
<tr>
<td>TGFβ</td>
<td>transforming growth factor beta</td>
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<tr>
<td>Th</td>
<td>T helper (cell)</td>
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<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-alpha</td>
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<tr>
<td>TLR</td>
<td>toll-like receptor</td>
</tr>
<tr>
<td>TPA</td>
<td>tissue plasminogen activator</td>
</tr>
<tr>
<td>UC</td>
<td>ulcerative colitis</td>
</tr>
<tr>
<td>UPR</td>
<td>unfolded protein response</td>
</tr>
<tr>
<td>XBP1</td>
<td>X box binding protein-1</td>
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Using a systems approach with self-limited inflammatory exudates to track and map tissue events, cell traffic, and identification of protein and chemical mediators, we identified novel families of potent bioactive lipid-derived mediators, coined the resolvins and protectins, in resolving exudates. Each of these pro-resolving mediators controls the duration and magnitude of acute inflammation in vivo with stereospecific actions in the picogram to nanogram range and are biosynthesized from essential fatty acids. The mapping of these endogenous resolution circuits provides new avenues to appreciate the molecular basis of many widely occurring diseases that are characterized by ungoverned inflammation. Currently, many of the widely used anti-inflammatory therapies are directed toward the inhibition of key enzymes and/or antagonism of receptors involved in the inflammatory response. Both selective cyclooxygenase inhibitors and anti-tumor necrosis factor-alpha (TNF-α) are examples of this therapeutic approach that are used with the goal of blocking production of local proinflammatory chemical mediators. Research in the author’s laboratory focusing on profiling and mapping of self-limited inflammation has uncovered novel mechanisms that terminate the local acute inflammatory response, as well as stimulate resolution and return of the tissue to homeostasis. Identification of these biochemical and cellular processes indicates that resolution of acute inflammation is an active programmed process at the tissue level. Therefore, rather than targeting inhibition or antagonism of inflammation, our research addresses the potential use of endogenous agonists of resolution to stimulate key regulatory points that naturally resolve inflammation.

In general, an acute inflammatory reaction in response to infection or tissue damage is characterized at least at the gross level by the classic cardinal signs of inflammation (heat, redness, swelling, and pain). In vivo experiments the temporal relationships are well established—namely, edema and the accumulation of leukocytes, specifically polymorphonuclear (PMN) leukocytes, followed by accumulation of monocytes and macrophages. These events in self-limited or resolving inflammatory reactions are coupled with release of local factors that prevent further or excessive trafficking of leukocytes, allowing for resolution. Early in
Bioactive Molecules That Target Inflammation

the inflammatory response, proinflammatory mediators such as prostaglandins and leukotrienes play an important role.

The progression from an acute inflammation to chronic inflammation, as in many widely occurring human diseases such as arthritis, periodontal disease, and cardiovascular disease, is widely viewed as an excess of proinflammatory mediators. Although mononuclear cells can sometimes contribute to proinflammatory responses, they are also critical in wound healing, tissue repair, and remodeling in a noninflammatory, nonphlogistic manner. Hence, it is possible that defects associated with mounting endogenous pro-resolving circuits and local autacoids could underlie some of the aberrant mechanisms in chronic inflammation.1-3

Complete resolution of an acute inflammatory response and the return of local tissues to homeostasis are the ideal responses and are necessary for ongoing health. Removal of leukocytes from tissues involved in the inflammatory response without leaving remnants of the host defenses and combat between leukocytes, invading microbes, and/or other initiators of inflammation is an ideal outcome. In our laboratory, we have focused on the question, “How is the acute inflammatory response regulated?” It is widely believed that simple dilution of local proinflammatory mediators is sufficient to “burn out” inflammation, with the subsequent responses ending passively. We found that on initiation of inflammation with TNF-α, there was a typical acute-phase response denoted by rapid PMN leukocyte infiltration preceded by both local prostaglandins and leukotrienes. Unexpectedly, the eicosanoids then underwent what we have termed a “class switch.” As the exudate evolved, the eicosanoid profiles switched and the lipid mediators made within this milieu changed with time. Leukotrienes (potent chemo-attractants) were deactivated and the transcriptional regulation of enzymes required for lipoxin and resolvins production was activated. This in turn attracted mononuclear cells and stimulated macrophages to take up apoptotic neutrophils within the contained inflammatory exudate site.1-3

The author and colleagues have made advances recently on the biosynthesis and functions of this novel genus of specialized pro-resolving mediators (SPMs). These new families of local chemical mediators were originally identified in murine exudates captured during the natural self-limited phase. The SPMs include three chemical mediator families termed resolvins, protectins, and the most recent addition, maresins—as well as the aspirin-triggered epimeric forms of the lipoxins, resolvins, and protectins, which are biosynthesized from essential fatty acids. Lipoxins are produced from arachidonic acid and resolvins and protectins are biosynthesized from omega-3 EPA and DHA. Specific members of each family possess potent multipronged anti-inflammatory, pro-resolving and antimicrobial actions in models of sepsis.6 The actions of SPMs proved to be potent, cell type-specific, and stereoselective with human cells and in many experimental animal diseases. These diseases include periodontal disease, skin inflammation, peritonitis, colitis, and ocular inflammation. In mice, overexpression of the murine 12/15-lipoxygenase (12/15-LO) (in humans these are two enzymes, 15-LO type I and 12-LO) protects from atherosclerosis and the associated vascular inflammation via local production of SPMs including lipoxin A4, resolvin D1 and protectin D1.4 This is a key enzyme in the biosynthetic routes to SPMs that can include transcellular biosynthesis within contained inflammatory exudates in vivo.

Toward human direct translation of these findings with SPMs, we used microfluidics in addition to animal models to monitor the single-cell targeted actions of SPMs in tandem with LC-MS/MS-based lipid mediator lipidomics to assess the actions of rare and transient intermediates within the lipidome. These microfluidic chambers have bioassay compartments of ~1 µl volume and can isolate leukocytes in less than 5 minutes from whole blood rather than the routine 2–3 hours.5 Endogenous formation of resolvins and protectins and their protective roles were recently confirmed and extended, for example, in murine ischemic renal injury6 and in obesity-induced insulin resistance and liver disease.7 The identification of endogenous SPMs biosynthesized during inflammation resolution indicates that the resolution of acute inflammation is an active programmed process at the tissue level. This changes the century-old concept that resolution of acute inflammation is a passive process. In addition, the resolvins (eg, RvE1 and RvD1) are potent analgesics possessing direct actions within the central nervous system and reduce peripheral inflammatory pain.8 Together these findings indicate that natural resolution pathways may underlie many prevalent diseases associated with uncontrolled inflammation and open the potential for resolution-based pharmacology.

The author acknowledges support of NIH grants DE019938, NS067686, GM038765 and DK07448.
critical regulation of cytosolic phospholipase A2 (cPLA2), which is the case for eicosanoids. But throughout the course of an acute inflammatory response, at least in the laboratory environment, the lion’s share of the substrate in this system comes on the back of albumin for edema and then transferred into the exudate. Thus, peripheral blood levels are critical, namely the circulating levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). So if essential fatty acids are deficient, we cannot regulate the turn-off time of the acute inflammatory response.

How can 5-LO induce both kinds of outcomes? Early in the inflammatory response, it utilizes arachidonic acid. At first it makes leukotriene B4 and lipoxin A4. Then it moves on and begins to accept EPA and DHA. We should consider the question, what happens when EPA and DHA are not there?

I also want to emphasize for this group of conference participants that 5-LO inhibitors have not shaped up to be as promising therapeutics for human disease as the field had thought it would. Because, I believe, that 5-LO is not only critical in initial host defense, but it is critical in the initial termination reaction by contributing to resolvin and protectin formation.

Q: So timing is everything. The question is, what induces the inflammatory mediator pathways in this response? If I understand you correctly, 5-lipoxygenase (5-LO), which can induce a proinflammatory leukotriene B4, likewise stimulates through resolvin. Is that a difference in substrate, or can the same enzyme induce a good and a bad outcome?

Dr Serhan: As you said, timing is everything in this regulation. I was prejudiced with respect to the arachidonic acid model being esterified with phospholipid and the
Inflammation and Nutrition in Chronic Disease

Gordon L. Jensen, MD, PhD

Over the last decade the central role of inflammation in the burden of chronic disease that afflicts mankind has been increasingly appreciated. There is also growing evidence that the pathophysiology of malnutrition that often accompanies disease invariably consists of a combination of varying degrees of undernutrition or overnutrition and inflammation leading to altered body composition and impairment of biological function. Multiple definitions for malnutrition syndromes are found in the literature, promoting widespread confusion. To address this concern and to specifically recognize the important contribution of inflammation to malnutrition, an International Consensus Guideline Committee recently proposed a simple etiology-based construct for diagnosis of adults in the clinical practice setting. The suggested nomenclature includes three categories of malnutrition syndromes:

- **“Starvation-related malnutrition”** when there is chronic starvation without inflammation (eg, in medical conditions such as anorexia nervosa).
- **“Chronic disease-related malnutrition”** when inflammation is chronic and of mild to moderate degree (eg, with organ failure, pancreatic cancer, rheumatoid arthritis, or sarcopenic obesity).
- **“Acute disease- or injury-related malnutrition”** when inflammation is acute and of severe degree (eg, with major infection, burns, trauma, or closed head injury).

Individuals may be diagnosed in one or more of these categories or may change from one to another. Development of supporting laboratory, functional, food-intake, and body-weight criteria and their application to routine clinical practice will require validation.

A host of medical conditions have been revealed to be inflammatory states, spanning a continuum from life-threatening cachexia/undernutrition associated with advanced malignancies or organ failures to obesity/overnutrition and many of its comorbidities. Periodontal disease is a chronic inflammatory state that may injure vascular endothelium and is in turn associated with an increased risk of cardiovascular disease. Organ failure syndromes (heart, lung, liver, and kidney) are chronic inflammatory conditions associated with elevated tumor necrosis factor and associated anorexia and erosion of body cell mass. Aging itself is likely a chronic

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To improve outcomes in the setting of chronic disease-related malnutrition, integrated multidisciplinary approaches that include tailored nutrition interventions are needed. Such approaches may include anti-inflammatory medications, anti-cytokines, glycemic control, modulation of the microbiota, physical activity/weight loss, anabolic agents, and appetite stimulants. Nutrition interventions to modulate inflammation may include specialized nutrition support, anti-inflammatory diets, functional foods, and application of specific nutrients such as antioxidants, omega-3 fatty acids, and vitamin D. It is likely that the complexity and variability of inflammation and nutrition interactions in humans will necessitate the development of individualized approaches that target specific aspects of inflammatory response. Some individuals are disposed to much more robust inflammatory responses than others. There is great potential to tailor interventions based on both genotype and phenotype. The primary objective must be to alter undesirable aspects of inflammatory response to protect body cell mass and vital organ functions.

References

1. Jensen GL. Inflammation as the key interface of the medical and nutrition universes: a provocative examination of the future of clinical nutrition and medicine. JPEN. 2006;30:453-463.

Q & A

Q: You have posed an association between inflammation and malnutrition. Is there any evidence that correcting malnutrition will interfere with inflammation, or is it purely a secondary phenomenon of the malnutrition? In your organ transplant consults, you really need to interfere with the inflammation.
Inflammation and Nutrition in Chronic Disease

**Dr Jensen:** Indeed nutritional resuscitation of malnourished patients can restore immune responsiveness and facilitate inflammatory response. I am proposing that we can use nutritional interventions to modulate inflammation. Certainly one does not want to promote rejection of an organ transplant but one also does not want to blunt an appropriate adaptive inflammatory response to an infection or injury.

I think the answer to your question lies in a multifaceted approach. Can we make inflammation go away by simply giving protein and calories? Generally not. On the other hand, we may be interested in using some micronutrients such as antioxidants and vitamin D and substrates such as fish oil that have potentially desirable anti-inflammatory effects. This is medical nutrition, in which we use nutrients as part of the treatment of a specific inflammatory condition, as opposed to classic nutrition support involving a primary focus on delivery of protein and calories to patients. In critical care scenarios, we increasingly talk about volitional underfeeding. Why would anyone consider that? The hypothesis is that robust feeding at traditional calorie goals may actually fuel inflammatory pathways.

**Q:** Is that a protective response in part? I understand that at least with some cancers, nutrition intervention might actually hasten cancer progression.

**Dr Jensen:** Yes, there have been legitimate concerns about whether we would be feeding the tumor with nutrition interventions, but clinically this typically has not been manifest. In addition if the host is unable to complete meaningful treatment for malignancy secondary to severe undernutrition, that also is not a desirable outcome. This discussion leads us to what I assume will be a repeated theme throughout this conference—the differences between physiologic inflammation, adaptive inflammation, and pathologic inflammation. Of course we have not had great tools to differentiate among these types of inflammation. By the time we are dealing with pathologic inflammation, it is too late. This is part of the challenge. Certainly, mounting a fever and raising the white blood cell count may be important adaptive responses. So this issue does take on a great level of complexity. This is where work with protectins and resolvins, for example, is tantalizing, because we might be able to manipulate aspects of inflammation that will be highly beneficial.

Western Diet as a Trigger for Inflammatory Bowel Disease

Karen Madsen, PhD

Inflammatory bowel diseases (IBD), consisting of Crohn’s disease (CD) and ulcerative colitis (UC), are chronic inflammatory conditions of the gut believed to occur in genetically predisposed individuals who are exposed to unknown environmental and microbial triggers. Genetic susceptibility to IBD is well-recognized, with approximately 20% of IBD patients reporting a family history of IBD and twin studies demonstrating higher concordance rates among monozygotic twins. Recently, a meta-analysis of genome-wide-association studies confirmed 11 CD genetic loci and identified 21 new genetic loci. The genes identified to date have provided insight into the pathogenesis of IBD by implicating defects in innate and adaptive immunity, epithelial barrier function, and autophagy.

**Role of the Environment**

While IBD genes have provided insight into disease pathogenesis, genetics has not completely explained the etiology of IBD. The majority of patients with IBD have neither a family history nor a known genetic defect. IBD has emerged predominantly in industrialized nations in the last century; as developing nations have become industrialized, the incidence of IBD in these countries has also risen. It is believed that environmental factors specific to modernization play an important role in the development of IBD. Potential environmental factors related to modernization include diet, a lack of exposure to particular microorganisms, increased stress, exposure to pollutants and microparticles, and lack of vitamin D (Fig 1).
Role of Gut Microflora

The gastrointestinal tract is densely colonized by a complex microbiota whose total number (>10^{14}) exceeds the number of individual cells in the human body. We have evolved over time with microbes, and it appears that we not only tolerate these organisms, but we absolutely require them for proper immune development and health. Nucleic acid-based approaches have shown that the normal intestinal microbiota is composed of four major bacterial phyla. These include the Firmicutes (mainly Clostridium coccoides and Clostridium leptum), Bacteroidetes (Bacteroides-Prevotella group), Actinobacteria, and Proteobacteria. The dominant intestinal microbiota of an adult is highly diverse and is composed of hundreds of different species. Increasing evidence indicates that elements of our modern lifestyle, including diet, hygiene, antibiotics, and urbanization are influencing the composition of our gut microbiota and may in fact be removing particular microorganisms that are essential for immunoregulation. Compared with healthy individuals, patients with IBD show altered gut microbial colonization. The most reproducible findings include decreased diversity, reduced stability, decreased Firmicutes (primarily C. leptum and C. coccoides groups), an increased number of enterobacteria, and the presence of unusual bacteria. IBD patients also have increased numbers of bacteria detected within or penetrating the mucus layer. In that animal studies have shown that only some bacterial strains induce gut inflammation while other strains prevent or downregulate inflammation, the concept of a dysbiosis in gut colonization of IBD patients, with a relative decrease in “protective” and corresponding increase in “inflammatory” strains, has arisen. However, whether these alterations in the gut microbiota are a cause or a consequence of disease, or whether these alterations exist in susceptible individuals before the onset of clinical disease, remains to be shown.

Endoplasmic Reticulum Stress and Inflammation

The epithelium in the gut provides a single-cell barrier between the commensal microbiota and hematopoietic cells and plays an active role in the immune response. Mucosal homeostasis depends on an active relationship between functional epithelial cells and underlying immune cells. Endoplasmic reticulum (ER) stress arises from situations where misfolded or unfolded proteins accumulate within the ER in cells. Environmental and/or primary genetic factors can promote ER stress in epithelial cells. Environmental factors to which gut enterocytes are exposed include bacteria and bacterial products, metabolic factors, drugs, hypoxia, food-derived factors, and inflammatory mediators (Fig 2).
occur in embryogenesis, during fetal development, or during the early postnatal period. Numerous dietary compounds, including curcumin, copper, diallyl sulfide, sulforaphane, selenium, and folate have been implicated in nutritional regulation of the epigenome. Western foods are deficient in several micronutrients, including selenium and folate, primarily due to the foods’ highly refined and processed nature. Little work has been done examining the effects of food components on key epigenetic mechanisms, particularly during early life, to determine how these may increase or decrease the risk of developing IBD in later life.

Role of Dietary Fat

Some epidemiological studies have shown an increased intake of dietary n-6 polyunsaturated fatty acids (PUFAs) and decreased intake of n-3 PUFAs to be associated with a higher incidence of IBD. Two of these fatty acids, linoleic acid (precursor of n-6 PUFA) and α-linolenic acid (precursor of n-3 PUFA) are considered to be essential in the human diet. Although it is recommended that a ratio of 4:1 (n-6:n-3 PUFAs) be consumed for optimal health, a more usual intake in the Western world are ratios of 15:1–16:1 n-6:n-3. Interestingly, this increased intake of n-6 PUFAs has paralleled the increase of chronic and autoimmune inflammatory disorders. Long-chain PUFAs are involved in numerous cellular processes, including membrane biosynthesis, energy metabolism, eicosanoid production, and signal transduction. Chronic inflammatory diseases are associated with increased production of the proinflammatory eicosanoids prostaglandin E2 and leukotriene B4, both of which are derived from the n-6 fatty acid arachidonic acid. Extensive evidence indicates that n-3 PUFAs, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), influence the immune system directly by altering the production and release of inflammatory eicosanoid mediators from arachidonic acid, as well as suppressing the production of inflammatory cytokines, and downregulating the expression of a number of genes involved in inflammation. Saturated fatty acids, including palmitate and oleate, induce ER stress and ER-stress-related apoptosis by triggering calcium signals and free radicals. In addition, a high-fat diet also alters the composition of the gut microflora. Thus, excessive intake of n-6 PUFAs and saturated fatty acids associated with a Western-style diet could potentially trigger the onset of inflammation in genetically predisposed individuals.

Epigenetic Mechanisms

Foods and food components can modify DNA through methylation, histone modification, and noncoding RNA to alter the structure of chromatin without altering the DNA nucleotide sequences. These modifications, termed epigenomic regulation, are inheritable and potentially reversible. Epigenetic modifications may

Fig 2. Genetic and environmental factors can induce ER stress in the intestinal epithelium and consequently inflammation. E. coli = Escherichia coli, ER = endoplasmic reticulum, TNF = tumor necrosis factor, IL = interleukin.

ER stress results in the activation of signal transduction pathways, termed unfolded protein responses (UPR), which alter transcriptional and translational programs. If ER-stress is prolonged, the UPR will induce cell death. In animal models, conditional deletion of a primary factor involved in ER stress (X box binding protein-1 [XBP1]) results in the spontaneous development of intestinal inflammation in the small intestine resembling human IBD. Both UC and CD patients show evidence of ER stress based on increased grp78 expression and XBP1 splicing. Furthermore, polymorphisms have been detected in the XBP1 gene that increase the risk of developing IBD. ER stress is being recognized as a secondary consequence of inflammation in the gastrointestinal tract, as well as possibly being a primary factor in the initiation of intestinal inflammation.
Western Diet as a Trigger for Inflammatory Bowel Disease

Role of Dietary Protein

The major sources of dietary protein in the Western diet are meat, cheese, milk, fish, nuts, and eggs. A recent prospective study involving over 60,000 women has demonstrated an association between enhanced animal protein intake and increased risk of developing IBD. These findings are similar to those of Shoda et al., who found a temporal relationship between the rising incidence of CD in Japan and the increasing dietary intake of animal protein. Depending on the quantity consumed, a variable proportion of heme and amino acids can reach the colonic lumen, where they can be metabolized by the resident microflora. This can result in the production of a number of possibly toxic end products, including hydrogen sulfide, phenolic compounds, and amines and ammonia. Increased levels of hydrogen sulfide are associated with UC. Another mechanism by which animal protein may trigger inflammation is through effects on intestinal energy metabolism. Chronic intestinal inflammation is characterized by energy deficiency with alterations in oxidative metabolism seen in epithelial cells and ER stress in enterocytes, mucus-producing goblet cells, and defensin-secreting Paneth cells. Interestingly, dietary iron has been shown to induce ER-associated stress responses and exacerbate inflammation in TNFΔARE/+ mice, while an iron-deficient diet completely prevented the development of inflammation in this mouse model of IBD. In addition, excessive macromolecular intake can also produce ER-associated oxidative stress. Overall, evidence appears to be accumulat- ing of a relationship between increased protein intake and an increased incidence of IBD, possibly through the induction of ER stress and/or alterations in gut microbial metabolism.

Conclusions

There is currently insufficient evidence to conclusively show a direct relationship between diet and IBD. However, the majority of analyses in humans have either depended on retrospective studies, which are prone to significant recall bias, or concentrated on individual foods or nutrients and have not taken into account either the genetic background of the individual or the presence of other environmental factors (eg, smoking). In addition, until recently, full analysis of the effects of diet on gut microflora was not possible, and thus a possible role for diet-induced changes in the microflora being the primary trigger for inflammation has been under-appreciated. With the advent of new molecular techniques, future studies examining a role for diet in triggering IBD should also include an assessment of the gut microflora and the patient’s genetic makeup.

References

Western Diet as a Trigger for Inflammatory Bowel Disease

18. Kim SC, Tonkonogy SL, Karrasch T, Jobin C, Sartor RB. Dual-association of 
gnotobiotic IL-10−/− mice with 2 nonpathogenic commensal bacteria induces 

effects of monocolonization with *Escherichia coli* strains O6K13 and Nissle 1917 
on the development of experimentally induced acute and chronic intestinal 
inflammation in germ-free immunocompetent and immunodeficient mice. *Folia 

defined bacterial cocktail induce intestinal inflammation in SCID mice 
reconstituted with CD45RBhigh CD4+ T cells. *Inflamm Bowel Dis.* 2007;13(10):1202-1211.

and perpetuate experimental colitis in rats and mice. *Infect Immun.* 
2001;69(4):2277-2285.

22. Kaser A, Martinez-Naves E, Blumberg RS. Endoplasmic reticulum stress: 

23. Zhang K. Integration of ER stress, oxidative stress and the inflammatory 


remodelling of our epigenomes by nutritional and metabolic factors and beyond. *Clin 

27. Shoda R, Matsuda K, Yamato S, Umeda N. Epidemiologic analysis of Crohn's 
disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and 

2003;56:67-70.

29. Simopoulos AP. Importance of the ratio of omega-6/omega-3 essential fatty 

30. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and 

31. Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-
2010;68(5):280-289.

32. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, et al. High-fat diet determines the 

33. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 
An obesity-associated gut microbiome with increased capacity for energy 

Animal protein intake and risk of inflammatory bowel disease: the E3N 

35. Hughes R, Magee EA, Bingham S. Protein degradation in the large intestine: 

36. Christl SU, Eisner HD, Dusel G, Kasper H, Scheppach W. Antagonistic effects of 
sulfide and butyrate on proliferation of colonic mucosa: a potential role for these 
2477-2481.

causes endoplasmic reticulum stress and spontaneous inflammation resembling 

38. Werner T, Hoermannsperger G, Schuemann K, Hoelzlwimmer G, Tsuji S, Haller 
D. Intestinal epithelial cell proteome from wild-type and TNFDeltaARE/WT mice: 
effect of iron on the development of chronic ileitis. *J Proteome Res.* 

kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed 
682-690.

Q & A

Q: We always relate the good bacteria with what we call bifidogenic activity. 
Do you think that bifidobacterium is the only one that plays a role in inflammatory 
bowel disease?

Dr Madsen: Absolutely not; several strains do. Dr Sartor is going to cover this in 
detail. But lactobacillus does specific things, and bifidobacterium also has specific 
qualities. The effects seen are dependent totally on the strain, and each strain 
is different.

Q: I have a question about timing. You said that the microflora is established 
relatively early in life, maybe by age 3 or 4 years, then you presented evidence that 
seemed to contradict that. In older twins, for example, there are environmental 
factors that change over time. Could you talk a little bit about how the microflora 
can change and how quickly that can happen?
**Western Diet as a Trigger for Inflammatory Bowel Disease**

**Dr Madsen:** One major change is not necessarily in which strains are present, but also in what the strains are doing—which genes are being turned on and what metabolism is going on. So a bifidobacterium, a lactobacillus, and a staphylococcus may all be living there in the gut but depending on diet and other things going on in a person’s life such as stress and inflammation the staphylococcus may be growing and the bifidobacterium may be decreasing. Specific bacteria are also changing what genes are being turned on, and changing metabolism, which, in turn, changes which metabolites are being released by that bacteria. They change what signaling molecules are being released and how they interact with the host.

**Q:** How quickly is this happening?

**Dr Madsen:** It happens instantly. As soon as we eat something different, we change this activity.

**Q:** Are there diurnal variations, circadian variations in this activity?

**Dr Madsen:** That is a good question. I do not know the answer. I would not be surprised if there were.

**Q:** An abstract was presented recently showing that the microflora was different during weekdays than it was on the weekends.

**Dr Madsen:** I bet it is diet. Probably there is an environmental effect; as we eat more during the day gene expression of microbes changes. One researcher looked at fed vs non-fed subjects and gene expression changed rapidly, literally within hours. I do not think diet composition changes significantly night to day. On the weekend, who knows what people do?

**Q:** Last year Abbott Nutrition had a conference on lean body mass, and we came away with that, as we get older, we need 25–30 grams of protein three times a day to maximize muscle protein synthesis. Is IBD a problem with the elderly or does it usually occur at some age younger?

**Dr Madsen:** It may be diagnosed later as you saw with the older woman cohort [Jantchou P et al. Am J Gastroenterol. 2010 May 11 (Epub ahead of print)], but disease onset is generally in young adults.

**Q:** So we recommend to young adults to build lean body mass and retain it. What do we recommend to an older population? It will not cause them to develop IBD?

**Dr Madsen:** Maybe we have to take measures to reduce ER stress and what goes along with it. Maybe there are ways to mitigate that with something else.

**Q:** I think the Eskimo diet is an interesting model—high intake of protein, n-3 fatty acids, and vitamin D. Have you looked at that population? Is there a significant IBD problem among Eskimos?

**Dr Madsen:** No, there is not.

**Q:** It is said that the native population of Alaska has a very low incidence of IBD, but is the cause socioeconomic? Is it diet? Is it genetics? How do we determine this?

**Dr Madsen:** It is possible that this intake is mitigated by the vitamin D and the n-3 fatty acids. This would be an interesting population to study.

**Q:** This is like the good cardiovascular effect of the wine-based French diet. There might be two mitigating factors that cancel each other out.

**Q:** I am interested in your point about the microbiotic differences between patients with IBD and those without. When we are born, microbes from our mothers populate our guts. Has anyone looked into going back to the mother of either adults or children with IBD and repopulating the patient’s gut with her microflora to see whether the disease can be reversed?

**Dr Madsen:** This is called fecal transplantation. The patient’s microflora is replaced with the whole ecosystem of someone else. Dr Borody, a physician in Australia, is having some success with fecal transplants, not necessarily using the mother’s flora, but using that of a significant other or somebody else who volunteers and is acceptable to the patient [Borody TJ et al. J Clin Gastroenterol. 2003;37(1):42-47]. It is hard to get this type of procedure past ethics boards in North America.
Humans coexist with an incredibly complex diversity of bacteria, fungi, and viruses that increases in both complexity and number in the distal relative to the proximal GI tract. The microbiota of the stomach and duodenum is predominantly characterized by aerobic Lactobacillus and Streptococcus and Candidus species while the distal ileum and colon are colonized by up to $10^{11}-10^{12}$ predominantly anaerobic bacteria that comprise up to 1000 species. These fecal bacteria consist of 16 divisions but the majority are Firmicutes (60%) and Bacteroidetes (17%), with smaller numbers of Actinobacteria (6%) and Proteobacteria (5%).

The gut microbiome is an integral part of our genetic landscape and humans can be viewed as a supra-organism composed of both human and microbial species, genomes, and metabolomes. Comparative studies show that Firmicutes and Bacteroidetes dominate across all mammals, but there are profound dietary influences such that carnivores have the fewest divisions with the highest concentrations of Firmicutes and herbivores have the most complex microbiota.

The ratio of Firmicutes and Bacteroidetes is altered by a diet leading to weight loss with a gradual decrease in Firmicutes and increase in Bacteroidetes over 52 weeks. In addition to compositional changes, diet dramatically alters bacterial species’ gene expression by activating those genes relevant to available dietary substrates. Finally, intestinal bacteria are efficient metabolic factories that ferment dietary carbohydrates and fiber to protective short-chain fatty acids such as butyrate, which is the primary metabolic fuel of the colonocyte. Conversely, other bacterial species can produce potentially toxic metabolites such as hydrogen sulfide and oxygen metabolites from other dietary substrates.
Rationale and Efficacy of Manipulating Intestinal Bacteria in Chronic Intestinal Inflammation by Probiotics, Prebiotics, and Diet

Bacteria Involved in the Pathogenesis of Inflammatory Bowel Diseases (IBD)

Bacteria are integrally involved in the pathogenesis of IBD by providing the chronic antigenic and toll-like receptor agonists that activate effector adaptive and innate immune responses, respectively, in genetically susceptible hosts (Fig 1).5,6


Developing evidence suggests that dysbiosis (abnormal microbial composition or function) can contribute to if not cause chronic intestinal inflammation.5,7 This inflammation can be caused either by an abnormal composition of enteric bacteria with an elevated ratio of aggressive vs protective species, defective production of short-chain fatty acids and other protective microbial products, or enhanced production of hydrogen sulfide and nitrates that block butyrate metabolism and disrupt the mucosal barrier. Available data show a selective decrease in Bacteroidetes and Lachnospiraceae, including Clostridia groups IV and 14A.5,7 Gnotobiotic rodent studies illustrate the essential nature of commensal enteric bacteria in chronic immune-mediated intestinal inflammation.5 Selected colonization of genetically susceptible hosts indicates that various bacterial species have differential abilities to induce or prevent experimental colitis, signifying that all bacteria are not equal in this capacity.4 In this setting some commensal bacterial species are aggressive, some are neutral, and some are protective, including multiple Lactobacillus and Bifidobacterium species and the commensal Faecalibacterium prausnitzii.9 Thus there is ample evidence that the relative balance of beneficial vs detrimental bacteria strongly influences intestinal inflammation vs homeostasis (Fig 2).

Fig 2. Relative balance of beneficial vs detrimental bacteria strongly influences intestinal inflammation vs homeostasis. E. coli = Escherichia coli.

Diet Affects Experimental Colitis

We observed that dietary iron, sucrose, fructose, and aluminum potentiates colitis in interleukin (IL)-10-deficient mice and that nonabsorbed oligosaccharides (prebiotics) attenuate colitis in HLA-B27 transgenic rats.10-13 We hypothesize that dietary constituents affect enteric bacterial composition, gene expression, metabolic activity, and mucosal immune function. These changes induce chronic intestinal inflammation or promote mucosal homeostasis or tolerance. Postulated mechanisms include preferential stimulation of growth of detrimental bacteria by dietary sucrose, fructose, and iron, while poorly absorbed oligosaccharides (prebiotics) foster growth and metabolic activity of beneficial bacteria with decreased production of short-chain fatty acids. Dietary aluminum, iron, and animal fat stimulate pathogenic mucosal immune responses. We have demonstrated that dietary iron alters bacterial composition by expanding luminal Klebsiella, Escherichia coli, and Citrobacter rodentium. These enteric commensal species require iron for
Rationale and Efficacy of Manipulating Intestinal Bacteria in Chronic Intestinal Inflammation by Probiotics, Prebiotics, and Diet

growth. Likewise, several adherent/invasive $E. coli$ strains that are capable of causing experimental colitis in knockout mice preferentially grow with fructose but are incapable of metabolizing sucrose. Conversely, the protective enteric species $Faecalibacterium prausnitzii$ does not grow with fructose, glucose, or sucrose but preferentially proliferates in the presence of maltose as a carbon source. Thus, it is quite likely that diet can contribute to the composition of intestinal bacteria with increased growth and function of aggressive species by refined sugars and iron and of protected bacteria by complex carbohydrates such as dietary fiber and commercially available prebiotics.

Clinical Efficacy of Probiotics in IBD

Probiotics have been used in multiple clinical trials in an effort to treat ulcerative colitis, Crohn’s disease, and pouchitis. These studies show some potential benefit of certain agents to prevent relapse of ulcerative colitis and possibly treat mild to moderate active ulcerative colitis, but no real benefit in Crohn’s disease. However, a dramatic improvement in preventing relapse (maintaining remission) of chronically recurring pouchitis by VSL 3, a combination of eight different probiotic species, was noted. Clearly there is a need for large, multicenter, double-blind, placebo-controlled trials for primary and adjunctive treatment of active ulcerative colitis and Crohn’s disease with probiotics, as well as for the use of these agents in preventing relapsing steroid-treated ulcerative colitis and Crohn’s disease and preventing pouchitis.

Certain conclusions can be reached: 1) Different probiotic species have different efficacies, 2) commercially available probiotics do not colonize the intestine over time despite chronic use, 3) components of probiotics can have biologic effects even when the parent organisms are nonviable, and 4) protected bacteria work by multiple mechanisms that include inhibiting growth and epithelial binding of pathogenic bacteria, improving epithelial barrier function and augmenting immunoregulatory effects by enhancing protective cytokines such as IL-10 and transforming growth factor beta (TGFβ) while blocking effector molecules such as tumor necrosis factor (TNF) and IL-12 p40. A relatively unexplored but exciting treatment option is to use genetically engineered probiotics or commensal bacteria to deliver protective molecules.

Use of Prebiotics and Diet in Intestinal Inflammation

Prebiotics are dietary substances, usually nondigested carbohydrates, that stimulate the growth and metabolic activity of beneficial enteric bacteria. These substances can prevent intestinal inflammation by stimulating growth of protective commensal bacteria such as $Bifidobacterium$ species; enhancing production of short-chain fatty acids such as butyrate that have protective activities; decreasing stool pH, which inhibits growth of detrimental bacteria; and enhancing water-holding capacity of the stool. The use of prebiotics in human IBD has been limited but several compounds, particularly fructooligosaccharides, including inulin, have demonstrable activity in experimental colitis. In a pilot study, fructooligosaccharides appeared to have clinical benefit in active ileocolonic Crohn’s disease with some immunoregulatory activities.

Dietary therapy of IBD could improve intestinal inflammation by several mechanisms: changing bacterial composition and metabolism, preventing bacterial adherence to mucosa, upregulating epithelial expression of toll-like receptors, inhibiting aggressive immune responses, and promoting epithelial differentiation. In the process, nutritional therapy could have the combined benefits of treating and preventing nutritional deficiencies, improving the host immune response, and improving microbial composition and metabolism, with the net effect of improving clinical outcomes.

Conclusions

Antibiotics, probiotics, and prebiotics, and the combination of all three approaches, have great potential to treat active IBD and to prevent relapse, but each patient subset may respond selectively to various agents. This selective response may result in the need to individualize treatment for each patient. To date, manipulating the intestinal microbiota in human IBD has not substantially altered the underlying disease process or changed the natural history of these disorders. It is likely that we have not yet identified the optimal mix of probiotics and/or prebiotics or that individual patient responses have obscured overall results in groups of heterogeneous patients. There is no doubt, however, that dietary manipulation offers the most physiologic and least toxic approach to treating IBD and has tremendous potential for long-term use. Finally, it is likely that multiple human inflammatory infectious and hypersensitivity disorders might respond to therapeutic use of probiotics, prebiotics, and dietary manipulation (Table).
Table. Potential Role for Probiotics, Prebiotics, and Nutritional Therapy in Multiple Human Conditions

- IBD (pouchitis, ulcerative colitis, Crohn’s disease)
- Irritable bowel syndrome
- Enteric infections
- Obesity
- Metabolic syndrome
- Nonalcoholic steatohepatitis
- Chronic obstructive pulmonary disease
- Cystic fibrosis
- Asthma, hypersensitivity disorders

References

Discussion

**Leader:** Refaat Hegazi, MD, PhD

**Dr Hegazi:** Thank you, Dr Jensen, for leading the initiative of defining malnutrition vs inflammation and where they overlap. When I was in practice, our group of six or seven clinicians was approached to define malnutrition, and there was no consistency of definitions among our group of experts. I think that we should not define malnutrition based just on patients’ weight because we have a lot of overweight patients who still can be considered malnourished. So the question to you is, how do we define acute vs chronic inflammation in the clinical setting? Is there any marker of inflammation that we can use now in clinical settings or do we continue to struggle with this?

**Dr Jensen:** Part of the reason for such widespread confusion in using this terminology is a fundamental lack of good practical indicators of both nutritional status and inflammation. To distinguish acute versus chronic inflammation, one can make use of crude indicators—e.g., fever, raised white blood cell count, and poor glycemic control during acute inflammation. Clinical judgment is a key part of the decision-making process to discern whether inflammation is present. Some of this judgment can be simply diagnosis-driven. For example, if patients have an acute flare of Crohn’s disease, they will have an acute inflammatory response.

One of the things that we have been encouraging, non-specific though it may be, is use of C-reactive protein (CRP) as a positive acute-phase reactant. If we combine that indicator with clinical judgment, it can be helpful. If you have a critically ill patient, you are going to see elevated CRPs, and you should observe a positive association of CRP levels with any major inflammatory event such as abdominal surgery or infection. Albumin and prealbumin are negative acute-phase reactants. That is, as long as there is active inflammation, they will stay reduced. After you have drained that patient’s abscess or you are not operating on him or her, the patient is not mounting fevers or exhibiting other evidence of active inflammatory response, the CRP will come down and albumin and prealbumin levels will recover.

But I think your question highlights a clear need to develop better laboratory indicators of both nutritional and inflammatory status. Of course, this discussion also highlights how inflammation and nutrition are intertwined and can be difficult to distinguish.
Discussion

**Dr Sartor:** Some organ-specific indicators exist in the gut as well. Fecal calprotectin and lactoferrin, which are neutrophil markers, are evidence of translocating neutrophils and correlate well with endoscopic disease. A problem with the systemic markers is that they are not sensitive disease activity markers. CRP is an acute-phase response protein produced by hepatocytes in response to interleukin (IL)-6 and probably indicates activation by either uptake of bacterial products in the portal vein or secondary cytokine activation. However, some people are genetically unable to make CRP responses, so a gut-specific marker is more direct and more sensitive.

**Dr Hegazi:** That is a good point. I was intrigued by the fact that GI dysfunction occurs in critically ill patients even if the origin of their disease is not the GI tract. Can we correlate these fecal markers, fecal calprotectin or lactoferrin, with systemic inflammation?

**Dr Sartor:** Not well. Calprotectin is a better marker for intestinal inflammation than for systemic inflammation, while CRP is better for systemic inflammation.

**Dr Hegazi:** I will explain where I am going with this. In critically ill patients, we see phases of acute inflammation: systemic inflammatory response syndrome (SIRS), mixed antagonist response syndrome (MARS), and compensatory anti-inflammatory response syndrome (CARS). Sometimes clinicians are asked whether we can define this specific progression of the patient because we know, for example, that interleukin 10 (IL-10) goes up in CARS. So with CARS maybe we should supplement the patient with something like an immune enhancer, while with SIRS we actually want to dampen the proinflammatory response. I think we are still missing these kinds of defining clinical end points that we can use in clinical application to define malnutrition. Moving forward we should look at this.

**Dr Greenberg:** If you are looking for biomarkers of inflammation CRP is obviously a useful one, but not the only one. I think the problem you face with the gut is that the proteins get degraded there, but I think you could probably look at the immune cell population in stool and define what is there. Those cells may be harder than proteins and cytokines, for example, as well as the cells that you would see. You probably have some signatures of what the cells are doing based on various characteristics of them.

**Dr Sartor:** Unfortunately, epithelial cell-activated macrophages and neutrophils do not persist very long either. Some markers of acute inflammation are fecal leukocytes, which are used clinically. These cells rapidly degrade after migrating into the stool, but calprotectin and lactoferrin are stable and persist for analysis. Harvesting intact intestinal cells requires a biopsy or resection.

**Dr Greenberg:** I do not think you can harvest them.

**Dr Sartor:** Clinical labs lose interest when you bring in a stool specimen for analysis.

**Dr Greenberg:** I think we will be able to do a lot of things much faster. For instance, I assume we will be able to do a genome signature on stool pretty quickly in the next 5 years. It is not difficult to do that—extract anything that is in there, look at a genome, and put it on a proper chip. It sounds as though we will find a pattern of the bacteria. Also, we can probably move on to the cells and say what they are making intercellularly. In the same way, when we do bacterial artificial chromosome analysis, we take cells out of people. This gives us a better understanding than when we look at blood cytokines, which do not always tell us very much.

**Dr Sartor:** But to do that we would have to isolate the cells, and their viability is poor, or look for mRNA in fecal material. However, RNA is degraded rapidly. Let us try it in animal models and see.

**Dr Greenberg:** DNA is not.

**Dr Sartor:** DNA is not going to be helpful in terms of activation of genes. Bacterial DNA is quite stable, but it remains to be proven that changes in the microbiome correlate with disease activity.

**Dr Greenberg:** Do you find polymorphonuclear leukocytes in the stool?

**Dr Sartor:** We do. They are not necessarily viable. That is what the fecal leukocyte test is, looking at neutrophils, and is the basis for calprotectin and lactoferrin assays.

**Dr Serhan:** With regard to the question about defining parameters of malnutrition and its relationship to inflammatory markers, is it productive to think about the optimal diet to have optimal resolution, if you will, of an inflammatory response in an ideal setting? And from that, to find deviations from optimum? It seems that it is difficult to pinpoint malnutrition because of so many components, so many organ systems. One organ system can be malnourished and another one enhanced by something. Look at the differences between a liver and the bone marrow, for example. I am sure people have tried to do this before—define optimal diets rather than subtractive.
**Dr Hegazi:** I think the idea is great. To sort out the source of inflammation in critically ill patients is probably problematic because of their multi-organ dysfunction. So many organs are either in an inflammatory stage or actually in failure stage. We should at least try to define who is in need of nutrition intervention or support, who will need the anti-inflammatory model. We have supplements and we have other formulas with fish oil, an antioxidant. From the clinician point of view, the main problem is just to define who is in need of this intervention in the time that the patient needs it.

**Dr Serhan:** In that particular case, we do not have a dipstick to determine which patients going into surgery are deficient in, say, omega-3 fatty acids, which many of us consuming the Western diet are. We would want to have a dipstick for that so we can treat them up front and take a proactive, preventive stance.

**Dr Hegazi:** Dr Sartor, I have a question about fructose and sucrose. I think some people will find this confusing because we previously mentioned fructooligosaccharides as a prebiotic preventive nutrient. Some people do not realize the difference between refined fructose and sucrose and fructooligosaccharide. This seems counterintuitive.

**Dr Sartor:** Location is probably the answer. I think a valid concern is, how do fructose and fructooligosaccharides work? How are you going to change fecal bacteria by giving something that is absorbed mostly proximally? Sucrose is broken down by sucrases, which are small intestinal brush-border enzymes, so there is fairly complete absorption of these refined carbohydrates proximally. My belief is that, first of all, some sucrose does get downstream, but probably a minority of what we ingest. So we are probably altering small bowel bacterial composition, maybe proximal small bowel, and then those come downstream. If the host cannot break down the carbohydrate substrate, as in the case of fructooligosaccharides and fiber, the material goes down to the distal ileum and colon where the anaerobic bacteria can then metabolize it.

So it is all about location. Our lab is looking at low-carbohydrate and high-carbohydrate diets in our mice to see whether we get blooms of bacteria proximally with these high-sucrose, high-fructose diets. This remains hypothetical; we do not have data yet to prove alterations in small intestinal bacterial concentrations. But if you are creating medical nutritional formulations, you might want to consider not loading them up with refined carbohydrates but instead using some of the more complex carbohydrates that have more of a prebiotic activity.

**Dr Jialal:** I am not a gastroenterologist; I am an endocrinologist. Basically the toll-like receptors (TLRs) have bacterial ligands as agonists. So I disagree with you slightly—you can tell me why I am wrong. The failure of probiotics might be that if you see them as ligands, then they signal and activate the TLRs and nuclear factor kappa B (NFκB) for inflammatory cytokines and chemokines. So when you are giving these bacteria, I think that you are not inhibiting anything. If anything, you are stimulating.

Another perplexing thing we see in metabolic medicine is that it is hard to measure endotoxin. But endotoxin levels in obesity, in diabetes, etc, increase, and they signal TLR4. So I am confused about probiotics, TLRs, and their benefit.

**Dr Sartor:** Because you are not a gastroenterologist, you can ask the key questions without preconceived notions. The answer again is location, location, location. Most of the TLR expression is on the basallateral membrane of the epithelial cell rather than on the luminal side. First, bacterial ligands in the gut may not activate as many TLRs as you might think. Dr Madsen has done some nice studies, so I will give my answer and then get the real answer from her. Second, following TLR ligation NFκB is activated, as is mitogen-activated protein kinase and other pathways. But NFκB activation clearly is protective in the epithelial cell. The evidence for that is that dominant negative transgenics of epithelial-specific IkappaB-alpha, or epithelial selective knockout of IkappaB kinase (IKK) gamma or beta components of the activation pathway of NFκB actually worsen disease. Blocking NFκB potentiates disease in dextran sodium sulfate-induced colitis and radiation-induced colitis. Conversely, NFκB in the lamina propria is proinflammatory.

Also remember that as you activate NFκB, you are not only inducing a number of proinflammatory molecules made by the epithelial cells, such as interleukin (IL)-8 and other chemokines in the mouse, but you are also activating protective pathways, such as IL-1 receptor antagonist and cyclooxygenase 2-dependent prostaglandin E2 (COX-2/PGE2).

So the take-home message, at least from the experimental data, is that epithelial NFκB is protective. Lamina propria NFκB is proinflammatory. Dr Madsen has found some secreted products of probiotics that actually activate NFκB.

**Dr Madsen:** TLR4 is downregulated and probiotics do not upregulate it. Some strains of probiotics will activate TLR2, which is a protective mechanism that maintains main gut homeostasis. Bacterial DNA from different species affects TLR9 signaling differently. If we take DNA from different strains of bacteria, we can
downregulate TLR9 and inhibit NFκB. If we take, say, a particular strain of lactobacillus, we can stimulate TLR9 signaling. Same for TLR5. Different flagellin from different species all signal through TLR5, but different strains will cause a different signaling pathway to happen within the cell. So an epithelial cell can tell the difference between different strains of bacteria based on TLR signaling.

**Dr Jialal:** My understanding is that this is like what we do with monocytes—we take human monocytes and activate with agonists. Is that not so in epithelial cells?

**Dr Madsen:** No, you do not see the same thing in a monocyte or a dendritic cell. Dendritic cells and monocytes can differentiate between live microbes. But TLRs are more on/off, as opposed to epithelial cells, which can alter signaling based on what strain it is.

**Dr Jialal:** Has anybody looked at probiotics and TLR expression in the gut to see why what I am offering as a plausible explanation does not work in Crohn’s or ulcerative colitis?

**Dr Sartor:** Certainly there have been studies showing that agents turn on and turn off different TLRs. Dr Madsen can probably respond to that more appropriately.

**Dr Madsen:** Actually, I would like to move away from TLRs because bacteria have to turn on gene expression to have an effect. Let us say they have to turn on a bioactive molecule like some of the ones that we have studied. They will turn that off based on what other strains are present. So a bacteria turns on the gene expression but it gets in the gut and comes in contact with other strains that cause it to turn off that gene expression. Otherwise it does not survive passage and then cannot have an effect. When we look for the probiotics in some patients, we cannot find them. So either a) they are not taking the probiotics and are lying to us, or b) the probiotics do not survive passage in those particular individuals. And that may be why it does not have an effect.

**Dr Jialal:** Why are steroids used in patients with inflammatory bowel disease? Does it have anything to do with NFκB?

**Dr Sartor:** Yes, among other things. Steroids do not exclusively act on NFκB. Steroids have a huge number of effects that go way beyond their effect on NFκB blockades. They may work by coming from the systemic side and hitting the lamina propria NFκB pathway, but certainly a number of other mechanisms are at work, as well. A preliminary clinical trial in Scandinavia using an oligonucleotide that is a competitive inhibitor of NFκB was not effective in ulcerative colitis, offering proof of principle that selective blocking is not effective, even though it did work in an inducible animal model [Neurath MF et al. Nat Med. 1996;2:998-1004].

**Dr Jialal:** Why do we find endotoxin in the plasma? We found it in children with type 1 diabetes and others with obesity and type 2 diabetes.

**Dr Sartor:** The gut is leaky and in a systemic inflammatory response, one of the first things seen is increased permeability of the epithelial barrier, demonstrated by sugar markers as well as macromolecule uptake. So there is a very clear translocation of viable bacteria and uptake of TLR ligands, including not only lipopolysaccharides, but also peptidoglycan and flagellin.
Targeting Inflammation in the Metabolic Syndrome

Ishwarlal Jialal, MD, PhD

The Metabolic Syndrome (MetS) comprises a constellation of features including abdominal obesity (measuring ≥40 inches in men, ≥35 inches in women), triglycerides ≥150 mg/dL, HDL-C (<40 mg/dL in men, <50 mg/dL in women), blood pressure ≥130/85 mmHg, and fasting glucose ≥100 mg/dL. Also included in the definition are patients on antihypertensive medication and patients taking drugs targeted at lowering triglycerides, and/or raising high-density lipoproteins (HDLs) such as fibrac acid derivatives and nicotinic acid.

The prevalence of MetS in the United States, according to the Third National Health and Nutrition Examination survey data, was approximately 24%. The prevalence in both Mexican American men and women was higher than in Caucasian individuals. Also, prevalence of MetS increased with increasing age such that in people over 60 years the prevalence exceeds 40%.

Disorders clustering with MetS include nonalcholic steatohepatitis, polycystic ovarian syndrome, obstructive sleep apnea, cholesterol gallstones, gout, and HIV treated with protease inhibitors. The central adiposity in MetS is largely manifest as an increase in visceral adipose tissue area. Also, there can be deposition of fat in the muscle contributing to insulin resistance, in the liver contributing to steatohepatitis, and also around the heart. The major reasons for the diagnosis of MetS are that it confers a two-fold increased risk for cardiovascular disease (CVD) and a five-fold increased risk of diabetes. The two common features of MetS include insulin resistance and inflammation. The Table on page 40 shows the biomarkers of inflammation that are increased in patients with MetS.
Jialal increases free fatty acid oxidation in the skeletal muscle. In sum, its effects include reduction in plasma glucose and free fatty acids, increase in insulin sensitivity, and decrease in hepatic glucose production. Adiponectin levels are lower in patients with obesity and insulin resistance, and indeed hypoadiponectinemia is a uniform feature of MetS. Thus, MetS is clearly a proinflammatory state.

Therapeutic strategies that have been successful in targeting inflammation in MetS include lifestyle changes. In the Diabetes Prevention Program, 7 in patients with pre-diabetes, it was shown that a weight loss of 5–10 pounds and exercise for 150 minutes a week resulted in a 58% reduction in the cumulative incidence of diabetes over 4 years. In a subsequent paper, Haffner et al 8 showed a significant reduction in hsCRP levels in both males and females in the lifestyle-change group compared to placebo at 6 months and 1 year. Furthermore, the prevalence of MetS in participants at 3 years decreased significantly in the patients in the lifestyle-change group from 51% to 42%, but it went up in the placebo group from 55% at baseline to 61% at 3 years. 9 Also, the incidence of MetS in participants without the syndrome at baseline decreased 41% in the therapeutic lifestyle-change group compared to the placebo group.

Numerous studies have shown that weight loss by various strategies including low-calorie diets and bariatric surgery significantly reduces CRP. In a recent publication, 10 we reviewed published studies and showed a significant correlation (R² 0.87, P <0.05) between percent CRP reduction and weight loss (kg). Case et al 11 showed that in 125 patients with MetS with a mean body mass index (BMI) of 40.7 kg/m², a very-low-calorie diet for 4 weeks was beneficial and the benefits were sustained at the end of the period of active weight loss (average of 16.7 weeks). The patients followed a protein-sparing, very-low-calorie diet with a total intake of 600–800 kcal that resulted in significant weight reduction. The benefits included a significant reduction in blood pressure, glucose levels, triglyceride levels, and cholesterol.

In one of the most instructive studies of MetS and diet, 12 Esposito and her colleagues studied the effect of a Mediterranean-style diet in 180 patients with MetS; 90 patients in the conventional-diet group and 90 in the Mediterranean-style diet group were followed for 2 years. The Mediterranean-style diet included 50%–60% carbohydrate, <30% total fat, <10% saturated fat, encouragement of fruits and vegetables (eg, walnuts, whole grains, and increased olive oil consumption). The intervention group consumed more foods rich in monounsaturated fat, polyunsaturated fat, and fiber, and had a lower ratio of omega-6 to omega-3 fatty acids. Total fruit, vegetable, and nuts intake (274 g/d), whole-grain intake (103 g/d),

### Table. Metabolic Syndrome: Evidence for Proinflammatory State

<table>
<thead>
<tr>
<th>Change</th>
<th>Biomarker</th>
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<tbody>
<tr>
<td>↑</td>
<td>Acute phase proteins (hsCRP and SAA)</td>
</tr>
<tr>
<td>↓</td>
<td>Adiponectin (high molecular weight)</td>
</tr>
<tr>
<td>↑</td>
<td>Proinflammatory status (IL-6, IL-18, and TNF-α)</td>
</tr>
<tr>
<td>↑</td>
<td>Chemokines (MCP-1 and IL-8)</td>
</tr>
<tr>
<td>↓</td>
<td>Anti-inflammatory cytokines (IL-10)</td>
</tr>
</tbody>
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hsCRP = high-sensitivity C-reactive protein, SAA = serum amyloid A protein, IL-6 = interleukin-6, IL-8 = interleukin-8, TNF-α = tumor necrosis factor-alpha, MCP-1 = monocyte chemotactic protein-1, IL-10 = interleukin-10

The best-studied biomarker is the prototypic downstream measure of inflammation, high-sensitivity C-reactive protein (hsCRP), which most studies report is increased in patients with MetS. Other biomarkers that support increased inflammation in MetS include decreased adiponectin, increased levels of interleukin-6 (IL-6), tumor necrosis factor (TNF), monocyte chemotactic protein-1 (MCP-1), low levels of interleukin-10 (IL-10, an anti-inflammatory cytokine), increased levels of serum amyloid A (SAA), and increased levels of plasminogen activator inhibitor-1 (PAI-1). Numerous studies using multivariate analysis have shown that hsCRP predicts subsequent cardiovascular events including myocardial infarction, stroke, peripheral arterial disease, and sudden death. 4 It is important to note that as the number of components of MetS increases, CRP levels increase significantly. In the Women’s Health Study, 3 CRP levels without any features of MetS were 0.68 mg/L compared to 5.75 mg/L in patients with all five features of MetS. The researchers also showed that a CRP >3.0 mg/L conferred a worse event-free survival in the presence of MetS.

The author and colleagues recently reviewed the literature on the evolving role of CRP in atherothrombosis. 6 In this detailed review, we discussed the in vitro and in vivo effects of CRP that support the hypothesis that CRP contributes to atherothrombosis. Furthermore, with regard to inflammation, it is important to note that adipose tissue is a rich source of adipokines such as TNF, IL-6, IL-8, PAI-1, SAA, adiponectin, and CRP. Adiponectin, a complement-related protein of 30 kilodaltons, is an important anti-inflammatory cytokine. It decreases lipid synthesis and glucose production in the liver, decreases triglyceride production, and increases free fatty acid oxidation in the skeletal muscle. In sum, its effects include reduction in plasma glucose and free fatty acids, increase in insulin sensitivity, and decrease in hepatic glucose production. Adiponectin levels are lower in patients with obesity and insulin resistance, and indeed hypoadiponectinemia is a uniform feature of MetS. Thus, MetS is clearly a proinflammatory state.
and olive oil consumption (8 g/d) also were significantly higher in the intervention group. This group lost significant amounts of weight. Furthermore, study results showed an improvement in endothelial function. The Mediterranean-style diet had a significant effect on biomarkers of inflammation, resulting in significant reductions in hsCRP, IL-6, and IL-18 (Figure). More importantly, the diagnosis of MetS at 2 years was reduced by 43% among those following the Mediterranean-style diet compared to those following the conventional diet (P<0.001).12

**Effect of a Mediterranean-Style Diet on Inflammation in Metabolic Syndrome**

<table>
<thead>
<tr>
<th>% change from baseline</th>
<th>Mediterranean Diet</th>
<th>Control Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsCRP</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>IL-6</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>IL-18</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

*P<0.05 vs control diet

Figure. Effect of a Mediterranean-style diet on inflammation in Metabolic Syndrome. hsCRP = high-sensitivity C-reactive protein, IL-6 = interleukin-6, IL-18 = interleukin-18

In 132 obese subjects with MetS, Seshadri et al13 showed that a low-carbohydrate diet compared to a conventional diet resulted in significant decreases in weight-loss-adjusted CRP levels in patients who had high CRP levels at baseline. Also, Roberts et al14 showed that a short-term diet and exercise intervention had a beneficial affect on biomarkers of oxidative stress in inflammation in obese men with MetS (n=31). The men were placed on a high-fiber, low-fat diet with daily aerobic activity for 3 weeks, which resulted in significant reduction in myeloperoxidase, F2-isoprostanes, and CRP and matrix metalloproteinase-9 (MMP-9) levels.

Thus, abundant data suggest that therapeutic lifestyle change including exercise and weight loss is anti-inflammatory in patients with MetS. However, in patients with MetS who fail at therapeutic lifestyle change, pharmacologic therapies can reduce inflammation. The author and colleagues looked at the effect of simvastatin therapy in 50 patients with MetS.15 The patients were randomized to placebo or to simvastatin 40 mg/d, and we showed a 32% reduction in low-density lipoprotein cholesterol (LDL-C), 42% non-HDL-C, and 36% reduction in hsCRP levels compared to placebo. Also, we looked at monocyte-cytokine release and showed that in both the basal state and following priming with lipopolysaccharide, IL-6 levels and TNF-α levels were significantly reduced compared to placebo. We also showed a significant reduction in nuclear factor kappa B p65 (NFκB p65) binding to the nucleus in the monocytes of the patients with MetS on simvastatin compared to placebo. This is the most direct evidence on this important transcriptional factor that appears to be the gateway to inflammation and clearly shows that simvastatin therapy is anti-inflammatory in patients with MetS. Szapary et al16 studied 60 patients with MetS given pioglitazone (PIO) 45 mg/d or placebo. This study shows that PIO, a peroxisome proliferator-activated receptor (PPAR-gamma) agonist, resulted in a 31% median reduction in hsCRP, a 10% decrease in resistin levels, and a 311% increase in adiponectin. All these effects were statistically significant. In a study by Hanefeld et al,17 researchers showed that in patients with coronary artery disease and MetS that PIO reduced CRP levels, and confirmed our findings that simvastatin lowers hsCRP levels. However, they did not show an additive effect of simvastatin-PIO combination therapy.

In conclusion, patients with MetS are in a proinflammatory state as evidenced by numerous biomarkers of inflammation. Importantly, therapeutic lifestyle changes including weight loss and aerobic exercise significantly reduce biomarkers of inflammation, the best studied being hsCRP levels. In patients who do not optimize CRP levels and dyslipidemia with therapeutic lifestyle changes, clinicians can consider using statin therapy since it lowers CRP and has a beneficial effect on the lipid profile. A developing evidence base supports use of statin therapy in patients with MetS.18

Finally, emerging data indicate that PIO might have beneficial effects on the progression of atherosclerosis and also might become a viable strategy with regards to the inflammatory burden in patients with MetS. However studies in patients with this syndrome are urgently required.
Targeting Inflammation in the Metabolic Syndrome

Acknowledgements

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References


Q & A

Q: It is interesting looking at the data on a portion side. I am concerned about this, too, but the amount of weight adult Americans gain in their life is very small—a really small amount of pure fat a day. I look at these data and consider whether something is going on here that could be explained other ways. For example, perhaps we should look not just at body weight and core intake, but also at fitness. Do some of the data you referred to address that issue? Some interesting information is emerging looking at fitness after accounting for body weight, and fitness seems to be driving the mortality outcomes more than body mass or particular body compartments per se.

Dr Jialal: I am not sure I agree with what you say. I think that poor diet and lack of exercise act in concert and conspire. According to 3-day dietary records, the patients I see in my clinic and in our studies are eating a lot of saturated fat, very little fiber, and very little n-3 fatty acids. Sometimes we look at pharmacological strategies in these patients instead of dietary strategies. But BMI is really a poor measure of adiposity. If I were given one choice, I would choose computed tomography (CT) to look at visceral and adipose tissue mass. Somebody showed a slide of a wrestler with a BMI of 42 who was totally healthy, and a slide of another
person with a BMI of 32 whose visceral and adipose tissues were significantly increased.

I think genetic factors are not the story. We are inducing inflammation with various adipose tissue signals and other signals. With CRP we have seen that genetic studies have been generally negative. And if you believe in Mendelian randomization for human disease, then you would say that CRP is not important. But the Jupiter study showed a great benefit with a concomitant reduction of CRP and low-density lipoprotein cholesterol [Ridker PM et al. Lancet. 2009;373:1175-1182]. So although we think all the answers lie in DNA and genome and genetic factors, we ought to step back and appreciate the environmental factors in these chronic Westernized diseases. We are causing these diseases and inducing them on ourselves.

Q: Which way do we go with people with pre-diabetes? Metformin or diet and exercise? Metformin provides rapid action, but diet and exercise require compliance.

Dr Jialal: This is a controversial area. The definition of pre-diabetes is a blood glucose level of 100 to 125 or between 140 and 199 in a 2-hour glucose tolerance test. The easy answer to your question is that the US Food and Drug Administration (FDA) has not approved any drug for the treatment of pre-diabetes. But you are correct: Metformin has definite benefits, as I showed you in the study in the diabetes prevention program [Orchard TJ et al. Ann Intern Med. 2005;142(8):611-619] and it was safer than the glitazone troglitazone that was discontinued because of liver toxicity. At this moment all the drug companies are trying to introduce drugs for pre-diabetes. They are trying to use glucagon-like peptide 1 and other drugs that lower glucose levels, such as sulfonamides.

Q: What do we tell patients to do in terms of diet?

Dr Jialal: Dietary reduction in calories, especially for those with a BMI over 27. The reduction in calories is the working concept of a diet. The other thing is to increase fiber—30 to 40 grams of dietary fiber per day. Increase complex carbohydrates, reduce saturated fat to less than 10%, and increase monounsaturated fats. In addition, I tell my patients to walk for at least 30 minutes most days of the week.
Obesity is associated with increased risk of developing insulin resistance and diabetes. A body of data has suggested that obesity promotes inflammation, which has become increasingly implicated as an important etiologic protagonist in the development of insulin resistance. An important initial clue to a potential role for inflammation was the observation that adipose tissue expressed two cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6. Importantly, the level of cytokine expression in adipose tissue of obese humans correlates with systemic insulin resistance. To confirm a role for cytokines in obesity-associated insulin resistance, TNF-α knockout mice fed a high-calorie diet were found to have increased insulin sensitivity compared to obese wildtype mice. This study provided important confirmation of a role for inflammation in obesity-associated insulin resistance.

Critical steps in linking obesity-associated inflammation to insulin resistance were important studies examining the effects of salicylic acid. Salicylic acid is different from aspirin but has similar anti-inflammatory effects. Salicylic acid was found to improve insulin resistance in both animal models of mice and humans. More recent studies suggest that administration of salicylic acid improves diabetes in obese humans.

To more precisely define the mechanisms underlying the interaction between obesity, inflammation, and insulin resistance researchers turned to additional mouse models. To determine specifically how inflammation is regulated, what tissues and cells contribute to obesity-associated inflammation, and finally the mechanism(s) by which salicylic acid functions to ameliorate obesity-metabolic dysfunction researchers turned to mouse models in which gene expression could be ablated in the whole body or specific tissues. The intracellular kinase, IKK-β, has been identified as an important target of salicylic acid anti-inflammatory actions in early studies. As a result, researchers utilized IKK-β knockout mice to investigate a role for this protein in obesity-associated insulin resistance and inflammation. Mice with a total body heterozygous ablation of the IKK-β were mated to a mouse model of genetic obesity or fed a high-fat diet as a model of diet-induced obesity and were found to have significant protection against obesity-associated inflammation and insulin resistance. To determine the relative contributions of different tissues in
promoting insulin resistance, tissue-specific ablation of the IKK-β gene mouse studies were undertaken. Ablation of IKK-β in liver was found to reduce obesity-associated insulin resistance in the liver but not in other tissues.14 However, ablation of IKK-β in myeloid/macrophage cells was found to reduce obesity-associated insulin resistance in both liver and skeletal muscle cells.14 These data suggested, for the first time, that the immune cells, macrophages, were important protagonists in the development of obesity-associated inflammation and insulin resistance.

Although it had previously been established that adipose tissue becomes inflamed with obesity and produces increased levels of mediators, the cell or cells in adipose tissue that contributes to the inflammatory state with obesity was not clear. A critical link to understanding the development of inflammation in adipose tissue in obesity was the publication of two papers in the Journal of Clinical Investigation from the laboratories of Anthony Ferrante and Hong Chen.15,16 Both laboratories noted that with obesity there was a dramatic influx of macrophages. The macrophages were found to be critical producers, if not the predominant producers, of inflammatory mediators and cytokines. The number of macrophages found in adipose tissue was directly related to fat cell size, suggesting a correlation between obesity and adipose tissue inflammation.

The potential mechanisms and role for macrophages in adipose tissue in the obese state were unclear. In collaboration with Saverio Cinti, we used immuno-histochemical techniques to identify the orientation and potential role for macrophages in adipose tissue.17 Initially, we focused on the arrangement of macrophages in adipose tissue. Immunohistochemical studies in two dimensions revealed that the macrophages in adipose tissue from obese mice and humans were found to localize in a specific ring-like orientation around what appeared to be adipocytes.

If one extrapolates our observations in two dimensions to three dimensions one can visualize macrophages forming a crown around an adipocyte. This led us to coin the phrase crown-like structures (CLS) as aggregates of macrophages that surround a presumptive adipocyte in adipose tissue. With obesity, we found a dramatic increase in these CLS. To provide further understanding of CLS, we performed electron microscopic studies, which revealed that the macrophages in the CLS were surrounding the remnant lipid droplets of dead adipocytes. Thus, with obesity, there were increased rates of death of adipocytes, leaving behind a remnant lipid droplet. Since the lipid droplet is triglyceride, which is insoluble, we hypothesized that the body possibly recognizes the lipid droplet as a foreign body and generates in a sense a foreign body reaction. The macrophages thus migrate into adipose tissue to surround, sequester, and scavenge the lipid droplets. As a result of this process the macrophage accumulates triglyceride.

To address whether it is the obese state or adipocyte cell size that is more closely associated with the occurrence of adipocyte death, we turned to a specific knockout mouse. Hormone-sensitive lipase (HSL) is an important lipase that regulates adipocyte lipolysis. As a result of decreased lipolysis, HSL knockout mice have increased adipocyte cell size in the absence of obesity. Interestingly, we found that the adipose tissue of HSL knockout mice had significantly increased rates of adipocyte death and increased expression of macrophages along with increased levels of the inflammatory cytokine TNF-α compared to the adipose tissue of control wild type mice. These studies suggested that adipocyte death promotes recruitment of macrophages into adipose tissue to sequester and scavenge the remnant lipid droplet in adipocytes.

To address the relationship between the occurrence and rate of adipocyte death and systemic insulin resistance, mice were fed a high-fat diet as a dietary model of diet-induced obesity and were investigated at different time points after initiation of the diet.18 At selected time points after the initiation of a high-fat diet fasting glucose and insulin were measured, insulin tolerance tests were performed, and serum and adipose tissue and liver samples were harvested from the animals. A critical observation was that the appearance of a significant number of adipose tissue CLS correlated with the onset of systemic insulin resistance. In parallel with the number of CLS there was a significant increase in the number of macrophages and cytokines and monocyte-chemoattractant protein-1 (MCP-1) in adipose tissue. MCP-1 is thought to be an important protein that is secreted into the blood to recruit macrophages from the bone marrow. From immunohistochemical studies we found that those macrophages that localize around the remnant lipid droplet and sequester lipid also produce significant levels of cytokines and inflammatory mediators. In general, adipocyte death was increased within intra-abdominal tissues but not in subcutaneous tissues. Only after an extended period of high-fat feeding was inflammation increased in subcutaneous tissue. An important observation of our studies was that the level of adipocyte death correlated with liver weight and steatosis. These data suggested that either the death of adipocytes provided fatty acids to the liver directly or potentially by promoting increased cytokine levels, which directly promotes lipolysis and inhibits insulin’s antilipolytic actions, promoting increased fatty acid flux to the liver. The appearance of adipocyte death and the formation of CLS appeared to correlate with the level of hepatic steatosis and systemic insulin resistance in mice. Thus adipocytes may act as initiating agents in promoting adipose tissue inflammation and/or promoting a feed-forward
cycle of macrophage recruitment to adipose tissue. The increased level of adipocytes and adipose tissue inflammation appears to have an important role in the development of obesity inflammation and insulin resistance.

In summary, a number of studies over the last several years have suggested that obesity is associated with a chronic, low-grade inflammatory state. In adipose tissue the inflammatory state is manifested in increased rates of adipocyte death, a dramatic influx of macrophages, and increased production of inflammatory mediators that contribute to the development of systemic insulin resistance and hepatic steatosis. Future studies will define further the pathways that promote inflammation and metabolic dysfunction in animals and humans. The development of drugs and nutritional therapies that impact obesity-associated inflammation and consequently metabolic dysfunction will be a focus of therapeutics in the 21st century.

References


Greenberg

Also, in obesity subcutaneous fat tends to develop by hyperplasia, but not other depots, which are more limited. The unique thing about adipose tissue is that each depot is like a mini-organ. Women tend to put fat in their thighs and their buttocks—deposition that is thought to be relatively protective against the development of insulin resistance in diabetes. Men tend toward central fatness, which may be worse. And it is not subcutaneous; it is deep fat. Is this merely a reflection of alterations in insulin sensitivity? This is a complex study.

Q: I was interested in the differences in the depots because you showed crown-like structures in one depot in the mouse and not in the other.

Dr Greenberg: You can get those structures in them all, but it takes a long time.

Q: Presumably the size of the adipocytes is the same in the two.

Dr Greenberg: No, the ones not getting the macrophages tend to be a little smaller.

Q: So you still think it is driven by the size?

Dr Greenberg: I do not know whether it is all driven by size. It also could be the way they grow. There is hypertrophy and hyperplasia and the body’s responses.

Q & A

Q: What do you mean by M2 macrophage? Also, I would like for you to talk about retinol-binding protein 4 (RBP4) and Barbara Kahn’s work on insulin resistance.

Dr Greenberg: Initially it was thought that some classic markers exist for M1 macrophages but not for M2. This is a bit like T helper cells Th1 and Th2 in the T-cell world; the populations clearly are mixed. M1 macrophages are known to be proinflammatory, and because we are metabolic scientists entering the world of inflammation, which is complex, we did not get it quite right. Now we have bacterial artificial chromosome (BAC) analysis, and that is much better than real time. Real time just tells us the number of times we see a gene, but one cell could have many copies of that gene.

Q: I am asking about this is because of collagen 3 and 6 deposition. I wonder whether they are good guys or bad guys.

Dr Greenberg: Collagen 6 is a bad guy. It is produced by adipocytes. One researcher showed that in knockout animals in which adipocytes do not make collagen 6, the animals became more obese on a high-fat diet, but although the fat cells got larger there was no inflammation. The hypothesis to explain this was that the collagen 6 was constraining the adipocytes; it did not allow them to get too big. If you remove that constraint, the cells can get even bigger and without inflammation. Many years ago, research showed that fat cells get only so big. They do not keep getting bigger, partly because some cells undergo hypertrophy and some undergo hyperplasia, which would be protective. Also, if they get bigger, they are more susceptible to cytokines and inflammation, which are anti-lipogenic and inhibit the uptake of glucose.

RBP4 appears to be an exciting protein. It is another adipokine. Barbara Kahn and colleagues showed that its levels seem to correlate with one of the mediators of insulin resistance [Graham TE et al. N Engl J Med. 2006;354(24):2552-2562].

Q: What is the average half-life of an adipocyte and is that reduced in the inflammatory process or is it increased? I am trying to get the dynamics of this interrelationship.

Dr Greenberg: That field is a little muddy. It is likely that turnover of macrophages increases with obesity. Although we see more turnover in obesity, there also are more cells. The average life? Some studies say years, some studies say a year.
Obesity is universally accepted as the culprit of insulin resistance and type 2 diabetes (T2D) but the underlying molecular mechanisms have not yet been fully established. Recently, inflammation was recognized to be the pathogenic mediator of not only these diseases but also metabolic syndromes such as cardiovascular disease (CVD). The first line of evidence indicating a relationship between inflammation and insulin resistance/T2D came from epidemiological studies. Independently, we found that obesity induces inflammation via the IkappaB kinase/nuclear factor kappa B (IKKβ/NF-κB) pathway and that pharmacological or genetic inhibition of IKKβ reverses obesity-induced insulin resistance in animals. We and others then showed that NFκB-dependent proinflammatory cytokines, including tumor necrosis factor-alpha (TNFα) and interleukin (IL)-6, mediate obesity-induced insulin resistance, and that anti-inflammatory cytokines such as IL-10 counter-regulate inflammation-induced insulin resistance. These studies together showed that inflammation that is activated by obesity via the IKKβ/NF-κB pathway mediates the development of insulin resistance and T2D.

This hypothesis has been tested further by treating humans with high doses of salicylates as an anti-inflammatory intervention; the salicylates were found to not only suppress inflammation but also to improve glucose homeostasis. The latter observations have led to the NIH-sponsored “TINSAL-2D: Clinical Trial Targets Inflammation Using Salsalate in Type 2 Diabetes” clinical study. Stage 1 of this trial was finished recently and the analysis of its data has confirmed the results obtained by the small clinical studies that were performed previously.

Although these studies have established the link between inflammation and insulin resistance in obesity, the underlying molecular mechanism by which inflammation participates in the development of obesity-induced insulin resistance is not yet fully understood. Two questions that we have been interested in are, “Where does obesity-induced inflammation occur?” and “Where do salicylates suppress inflammation?”

We approached these questions by directly examining the obesity-induced activation of NFκB in various tissues and cells by using NFκB-GFP transgenic
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mice. These mice express green fluorescent protein (GFP) in direct proportion to the degree of in vivo NFκB activation, which can be quantified by various techniques. Our initial analyses revealed that the liver and adipose tissue exhibited increased NFκB activity by obesity and that, in adipose tissue, adipose tissue macrophages (ATMs) were the primary cells to show significantly up-regulated NFκB activity by obesity, which is in agreement with numerous published studies.

However, we found that circulating immune cells, in particular circulating monocytes, showed the highest levels of obesity-induced NFκB activation. In addition, obesity also elevated the numbers of the classic inflammatory Ly6C+ monocyte subpopulation. Interestingly, the Ly6C+ monocyte subpopulations were completely different from the NFκB− monocyte subpopulation. Since monocytes are macrophage precursors, it is possible that circulating monocytes may regulate ATM numbers and functions in obesity. Indeed, our transfusion experiments showed that the monocyte subpopulations differentially regulated the migration of monocytes into adipose tissue and the induction of NFκB activity in differentiated ATMs. Moreover, salicylate treatment decreased both monocyte subpopulations along with decreasing ATM numbers, but without altering the molecular signatures of ATMs. This suggests that circulating monocytes may systemically regulate ATM inflammation by regulating ATM numbers and its fate in adipose tissue and that salicylate treatment may reverse these processes by specifically suppressing monocyte activation, thereby improving obesity-induced inflammation and insulin resistance. This further suggests that circulating monocytes may be an important therapeutic target for the treatment of obesity-induced inflammation and insulin resistance.

References


Q & A

Q: Local insulin resistance, whether it starts in the muscle or in the liver, always generates a big discussion. If I understand your presentation, it has to start in the liver because that is the home of these cells. Is that correct? Or did you do time courses of these processes and examine how they relate to insensitivity?

Dr Lee: When we did a time course of high-fat diet feeding, we found that the first tissue to show NFκB activation is always fat. For the liver, NFκB expression is always nice, and therefore, we initially thought that the liver could be the primary target or initiating site for insulin resistance. But in my current view, it may not be. I would say adipose tissue would be the primary site.

Q: I think you are right, Dr Lee. Data from several labs suggest that liver insulin resistance may be an early problem but may not be related to inflammation. I think when the inflammation comes into the liver, it makes problems much worse.

Dr Lee: Much worse. I believe that obesity-induced inflammation is not the only mechanism to cause insulin resistance. I also would like to add that most people want to categorize adipose tissue macrophages as black and white, but I think that this is gray scale, somewhere from M1 to M2, and the spectrum between two phenotypes. Also, I would emphasize that muscle is important for glucose homeostasis, as well as insulin resistance although we do not think it develops inflammation by obesity. What do we know about insulin resistance? Several hypotheses have been offered, and I will not say which one is right and which one is
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wrong. However, I believe obesity-induced inflammation is one of the mechanisms to induce insulin resistance and type 2 diabetes in certain tissues.

Q: What is the status of the phase 3 clinical trial with salsalate to which you alluded?

Dr Lee: I was told last week that the last patient was recruited. So a year from today, more or less, the study will be finished. Then probably it will take 2 to 6 months to see the results. We also are collecting circulating immune cells from patients participating at the Joslin Center in Stage II because we did not have a chance to do that in Stage I. So we will have a chance to look at what type of changes in circulating immune cells are regulated by the salsalate.

Inflammation and Wasting in Chronic Kidney Disease: Partners in Crime

Peter Stenvinkel, MD, PhD

Since the first reports in the late 1990s connecting uremic inflammation with a wasted, fatigued and calcified phenotype that led to premature death almost 4000 publications on inflammation in chronic kidney disease (CKD) have appeared on Medline. What was described as a “novel” risk factor 10 years ago has evolved into an established finding in patients with end-stage kidney disease. Thus, inflammatory biomarkers such as high-sensitivity C-reactive protein (hsCRP), white blood cell count, and interleukin (IL)-6 should no longer be considered “novel” risk factors. During the last decade much progress has been made in elucidating the molecular mechanisms that lead to inflammatory reactions. We have also learned a lot about the characteristic inflammatory profile of uremic patients, which may result from both retention of inflammatory mediators and increased tissue production.

Although inflammation is present in the majority of dialysis patients substantial differences exist in the prevalence comparing European/North American vs Asian dialysis patients. Given those differences and the fact that Asian dialysis patients treated in the United States also have a markedly lower adjusted relative risk of mortality than Caucasians, it is not surprising that many studies show that the inflamed uremic phenotype is not only associated with environmental but also with genetic factors. Although several factors have been implicated in uremic inflammation the exact causes have not yet been fully elucidated (Fig 1).
Prospective studies in patients on hemodialysis and peritoneal dialysis and following kidney transplantation show that even a single measurement of inflammatory biomarkers is an independent predictor of poor outcome. Furthermore, inflammation has been identified as a strong prognosticator of sudden death in patients with end-stage kidney disease, which indirectly supports a link between persistent inflammation and an imbalance between the sympathetic and parasympathetic nervous systems. Prospective studies have compared the predictive value on mortality of different inflammatory markers in dialysis patients, demonstrating that IL-6 was a better prognostic marker than other molecules, including CRP. Furthermore, the mortality prediction power of the combined inflammatory burden of a number of commonly measured cytokines and adhesion molecules was identical to that provided by the sole measurement of IL-6. Thus, data suggest that IL-6 is the best option for risk stratification in dialysis patients, including in early stages of CKD and particularly in the context of clinical studies. Several recent studies suggest that persistent inflammation magnifies the risk of poor outcome via mechanisms related to self-enhancement of the inflammatory cascade and/or exacerbation of the wasting and the vascular calcification processes.

The causes accounting for protein-energy wasting (PEW) include a spectrum of conditions in which chronic inflammation is a common feature such as HIV, tuberculosis, congestive heart failure, obstructive pulmonary disease, malignancies, and septicemia. PEW leads to anorexia, progressive weight loss, and depletion of both adipose tissue and skeletal muscle and is present in many patients with advanced CKD in whom it predicts poor outcome. Among the multiple factors known to promote PEW, inflammation plays a crucial role. In fact, PEW and inflammation are often interrelated and thus partners in crime. Muscle mass is inversely correlated to both IL-6 and CRP in hemodialysis patients, even after adjustment for demographics. Also, the declining markers of muscle mass during a 1-year period in hemodialysis were also associated with higher IL-1β concentrations and some studies show associations between the loss of appetite and higher levels of inflammatory markers. In dialysis patients, levels of IL-6 and tumor necrosis factor-alpha (TNF-α) progressively increased as appetite scores got worse. The skeletal muscle-derived IL-6 may contribute to both the production of IL-6 and oxidative stress during hemodialysis sessions and stimulate muscle protein breakdown and promote cancer-related wasting. Thus, increased levels of IL-6 have been related to various markers of wasting in uremic patients, indicating an important role for this cytokine in the development of PEW and muscle catabolism. As IL-6 also inhibits the secretion of insulin-like growth factor (IGF)-1, decreased IGF-1 signaling may also be involved in the sarcopenic process. However, signs of

Fig 1. Causes of altered cytokine balance in chronic kidney disease.

Not surprisingly intercurrent clinical events such as infectious complications are the most important factors predicting elevated CRP also in the uremic milieu. As reduction of kidney function per se seems to be associated with an inflammatory response in both mild and advanced CKD, even small changes in residual renal function may have an impact on inflammation in the context of kidney failure. It is hypothesized that retention of circulating cytokines, advanced glycation end products (AGEs), and pro-oxidants contribute to a proinflammatory milieu when renal function declines. Moreover, plenty of data exist regarding the direct involvement of the dialysis procedure on inflammation. The interaction of circulating monocytes with nonbiocompatible membranes, blood contact with nonsterile dialysate solution, use of unpure dialysate, the extent of convective transport, and frequency and duration of dialysis also may contribute to the inflammatory process.

Of the acute phase proteins and plasma markers of vascular inflammation CRP has emerged as the most widely used inflammatory marker of risk and is the most used inflammatory biomarker in the clinical setting. Based on CRP measurements, and due to the multiple sources of inflammation inherent to uremia and its treatment, inflammation is a major feature in advanced CKD worldwide.
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PEW are not inherent only to dialysis patients with a body mass index (BMI) <20 kg/m², but also to patients with normal (20–25 kg/m²) and high (>25 kg/m²) BMI. In each of these BMI categories, CRP and IL-6 concentrations have been demonstrated to be higher in wasted patients than in nonwasted patients, suggesting that presence of overweight does not exclude the occurrence of PEW. Several additional mechanisms exist by which inflammation can lead to muscle wasting in CKD patients, such as increased insulin resistance, activation of adenosine triphosphate (ATP)-ubiquitin proteolytic pathway, increased energy expenditure, and anorexia.

Because persistent inflammation also may be the culprit in other commonly observed pathophysiological alterations in CKD, it has been advocated that inflammatory markers should be monitored regularly and therapeutic attempts made to target this silent killer. As a first step physicians should evaluate and treat intercurrent events and comorbidities such as periodontal disease, failed kidney transplant, silent ischemic heart disease, inflammatory diseases, and volume overload that may promote inflammation (Fig 2).

The second step is to evaluate and, if possible, handle potential dialysis-related causes of inflammation such as bioincompatible dialysis fluids, unpure dialysate, thrombotized fistula or grafts, infectious complications of the vascular access, and last but not least, the use of central venous catheters. As a last step, physicians could consider possible anti-inflammatory treatment strategies. Indeed, small and uncontrolled interventional studies have shown that physical training, nutritional intervention (such as gamma-tocopherol, genistein, and soy) and pharmacological immunomodulatory treatments (such as cholecalciferol, ACE-inhibitors, statins, and sevelamer) may have an anti-inflammatory potential in dialysis patients. Although much has been learned during the last decade about the complex inflammatory interactions that exist in patients with advanced CKD intervention, randomized, placebo-controlled trials have not yet been initiated to prove the concept that persistent inflammation is not only a driver of many features commonly observed in uremia (such as PEW, depression, accelerated atherosclerosis, and vascular calcification) but also a causal mortality risk factor. Given the natural variability of inflammation biomarkers in the uremic milieu, such intervention studies do require large study populations.

**References**

3. Carrero JJ, Stenvinkel P. Inflammation in end-stage renal disease—what have we learned in 10 years? *Semin Dial.* 2010, in press.
5. Stenvinkel P, Lindholm B. C-reactive protein in end-stage renal disease: are there reasons to measure it? *Blood Purif.* 2005;23:72-78.
Discussion

Leader: Tracy R. Smith, PhD, RD, LD

Dr Sartor: I was struck with the parallels between chronic intestinal inflammation and metabolic syndrome with the main difference being that intestinal inflammation such as inflammatory bowel disease (IBD) is T cell activated and metabolic syndrome seems to be a more innate immune response. There has been an explosion of Genome Wide Associations searches for common genes. So this is a two-part question: First, what genes seem to be associated with a propensity toward developing the metabolic syndrome that may or may not be dependent of type 2 diabetes genes; second, do some of these genes fall into the category of those shared between multiple inflammatory situations such as Crohn’s disease, rheumatoid arthritis, psoriasis, and even type 1 diabetes?

Dr Jialal: The only one I know about is the peroxisome proliferator-activated receptors (PPARs) gamma mutations. But your question was specifically about diabetes. We did not mean to imply that it is a disorder we need to mitigate. I think there are T-cell biology abnormalities also in metabolic syndrome. We are just scratching the surface in regard to that.

Dr Sartor: So no real genes?

Dr Greenberg: Are you asking for genes that are different?

Dr Sartor: Genes that may be independent of type 2 diabetes. If so, do they correlate with other inflammatory conditions? Some central pathways are similar.

Dr Greenberg: Right. Obviously, however, the effort to identify genes for diabetes is greater than that for metabolic syndrome because diabetes historically has been considered a disease; metabolic syndrome is a syndrome and kind of a newcomer on the path here. Two issues are associated with diabetes—insulin resistance and a relative inability to secrete an efficient amount of insulin. Many of the type 2 diabetes genes are pancreatic genes. This is separate from metabolic issues and metabolic syndrome. Colleagues and I worked on a perilipin polymorphism that was protective against obesity and diabetes in humans because it regulates body weight. So I think inflammation is investigated much less in this area of genome research.
Dr Lee: Many genes have been identified as “diabetes genes” but the risk factors with mutations in these genes are between 1.2-fold and 1.5-fold. In other words, whatever the mutation is, a single mutation of these genes will not cause diabetes 100% of the time, except those obvious mutations such as in the insulin receptor gene. It may require abnormalities in 20 or 30 genes to induce diabetes 100%. So it is not clear whether we can ever find any pure diabetes genes.

Dr Sartor: No one has talked about autophagy, an important pathway during starvation and preservation of cells. Have there been any investigations of autophagy in metabolic syndrome?

Dr Greenberg: Colleagues and I did some work on autophagy. I should explain what autophagy is, because not everyone knows. Protein can be metabolized two ways. One is through the propiazone and the other is through the lysis zone. Autophagy is metabolism via the lysis zone. If we block autophagy in pre-adipocytes, they do not differentiate into adipocytes. So this has nothing do with being an adipocyte. That is the problem with knockout models. Autophagy clearly has a role in removing or scavenging lipid from the droplets into the mitochondria for disposal. A recent paper by Gokhan Hotamisligil and co-workers in *Nature Medicine* [Erbay E et al: 2009;15(12):1383-1391] indicates that there is incomplete autophagy in mice liver that is clearly linked to endoplasmic reticulum (ER) stress. One of Hotamisligil’s favorite themes has been promotion of inflammation by ER stress.

Dr Make: Let me gather together some of the comments made previously about obesity and inflammation and focus these more narrowly to the lungs. These prior comments were related to diabetes and liver and muscle. What about inflammation obesity that seems to be systemic, getting into other organs, particularly the lungs? Obese patients with asthma, for example, seem to have worse disease that is less well controlled than those who are not obese. And there seems to be a relationship between diabetes and reduced lung function.

Dr Lee: A strong association exists between lung disease and obesity without doubt, but I do not remember anybody in our field actually studying the lungs. I saw a poster at the AAAI meeting presenting studies of immune cell profiles in the lung from overweight mice. I was surprised to see that the profiles were similar to those of the adipose tissue.

Dr Jialal: I mentioned sleep apnea previously.

Dr Greenberg: Some data indicate that asthma is linked to obesity.
Dr Serhan: In your experience, there are differences in the practices of peritoneal dialysis in the United States. Do you have a feeling for how much inflammation is provoked by peritoneal dialysis compared to membrane dialysis?

Dr Stenvinkel: Peritoneal dialysis also provokes inflammation. Of course, these patients are subject to increased risk of complications such as peritonitis, but bioincompatible dialysis solutions also may contribute to inflammation. You should remember that in peritoneal dialysis, we are giving patients about 50 kg of glucose every year. This also may promote inflammation. In fact, peritoneal dialysis is an excellent way to, in a short time, study effects on fat mass increase because many peritoneal dialysis patients will gain 5 to 6 kg in pure fat in just 12 months of treatment.

Dr Serhan: You previously pinpointed the cut-off with glomerular filtration rates. Are there any data to suggest that inflammation mediates these rates?

Dr Stenvinkel: That question is hard to answer. We know that inflammation is associated with progression of chronic kidney disease, but we do not know whether inflammation causes loss of renal function or is the effect of renal function loss.

Dr Jialal: Many are confounded about the phosphatonin, and I know you did not discuss fibroblast growth factor 23 (FGF 23). Studies show that FGF 23 predicts mortality in cardiovascular events in renal disease but it is phosphaturic that is supposed to be good for kidney disease.

Dr Stenvinkel: It seems like the human body has many defense systems to protect itself from increasing levels. FGF 23 may be the most important protective system. Unfortunately, some evidence suggests that FGF 23 itself has a detrimental effect on the vasculature that promotes endothelial dysfunction. And as you say, quite a strong independent association exists between elevated FGF 23 and vascular events in these patients.

Dr Smith: Dr Jialal, I have a question about the metabolic syndrome in patients who are hospitalized for other diseases, other reasons. What are hospitals doing about that? Do they treat these patients differently? If not, what can be done?

Dr Jialal: This is also not well studied. I can tell you that cardiologists take this seriously. Patients with acute coronary syndrome and metabolic syndrome do worse than those without metabolic syndrome. People with diabetes also fare worse with certain surgeries. Most people with diabetes in the United States, say 75% to 80%, have metabolic syndrome.

Dr Smith: So are primary care physicians looking at the risk factors for it? Are they telling patients that they have metabolic syndrome, or are people asking their physicians about it?

Dr Jialal: That is a good question. As Dr Stenvinkel said, there will be no magic bullet for metabolic syndrome. We thought that rimonabant, a blocker, was going to be the one, but we were disappointed because of side effects. The syndrome is early diabetes, mild hypertension, central adiposity inflammation, and insulin resistance, so I think it is highly unlikely that one drug will solve the problem. As I emphasized previously, numerous studies have shown that weight loss through diet and exercise reverses metabolic syndrome. Endocrinologists and I think cardiologists pay attention to this. I am not sure how much primary care physicians with their busy practices and reimbursement issues care about metabolic syndrome.

Dr Beck: At our university we have a lot of interventionists who try to work with primary care physicians. When the interventionists talk to these physicians, the physicians say that even if they counsel patients to diet and exercise, they do not do it. The interventionists have started to work with dental professionals as well, especially with pediatric dentists, because the pediatric population is rapidly becoming obese. So how do we get information about metabolic syndrome to primary care dentists and physicians so they can provide something for their patients? Right now they have nothing to offer because patients will not exercise or change their diets. We do not have a drug, there is no magic bullet. So physicians are throwing up their hands.

Dr Make: One of the most difficult issues facing health care practitioners is changing diet and exercise behavior in our patients. We frequently highlight advances in new medications and not as commonly the importance of behavior change. If a primary care physician were to ask you to suggest one dietary change other than reducing calories that they could recommend to their patients with metabolic syndrome, what would it be?

Dr Greenberg: Take at least two fish pills a day.

Dr Jialal: No reduction in calories? The question is so hard to answer. I would say reduce saturated fat and increase fiber intake. I would add exercise at least 30 minutes most days of the week. And cook in olive oil.
Dr Make: I appreciate that, and I understand the difficulty that our patients have making these changes, which is why I ask us to focus on one thing we can suggest.

Dr Jialal: Weight loss is the answer.

Dr Smith: There is so much controversy about which way to lose weight, which diet, which exercise. Would you recommend doing it any way you can?

Dr Jialal: I am biased also. I am a spokesperson for the American Heart Association, so I have to be careful what I say. Basically, the mantra of the Association is to reduce saturated fat, increase fiber, reduce trans fat, and reduce total calories by 500 a day. But studies have shown that all these diets followed over a long period show weight loss benefits. People are talking now about bariatric surgery as a treatment for diabetes. I am not saying bariatric surgery is the answer to diabetes, but bariatric surgery cures diabetes for at least 2 years. If we could understand the pathobiology of that we might have better strategies than just calorie reduction. I do not know where we will be in 5 or 10 years, whether we will understand more about the signaling between the brain and gut and whether we will have therapies targeting that signaling with regard to weight loss.

Dr Serhan: Dr Stenvinkel, I like the point that you brought up about Yogi Bear. The question is, is the bear a couch potato? If not, why not? Does it have a salvage pathway for nitrogen? I always thought hibernation just involved a lower metabolic rate, lower turnover, but you imply that there is a specialized scenario there that we might be able to borrow and use for all these diseases.

Dr Stenvinkel: I also thought that it was just that the resting expenditure decreased markedly, but there seems to be some mechanism that salvages muscle in these bears during these 5 months of rest. When they wake up in the spring after hibernation they can actually go hunting immediately without having lost too much muscle. If human beings, like black bears, were confined to bed for several months we would lose most of our muscle mass. It is amazing. I think the science of biomimicry can teach us interesting novel aspects.

Dr Sartor: I think that needs to be explored. The bear does not lose protein, but it does lose weight. So preferentially it is losing fat rather than muscle. This could be tremendous; exactly what we want—to build up muscle and prevent sarcopenia while preferentially using fat. Is there any information about which metabolic pathway preferentially uses lipids rather than protein as an energy source? I wonder how to get glucose out of a lipid.

Dr Jialal: Marmots and other less fierce creatures also hibernate. I do not know whether they have a similar pathway, but this could be the answer.

Dr David Beno [Abbott Nutrition]: I have a question for the whole group. We are talking a lot about biomarkers and markers of inflammation. How can we use this potential stratification of patient populations in clinical trials? For example, in patients with kidney disease, can we nutritionally modify some of these markers of inflammation and have a better probability of success in our clinical trials? How do you think we should do this and how could we approach regulators so that we can do it?

Dr Szefler: That is a good question. This will require a philosophy change. In my experience of watching drugs develop, the general philosophy is to develop a drug for a disease, get a drug, and use that drug. I guess the term “personalized medicine” tries to take that into account, directing therapy based on a patient’s individual characteristics, including biomarkers and genetics. We are in an age of discovery around biomarkers. Which ones have relevance, do they predict disease, do they predict prognosis, and are they useful in monitoring therapeutic response? Are they universal or do they point to one feature of the disease? We have had a fair amount of experience with that in asthma, cardiology, and cancer chemotherapy. If we can begin to relate biomarkers to phenotypes, we can become more selective in choosing medications or other interventions, for example, environmental controls. The pharmaceutical industry still develops drugs that fit a certain disease category to make it simple for clinicians. But we are starting to move to an era that is a little bit more complex, when we start to apply biomarkers to select specific treatment based on disease presentation. We are moving there, and it sets up a different paradigm for studies.

Dr Serhan: I think you are right about the need for a change in philosophy. But because of how biomarkers are “annotated,” we have big issues. There is a lot of misannotation. We certainly do not appreciate the function of proteins in one location versus another in the body. So I want to draw your attention to thinking about a functional biomarker as I showed you previously on a single cell monitoring from an individual from one drop of blood. I think we have to stop and think of those types of functional monitoring for an outcome when we want to have a therapeutic modality.
Dr Jialal: In cardiovascular disease and inflammation I think the number-one spot would be high-sensitivity CRP. I am wearing my hat as a clinical pathologist because it is a calibrated assay. It is precise, robust, and available on most platforms, including that of Abbott’s diagnostic division. For years CRP was used by rheumatologists and surgeons for diagnosing inflammation. I am not saying it is the only biomarker; I am saying if you think about all the qualities of an ideal biomarker, this comes the closest. It also predicts stroke. I think CRP also is a pretty good marker in chronic kidney disease, even though there are others, and it predicts cardiovascular disease in diabetes. I am not convinced that CRP causes diabetes. I would like to see better data. I am willing, however, to put my head on the chopping block and say that the best marker for cardiovascular disease right now is CRP, but it is not the only one. We are learning about new markers such as functional assays and SAA—serum amyloid A assays.

Dr Pamela Anderson [Abbott Nutrition]: The Institute of Medicine (IOM) recently published their biomarker report and interestingly the Institute decided that CRP was not a sufficient biomarker for use in primary prevention of cardiovascular disease [Michael CM, Ball JR, eds. Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease. Washington, DC: The National Academies Press, 2010]. What you said previously, Dr Jialal, was correct. You said CRP was a predictor of risk, and the IOM agrees with that. The analysis met the qualification step, but the marker was nonspecific for cardiovascular disease; that is, we cannot determine where it is in the specific pathway for cardiovascular disease.

Also, back to Dr Beno’s question, if CRP is off the table as a marker for cardiovascular disease, it certainly is on the table for inflammation. What would be the particular biomarkers that we should choose for inflammation? It sounded to me like CRP was one of the main ones but there could be others.

Dr Jialal: The evidence for CRP as a biomarker for cardiovascular disease is based on the Jupiter study. In that study, patients with a high CRP level and a normal low-density lipoprotein (LDL) value of 180—healthy people treated with a statin—showed a 44% reduction in cardiovascular events, including the group that had only an elevated CRP and none of the other features [Ridker PM et al. Lancet. 2009;373(9670):1175-1182].

Dr Anderson: You are more knowledgeable about this than I am, but the IOM does discuss the Jupiter trial.

Dr Jialal: A lot of us subscribe to the hypothesis that inflammation is critical to atherosclerosis and acute coronary syndrome. If you do not subscribe to that hypothesis, then you can just say CRP is nonspecific and not causal. I believe CRP participates in atherosclerosis, but that is work in progress. Others have shown that these can predict cardiovascular events. We know in animal models that CRP reduces myocardial infarct size following coronary artery ligation myocardial infarction. Also, studies show that concomitant reduction of LDL and CRP resulted in fewer cardiovascular events [Ridker PM et al. Lancet. 2009;373(9670):1175-1182].

Dr Serhan: What biomarkers did the IOM discuss?

Dr Anderson: They discussed about six biomarkers. The one I remember is troponin. They also talked about more controversial ones.

Dr Stenvinkel: CRP belongs to the pentraxin family. My colleagues and I have been studying long pentraxin such as pentraxin 3, which seems to predict outcome somewhat better in renal patients. A strong correlation also exists between the degree of albuminuria and pentraxin 3 levels. Pentraxin could be a potential new biomarker that should be tested in larger patient groups such as diabetic patients.

Dr Serhan: With regard to biomarkers, we have to think about implementation. As a basic scientist, I think the sensitivity of seeing a therapeutic-induced change in a rare component in a whole human system is difficult. And how early up front does something become an indicator? Namely, what is the timeline of the markers’ appearance? Many things temporally change with all sorts of circadian rhythms, and it is much more complex than we tend to think. A clinical diagnosis today from a lab is really a snapshot of averages.

Dr Jialal: In cardiovacular disease and inflammation I think the number-one spot would be high-sensitivity CRP. I am wearing my hat as a clinical pathologist because it is a calibrated assay. It is precise, robust, and available on most platforms, including that of Abbott’s diagnostic division. For years CRP was used by rheumatologists and surgeons for diagnosing inflammation. I am not saying it is the only biomarker; I am saying if you think about all the qualities of an ideal biomarker, this comes the closest. It also predicts stroke. I think CRP also is a pretty good marker in chronic kidney disease, even though there are others, and it predicts cardiovascular disease in diabetes. I am not convinced that CRP causes diabetes. I would like to see better data. I am willing, however, to put my head on the chopping block and say that the best marker for cardiovascular disease right now is CRP, but it is not the only one. We are learning about new markers such as functional assays and SAA—serum amyloid A assays.

Dr Pamela Anderson [Abbott Nutrition]: The Institute of Medicine (IOM) recently published their biomarker report and interestingly the Institute decided that CRP was not a sufficient biomarker for use in primary prevention of cardiovascular disease [Michael CM, Ball JR, eds. Evaluation of Biomarkers and Surrogate Endpoints in Chronic Disease. Washington, DC: The National Academies Press, 2010]. What you said previously, Dr Jialal, was correct. You said CRP was a predictor of risk, and the IOM agrees with that. The analysis met the qualification step, but the marker was nonspecific for cardiovascular disease; that is, we cannot determine where it is in the specific pathway for cardiovascular disease.

Also, back to Dr Beno’s question, if CRP is off the table as a marker for cardiovascular disease, it certainly is on the table for inflammation. What would be the particular biomarkers that we should choose for inflammation? It sounded to me like CRP was one of the main ones but there could be others.

Dr Jialal: The evidence for CRP as a biomarker for cardiovascular disease is based on the Jupiter study. In that study, patients with a high CRP level and a normal low-density lipoprotein (LDL) value of 180—healthy people treated with a statin—showed a 44% reduction in cardiovascular events, including the group that had only an elevated CRP and none of the other features [Ridker PM et al. Lancet. 2009;373(9670):1175-1182].

Dr Anderson: You are more knowledgeable about this than I am, but the IOM does discuss the Jupiter trial.

Dr Jialal: A lot of us subscribe to the hypothesis that inflammation is critical to atherosclerosis and acute coronary syndrome. If you do not subscribe to that
Current Asthma Management: Opportunities for a Nutrition-Based Intervention

Stanley J. Szefler, MD

Approximately 22 million Americans, including 6 million children, have asthma. It is one of the most prevalent chronic diseases of childhood. Asthma affects the patient, their family, and society in terms of school absences and missed work, along with a lessened quality of life and potentially avoidable emergency department visits, hospitalizations, and even death. While mortality related to asthma has declined, hospitalizations have remained at about 500,000 per year. Higher rates of hospitalizations have occurred, particularly among young children, especially with African Americans and Puerto Rican populations.

This review will indicate some of the recent accomplishments in asthma management, discuss some of the unmet needs in light of current management principles, and indicate the potential role of nutrition in future asthma management.

The Changing Faces of Asthma

Not more than 50 years ago, asthma was viewed as an episodic disease, and treatment was directed at relieving symptoms using short-acting bronchodilator therapy. It was then recognized that longer-acting bronchodilators, such as albuterol and theophylline, could serve to prevent symptoms, if they were administered on a regular basis.
control, responsiveness, impairment, and risk. \(^1,2\) Severity is defined as the intrinsic intensity of the disease process. Control is the degree to which the manifestations of asthma (symptoms, functional impairment, and risks of untoward events) are minimized and the goals of therapy are achieved. Responsiveness is the ease with which control is achieved by therapy.

Asthma severity and asthma control are both divided into two domains—impairment and risk. Impairment is the assessment of the frequency and intensity of symptoms, as well as the patients’ functional limitations, now or in the past, because of their asthma. Risk is the estimate of the likelihood of an asthma exacerbation, progressive loss of pulmonary function over time caused by asthma, or an adverse event from medication or even death. The assessment of severity and control provides guidance on the direction to take in conducting additional diagnostic evaluations, assessing environmental factors and adherence to the management plan, and consequently stepping up or stepping down medications.

Asthma Control: Unmet Needs
Asthma is a disease characterized by chronic inflammation, including allergen-induced airway inflammation. The inflammatory reaction consists of cell injury, release of mediators, vascular and cellular responses, and a repair response that can lead to acute and chronic airway obstruction. Persistent inflammation and airway remodeling are associated with poor asthma control. Medications such as inhaled glucocorticoids that reduce airway inflammation are the preferred treatment for asthma, but do not prevent progression.

The consequences of poor control include asthma exacerbations, airway remodeling and asthma progression, school absence, altered lifestyle, and potential evolution to chronic airway obstruction and possibly chronic obstructive pulmonary disease (COPD). Despite significant advances in asthma management during the last several years, some unmet needs still exist, including methods to diagnose asthma early, development of treatments that alter the natural history of asthma to prevent progression, new techniques to anticipate and prevent asthma exacerbations, and strategies to address health disparities in morbidity and mortality related to asthma. \(^3-5\)

Opportunities to Improve Asthma Control
Several potential methods can improve asthma outcomes by directing our management concept to a personalized medicine approach. \(^6\) This could include early recognition and early treatment of asthma, application of genetics and

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**Table. Summary of Events Describing the Interrelationship of Goals in Asthma Management to the Medications Available for the Treatment of Asthma**

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Goal of Management</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960s</td>
<td>Relieve bronchospasm</td>
<td>Epinephrine</td>
</tr>
<tr>
<td>1970s</td>
<td>Prevent bronchospasm</td>
<td>Albuterol, Theophylline</td>
</tr>
<tr>
<td>1980s</td>
<td>Prevent allergen-induced bronchospasm</td>
<td>Cromolyn</td>
</tr>
<tr>
<td>1990s</td>
<td>Prevent and resolve inflammation</td>
<td>Inhaled glucocorticoids, Leukotriene modifiers, Long-acting ß-adrenergic agonists, Combination therapy</td>
</tr>
<tr>
<td>2000s</td>
<td>Achieve asthma control</td>
<td>Anti-IgE</td>
</tr>
<tr>
<td>2010s</td>
<td>Personalized medicine, Early intervention</td>
<td>Biomarkers/genetics, Immunomodulators?</td>
</tr>
</tbody>
</table>
epigenetics to predict the risk for developing persistent asthma, utilization of biomarkers to monitor disease activity, and the development of new approaches to manage inflammation, including immunomodulator therapy. The latter could include a nutrition-based approach to asthma management.

**Possible Strategies for a Nutrition-Based Approach**

Recent attention has focused on nutrition as an important part of disease management through observations related to the deficiency of vitamin D, as well as the potential benefits of probiotics, antioxidants, and anti-inflammatory omega-3 fatty acids. The epidemic of obesity and its potential effect on health outcomes, including asthma, also is of interest.

Therefore, the potential benefits of a novel nutritional formula (NNF) were studied through a relatively small, but well-designed pilot feasibility study in children with mild persistent asthma. To date, it is the most comprehensive study of a nutritional supplement conducted in childhood asthma. This NNF contained eicosapentaenoic acid (EPA), gamma-linolenic acid (GLA), and antioxidants that reduced inflammation and improved oxygenation and clinical outcomes in critically ill patients.

Children, 6–14 years of age, with mild to moderate persistent asthma, on-as-needed albuterol alone, were randomized to receive daily NNF (n=23) or control formula (n=20) for 12 weeks, with multiple assessments of asthma control, spirometry, measures of airway inflammation, formula tolerance, and adverse events.

Daily consumption of either NNF or a control formula showed improvement in asthma-free days over time (P=0.04), but no difference was seen between groups (Fig 1A). However, the NNF group had lower exhaled nitric oxide levels compared with the control group at weeks 4, 8, and 12 (P<0.05) (Fig 1B). An overall group difference in log forced expiratory volume (FEV1) methacholine PC20 (the dose of methacholine that results in a 20% decrease in FEV1; the lower the methacholine PC20, the more reactive the airways) (P=0.05) was found in favor of the NNF group as well (Fig 1C), along with a nominal difference in FEV1 percentage predicted and sputum eosinophils between the two groups (Fig 1D and Fig 1E).

Significantly higher levels of EPA in plasma (P<0.01) and peripheral blood mononuclear cell (PBMC) (P<0.01) phospholipids in the NNF group compared with control group within 2 weeks indicated good adherence with daily NNF intake (Fig 2). No differences were noted in adverse events for NNF vs control after 12 weeks.
Future Directions

The next steps to take to establish the role of this nutritional formula as a cornerstone of asthma therapy include studies to replicate the published findings in a larger sample set, including more severe asthma, evaluation of the role of this nutritional formula in exercise-induced asthma, and consideration of an early intervention trial, including a possible prenatal application. This work will help to determine if such a nutrition-based approach could form the first step in asthma management as the preferred initial therapy of asthma with our current therapies, such as inhaled glucocorticoids, used as supplementary strategies to maintain asthma control.

References


COPD: Inflammation, Phenotypes, and Nutrition

Barry J. Make, MD

Long considered an unimportant disorder of older male cigarette smokers, chronic obstructive pulmonary disease (COPD) is the fourth most common cause of death in the United States and is now recognized as a leading cause of morbidity. More women now die of COPD than men. The high financial costs of the disease are largely associated with hospitalizations and management of acute exacerbations of the disease.1,2

The definition of COPD was modified in the past decade to include the importance of inflammation and comorbidities: “COPD is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases.”3

Inflammation in COPD

It is thought that the pathogenesis of COPD is inextricably linked to cigarette smoke-induced inflammation (Fig 1). Inflammation is because of multiple factors, including oxidative stress and the effects of proteinases. However, not all smokers develop COPD, suggesting that host factors, such as genetic predisposition, play an important role. For example, it recently was shown that extracellular superoxide dismutase expression and polymorphisms are associated with risk of COPD.4,5

COPD Phenotypes

Not all patients with COPD are alike.\(^5,10\) For example, prior to the widespread use of the term COPD, emphysema (marked by destruction of alveolae) and chronic bronchitis (defined by chronic cough and sputum production) were recognized as different presentations of the disorder. The differences between patients with COPD are referred to as COPD phenotypes, recently defined as “a single or combination of disease attributes that describe differences between individuals with COPD as they relate to clinically meaningful outcomes (symptoms, exacerbations, response to therapy, and rate of disease progression or death).”\(^7\) Fig 2 shows some of the features that are associated with different COPD phenotypes.

Fig 2. COPD phenotypes. Adapted from Frank Sciurba, MD.

Traditionally, COPD phenotypes are defined by using the presence and severity of airflow limitation. COPD clinical practice guidelines define COPD severity by the percent of predicted forced expiratory volume in 1 second (FEV\(_1\)).\(^3,11\) FEV\(_1\) is the single best predictor of mortality and generally correlates with other important clinical features, including shortness of breath, exercise capacity, and health-related quality of life.\(^12-14\) However, the strength of the correlation of FEV1 with clinical

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**Fig 1. Pathogenesis of COPD, illustrating the central role of inflammation.** Used with permission of Global Initiative for Chronic Obstructive Lung Disease (GOLD); www.goldcopd.org. Teaching slide set, Global Strategy for Diagnosis, Management and Prevention of COPD.

The earliest pathologic lesion in COPD is respiratory bronchiolitis, characterized by lymphocytic inflammation in the airway walls and lumen. This small-airway disease is a major contributor to airflow limitation in COPD.\(^6\) Inflammation also is present in patients with emphysema.

In a landmark study, Hogg and associates studied resected lung tissue from 159 patients with various stages of COPD.\(^7\) Patients with more severe disease, Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages 3 and 4, were nonsmokers undergoing lung volume reduction surgery (LVRS) for moderate-severe emphysema on chest computed tomography (CT) scan. Inflammation in airways <2 mm in diameter was found in all GOLD stages of COPD, including nonsmokers with emphysema. More severe airflow limitation was associated with increased inflammatory exudates and a higher percentage of airways containing polymorphonuclear neutrophils, macrophages, CD4 cells, CD8 cells, and B cells.

The importance of these pathologic findings was highlighted in another study by the same group, showing that occlusion of small airways by inflammatory exudates was associated with early death in patients undergoing LVRS.\(^8\)
features is modest, making other phenotypes perhaps more important than lung function alone.

Chest CT scans can identify different phenotypes, and quantitative measurements can capture the severity of emphysema and airway disease. CT scans have the potential to describe clinically meaningful phenotypes. Increasing emphysema is associated with COPD exacerbations. In the National Emphysema Treatment Trial, patients’ CT emphysema characteristics and exercise capacity were predictive of outcomes from LVRS; patients with upper-lobe-predominant emphysema and low-exercise capacity have markedly improved survival following LVRS. The Genetic Epidemiology of COPD Study, funded by the National Institutes of Health, is using CT scans to phenotype patients to find genetic associations with COPD.

Patients with exacerbations of COPD represent an important phenotype. Patients with more severe airflow limitation have more exacerbations. In one recent study, 22% of patients with stage 2 COPD, 33% with stage 3, and 47% with stage 4 had two or more exacerbations in 1 year. The mortality and morbidity of patients following a hospitalization for COPD is high. In a study of patients by the US Dept of Veterans Affairs, 1-year mortality was 21%, 5-year mortality was 55%, and 25% of patients were rehospitalized within 1 year.

**Nutritional Aspects**

Patients with COPD frequently have many other disorders, including most cardiovascular diseases, skeletal muscle dysfunction, osteoporosis, diabetes, and depression. These comorbidities may represent spillover of the inflammation in the lungs to the systemic circulation.

Low body weight carries a poor prognosis in COPD, and thus is seen as an important phenotype. Not only is body weight reduced in some patients with COPD, but decreased lean body mass and skeletal muscle dysfunction also are reported. Decreased protein synthesis, accelerated protein breakdown, and an increase in cytokines, including tumor necrosis factor, interleukin (IL)-1 and IL-6, are reported in underweight COPD patients.

COPD patients with these features are perhaps the best candidates for adequate macronutrient intake, as well as novel nutritional interventions, such as docosahexaenoic acid, eicosapentaenoic acid, gamma-linolenic acid, vitamin D, antioxidants, and omega-3 and omega-6 fatty acids.

**References**


Discussion

Leader: Stephen J. DeMichele, PhD

Dr DeMichele: I want to first thank Dr Szefler and Dr Make for excellent presentations and the overview of two major health burdens with chronic obstructive pulmonary disease (COPD) and asthma. I think they went over very nicely the complexities of the two diseases or COPD syndrome—how they treat it pharmacologically and some possible ways we could intervene with nutritional applications and treatment of the disease. I would like to open up the floor for questions.

Dr Jialal: You said that you see more cardiovascular disease in COPD patients. Do you see a biomarker for your patients in inflammation? Does it predict cardiovascular events in COPD?

Dr Make: A number of studies have looked at C-reactive protein (CRP) as a biomarker in COPD, but most have not followed patients long enough to know whether it is a biomarker for mortality, either total cause mortality or mortality related to cardiovascular disease. We routinely measure CRP in our patients. We also search more aggressively for silent cardiovascular disease in patients with COPD.

One of the problems of identifying cardiovascular disease in COPD is that patients are short of breath, and most physicians attribute that symptom to COPD, COPD patients often have silent cardiovascular disease, and cardiovascular disease is the second most common cause of death in patients with COPD. We routinely perform cardiac stress tests in our patients at National Jewish, because COPD patients are at high risk for cardiovascular disease.

Dr Jialal: Could you get a measure using noninvasive tests? With a computed tomography (CT) scan, you could get the calcium score for what it is worth. That is a noninvasive subclinical measure, and it will tell you about atherosclerosis.

Dr Make: In our National Institutes of Health COPD Gene Study, we are performing chest CT scans. Although our chest CT scans are un gated, we have found that they correlate with gated CT scan coronary calcium scores. Coronary calcium is a primary measure of cardiovascular disease in a grant application that we are submitting to look at cardiovascular disease in smokers with and without COPD.

Dr DeMichele: I have a question for Dr Szefler while we are on the CRP theme. Was CRP looked at in children with asthma, either mild, persistent, or severe, and if it was, has it played out in the adult situation as well?
Parents are very reluctant to use steroids in any way, especially continuously. They have concerns about growth. They have concerns about cataracts. They still have concerns about anything that is associated with prednisone therapy, as much as we have tried to reassure them. Then every once in a while, an article will come out that confirms the concerns rather than allaying the fears.

The long-acting beta-agonists have a black-box warning associated with them. Every year the US Food and Drug Administration (FDA) comes out with another reminder about that, so they are concerned. Even leukotriene antagonists, which we thought were very safe, had a behavioral signal come out a little more than 1 year ago. Even though the literature is reassuring, you still hear concerns about that possibility.

Parents and patients really do have concerns about medication adverse effects, and they try to minimize the use of medications. In asthma, the asthma therapy is not always taken as prescribed. It often is parent driven in terms of using intermittent therapy when the child is having symptoms, rather than continuous as a preventive measure. Parents have many concerns, and they are very open to other alternative management mechanisms, including diet. We found that parents are very receptive to discussions about nutritional intervention as an alternative to medications.

Dr Madsen: As a mother with asthmatic children, I would have given anything to have had a nutritional intervention, rather than having to go on the drugs.

Dr Hébert: My question has to do with adaptation. This has come up several times, most recently in Dr Make’s presentation. You said the short-term effect of the physical activity was pro-oxidative. It seems to me that many times we have these exposures that produce a pro-inflammatory effect in the short term, but in the longer term, they are anti-inflammatory.

How does that change matter in terms of acute vs chronic responses, given the types of disease outcomes we are talking about here?

Dr Make: Longer-term exposure to exercise, pulmonary rehabilitation, is not shown to uniformly increase oxidative stress.

Dr Mazer: With respect to this study that is going on with the 10,000 patients, are you collecting data on secondary inflammatory conditions, obesity, and arthritis that these patients might have to help sort out the subgroups?

Dr Make: We are collecting a history, at least a patient-recorded history of a physician diagnosis of a number of other diseases.

Discussion

Dr Szefler: I cannot think of specific publications on that topic. It certainly has not hit the forefront as an important biomarker. The one that has is exhaled nitric oxide, which is considered a biomarker of allergic airway inflammation.

Dr Suzette L. Pereira [Abbott Nutrition]: I have a question regarding patient exacerbation. Is it related to smoking? Do smokers have more of these events? Could a biomarker predict who might have more of these events than others?

Dr Make: The best predictor of COPD exacerbations is the presence of a past exacerbation. An exacerbation in the past year is predictive of another. Other predictors include depression, worse lung function, and oxygen therapy.

Dr Pereira: Dr Szefler, you mentioned prenatal predictions in asthma patients. Could you elaborate a little more on that? Do you really have a way to do that?

Dr Szefler: Family history, particularly maternal history, is a good one in terms of asthma. The asthma literature contains only a few long-term studies. One of them was developed in Tucson, and Fernando Martinez was involved in that [Castro-Rodriguez JA et al. Am J Respir Crit Care Med. 2000;162(4 pt 1):1403-1406].

Something that caught everybody’s attention was when Dr Martinez and his colleagues in Tucson presented an asthma predictive index. It almost was like the rheumatic fever cardiac predictive index in that it includes major and minor criteria. The index for predicting asthma actually is better if you did not have any of those indicators for not having the disease, but it was still in that positive direction.

I think we are moving toward this. A study going on now in Australia is looking at omega-3s and diet to see whether they will predict the evolution of asthma disease.

Dr Smith: Dr Make, what is the prevalence of obesity these days in COPD? Is it different with chronic bronchitis and emphysema? Is it growing along with the rest of the population?

Dr Make: We used to think that most patients with COPD had normal or low body weight, but that is not true. I think many patients are obese, and obesity is more common than a very low body mass. Possibly 10% of COPD patients have a body mass index (BMI) above 36.

Dr Riley: Dr Szefler, how open were the families in your trial to looking at a nutritional intervention for asthma?

Dr Szefler: Very open. I think all the medications that we have in asthma have had flags of concern, starting with inhaled steroids.

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**Discussion**

**Dr Serhan:** I think I missed the point of the lead-in time. How long was that before you started to see changes in presumably keratin 1 levels?

**Dr Szefler:** We saw changes in terms of the fatty acid composition within the first measures that we obtained in the study. The markers of inflammation already were heading in that direction after 1 month.

**Dr Serhan:** Obviously from my presentation, you could tell that I would advocate making sure that we had enough essential fatty acids in the diet. My question is about the source and the quality control of the essential fatty acids that are going into these solutions. Do you have concentrates of marine oils? How would one prevent auto-oxidation? What about storage and those sorts of routine issues?

Are there any reliable markers of inappropriate auto-oxidation of the substrate in vivo? In other words, if you had an auto-oxidation product of eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), could you mark that in vivo?

**Dr Szefler:** Dr DeMichele probably should answer the question in terms of quality control. In terms of clinical management, ideally if you could track and monitor those biomarkers or nutrition indicators and have certain desired or target levels, you could begin to identify biomarkers possibly associated with more beneficial effects, particularly if you were reducing exacerbations. Currently, I do not think normals for these measures exist. It would trigger a whole new field in terms of looking at fatty acid ratios or monitoring to define an appropriate amount in the diet.

We have touched on that issue a bit with vitamin D. As that literature emerged, people asked about the normal level and about the clinical relevance for a certain level. I think we would cross that same bridge in terms of fatty acids.

**Dr Serhan:** Before you answer that, would you consider performing a bronchoscopy in a normal person to obtain relevant functional biomarkers?

**Dr Szefler:** It is a great question. All of these different markers have sort of come into play. You have aspects of quality control, standardization of management, and measurement. You know of laboratory assessments. You can look at it in terms of percentages of cells, and you kind of have an internal standard to compare it to in order to define normal and abnormal ranges.

**Dr Serhan:** And a solid standard set of measurements.

**Dr Szefler:** Right. That is a struggle in terms of the biomarker measurements and lavage fluid. Because like lavage fluid, you do a bronchoscopy, you put fluid in, and depending on how much fluid you get back, you have to have a common denominator.

The eosinophils are actually a very interesting biomarker. To me, they are the most successful. Published studies have applied titration of inhaled steroid dosing against that biomarker, sputum eosinophils, and they are the most successful in terms of reducing asthma exacerbations, even more successful than exhaled nitric oxide. So, it is a potential marker to measure over time, which could have impact that would reflect disease activity.

**Dr Make:** Would you agree with that in terms of the standardization?

**Dr Make:** We have the same problem in COPD with standardization and repeatability of biomarkers.

**Dr Szefler:** The National Institutes of Health has taken that problem on for further discussion. It has assembled an asthma outcome measure taskforce, and I chair the biomarker committee. Those issues that you mention are exactly the type of questions that have surfaced in our discussions. With some of our asthma networks, we have worked at standardizing procedures, so that type of outcome measure will become more uniform for future studies.

**Dr DeMichele:** This formula was built on what we have done in the intensive care unit, feeding elevated levels of fatty acids with antioxidants. Those patients are under ventilation. They are under oxygen tension. We have looked at peroxide levels and what may go on if we excessively feed these fatty acids and not provide enough antioxidant support.

We use that similar model here orally, lower dose, but with sufficient external antioxidants, not only to cover the in vivo protection, but also to sort of replenish the children who have low baseline levels of some of these antioxidants in their blood.

**Dr Serhan:** So the original source is a marine extract?

**Dr DeMichele:** We use a well-qualified fish oil in our products here. We have our internal quality assurance system. It is processed, so everything is protected. It is not in our laboratory setting. It is more of a commercial processing-type setting.

**Dr Serhan:** Right. The reason I asked that is because of the considerable concern in the literature about the fatty-acid composition of different fish populations, depending on where they are from—concerns about whether they are farmed and
Discussion

about what they are eating. If they are eating chicken, you have a different fatty-acid composition.

However, I do want to make a statement relative to my own interests and what I presented. What we have learned with time is that we really like to see use of agents (ie, drugs) that stimulate resolution, and I hope I was able to make that point. Likewise, a number of groups have shown that many clinically used agents are resolution toxic. Several of them are resolution friendly. One group in the UK at Edinburgh shows steroids stimulate macrophage phagocytosis and cleanups.

If we want to think about preventive therapeutics, we still need to think about ways of avoiding that background, because it could confound data sets if we are taking something that is “resolution toxic.” We may not recognize that within the population.

Dr Stenvinkel: I have a question for Dr Make. I am intrigued by the similarities that seem to exist between patients with COPD and congestive heart failure and dialysis patients with regard to the protective effect of high BMI.

Consistent findings also are found in the renal population with high BMI being protective for premature death. Several studies show that high, not low, adiponectin levels predict poor outcome in dialysis patients, perhaps a reflection of loss of fat stores during the process of wasting. However, an intriguing paper in Nature Medicine [Qi Y et al. Nat Med. 2004;10:524-529] showed that if you give intracerebroventricular adiponectin, energy expenditure increases markedly. The mice lose body weight, suggesting that the adiponectin also may serve as a driver of wasting and the wasting process.

What is the current status of adiponectin as a predictor of poor outcomes in your COPD patients?

Dr Make: That is a great question. I will put it on my list.

Dr Greenberg: Have you looked at myostatin? I would put it pretty high on my list, more so than adiponectin. You have essential adiposity, right? So myostatin is a really good candidate.

Dr Stenvinkel: That is quite different in renal patients, because adiponectin is retained due to reduced renal clearance.

Dr Greenberg: That is a different thing, a good point. Everything is changed in renal patients because of the renal excretion.

Dr Jialal: Just measuring total adiponectin is not sufficient because we have circulating trimers, hexamers, etc. It is not an easy molecule to measure and use as a biomarker. I do not agree that the ratio of high molecular weight to total adiponectin is the best biomarker in regard to adiponectin and just measuring total adiponectin. You better use caution. In renal disease, it is elevated. So everything is elevated.

Dr Stenvinkel: Volume retention seems to promote adiponectin retention as brain natriuretic peptide upregulates this hormone. The relationship between adiponectin and volume status and renal function in our patients is complicated.

Dr DeMichele: We heard about how obesity is related to inflammation. Dr Make and Dr Szefler, what role does obesity play in COPD or asthma in terms of the induction of it or with lung function? The lung is unique, because it is exposed to the external environment, to all these inflammatory triggers. How does obesity play a role in either disease?

Dr Make: I was going to surmise that the other 40% of the cells that Dr Lee injected went to the lungs.

Dr Lee: I would say that is correct.

Dr Szefler: Obesity is considered a comorbid feature of asthma. Dr Smith’s question relates to asthma, as well. We would find that population statistics have shown the increasing proportion of obese asthmatics is similar to the increased proportion that is seen in the general population. Obesity plays a role in asthma management, because you are adding body mass and restricted lung volumes. It is going to play a role in terms of symptom management. It is controversial as to whether or not the proposed inflammation related to obesity enhances the inflammation in asthma. Nothing I have seen to date substantiates that, either as an origin of asthma factor or contributing to worsening inflammation.

However, it is definitely a factor to deal with in terms of asthma management, because the patients who are more obese are going to exercise less and thus become more sedentary.

Dr Make: I think you can hypothesize that macrophages are increased in obese patients or overfed mice. They are a key player in driving inflammation in COPD, and these cells may wind up in the lung and increase inflammation. That is a hypothesis. I have no information, but it sounds plausible.
**Dr Jialal:** Pickwickian syndrome we learned about in medical school—obesity with lung disorder. What kind of lung disease do they get?

**Dr Make:** We traditionally have placed obesity without airflow limitation in the category of a restrictive respiratory system disease because of its effects on the chest wall and restricting lung expansion. However, we should consider obesity a lung disease associated with inflammation, with or without COPD or asthma.

**Dr Jialal:** Not emphysema.

I want to make a cautionary note about beta-carotene. I notice that in your supplements you use beta-carotene. Beta-carotene was shown to increase lung cancer in smokers in the Beta-Carotene and Retinol Efficacy Trial (CARET) and the Alpha-Tocopherol and Beta-Carotene (ATBC) study.

**Dr Hawley Linke [Abbott Nutrition]:** I think we had a discussion a couple weeks ago about moving these formulas from one area to another. We are going to need to watch for what the essential components are and what are not. The one question we have, depending on the study, is whether or not to include vitamin D, if it is not already in there. When you get to a formula, you are going to need to tease down the individual components and say what is potentially helpful and what is not. It is an interesting area.

Dr DeMichele also had mentioned that just providing this type of nutritional supplement may actually have a satiety factor and perhaps even result in prevention of obesity, in terms of having necessary calories—good calories rather than bad calories.

**Dr Jialal:** My caution was with regard to COPD, because I was on the panel for the Institute of Medicine, and we came down very strongly against recommending beta-carotene in smokers.

**Dr Matthew Kuchan [Abbott Nutrition]:** I am interested in whether you have either contemplated linking, or have actually linked, dietary influence on your factors of interest in young infants or animals to the later risk of asthma.

**Dr Serhan:** The answer to the first question is easy. No, because the work that I presented was conducted just in animal studies. The first real trial of principle has just gone into phase 3 for ocular inflammation. All those questions linking dietary intake in terms of level of the pathway are ongoing.

**Dr Szeffler:** Unfortunately, that is not a good animal model for the natural evolution of asthma. The animal models are excellent tools to examine inflammation pathways. The animal models used to mimic asthma really are allergen-induced airway inflammation models and do not necessarily reflect the chronic inflammation associated with the natural course of asthma.

Good ways of doing an animal study that would support this in terms of altering the evolution of asthma do not exist.

**Dr Serhan:** I agree entirely. It is useful for mechanistic studies to get good ideas about which direction the air flows in, but only to provide the information necessary to get the work done in humans. That is why we look for translation as much as possible.

However, the type of questions that you are asking really are related to the root question in respect to essential fatty acids. Knowledge about the minimum daily requirements does not exist. We need to have that base level to start with, then we can start to think about tissue and organ distributions and challenge provocations in different populations. I am sure these differ significantly among children around the world.

**Dr Kuchan:** Do you believe there is any credibility in the literature that links increasing rates of Cesarean deliveries and the impact of Cesarean deliveries on the infant microbiota to the increasing incidence of allergy and asthma?

**Dr Szeffler:** Most of the studies are epidemiologic, poorly controlled, cross-sectional, and not prospective studies. They are all hypothesis generating. The available literature is thus provocative, but not confirmatory.

**Dr Make:** A good target for basic research?

**Dr Szeffler:** In the right model.
Structured Panel Discussion

Leader: Refaat Hegazi, MD, PhD

Dr Hegazi: In this panel discussion, I want to connect the dots a little. It is amazing that we have seen so many different diseases and organs, but I think we share a common background.

However, do we have a definition of inflammation yet? Do we have the same definition of inflammation as pathologists? They will say this is acute inflammation based on what they see. Probably they will look at leukocyte infiltration, and the type of infiltrating cells that they see will define whether it is acute or chronic. If they see more plasma cells and lymphocytes, they will say it is chronic inflammation. However, what we have been talking about for the last 2 days would have us define it as a different type of inflammation—probably systemic inflammation, regardless of the origin of the injury.

As a result of our conference, is there a need to better tune this kind of clinical definition of inflammation vs a pathological definition of inflammation? How can nutrition intervene with the inflammation, systemic inflammation vs acute and chronic inflammation? This is what interests us as nutrition experts and others working in the field of nutrition. I would like to get opinions from different disciplines, probably starting with the gastrointestinal perspective.

Dr Sartor: You are posing more philosophical questions rather than purely scientific ones. If you ask me my definition of inflammation, I think that it is both pathologic and immunologic. By histologic definition, it is an infiltration of several types of leukocytes, which are both innate and at times adaptive in nature, combined with activation of a profile of cytokines and other mediators that are characteristic of activated effector immune cells.

I would not pin myself down to say tumor necrosis factor (TNF) or interleukin (IL)-1 are universally stimulated, because each type of inflammatory process is associated with a characteristic profile that may be different in each situation. We need to think about inflammatory processes in an organ-specific way, as well as the systemic manifestations of either an organ injury or inflammation, and perhaps a more systemically mediated condition that may have no single-organ defining characteristic. However, TNF and IL-1 belong to fairly central immunologic pathways that are conserved in most inflammatory processes, regardless of the organ involved. Enhanced mucosal permeability from gut injury/inflammation leads
to leakage of bacterial products into systemic circulation, which activates cells to secrete cytokines. This can then stimulate secondary inflammatory responses in downstream organs, such as the liver, and spill over into the systemic circulation.

Dr Hegazi: I probably should restate my question. If we have an inflammatory bowel disease (IBD) patient who has comorbidities, such as obesity or insulin resistance, do we focus on the primary gastrointestinal (GI) disease and GI inflammation or take care of the superimposed systemic inflammatory response, in addition to the GI source of inflammation?

Dr Sartor: I think the answer is whether the systemic inflammatory response is viewed as an independent or secondary phenomenon. If it is a secondary phenomenon, then you have to attack the underlying problem, which in this case is the gut-specific, organ-specific disease. That is merely the portal of entry for all those systemic manifestations. Then you treat the systemic manifestations by interrupting that target inflammation.

On the other hand, if you have a type 2 diabetes patient who has Crohn’s disease or ulcerative colitis, then you obviously need to think more globally and treat both processes. Now, it will not hurt to treat the systemic manifestations while you also are treating the underlying process, but in theory, you could selectively treat the underlying process if the systemic manifestations are secondary.

Why do we not see systemic inflammatory processes in IBD more often? A subset of our patients is obese, but they do not necessarily have type 2 diabetes. Why do we not see more of the systemic metabolic syndrome in that setting? Many of the cytokines are the same. Macrophages are activated. It is more of an M1 macrophage profile, but clinically I do not see metabolic syndrome often. We see fatty liver quite a bit, probably with steroids, as well as malnutrition, but rarely to the point of steatohepatitis with a fibrosing complication—the true nonalcoholic steatohepatitis (NASH).

Dr Hegazi: With respect to renal disease, when a patient has comorbid conditions, such as acute renal failure or chronic kidney insufficiency in the setting of diabetes, which one do you focus on more, the diabetes and glycemic control or the chronic renal insufficiency-related inflammation?

Dr Sartor: Renal disease is complex. If we exclude those cases of localized renal inflammation, which is usually associated with nephritis, we have systemic inflammation. That is the type of inflammation we are mostly interested in as it predicts poor outcome.

In chronic kidney disease, we see a very complex situation because it seems like decreased renal function may increase inflammatory activity. Although as I said previously, some patients, even those with advanced chronic kidney disease, may have completely normal C-reactive protein (CRP) levels. Of course, comorbidities are the most common course of inflammation, and many of our patients indeed have diabetes.

It also seems that volume status is important. Usually you find more inflammation in patients who are volume overloaded. Infectious complications also are common in this patient group. Then we have the third course, which is the specific treatment, dialysis treatment, which adds on inflammation to the impact of chronic kidney disease and comorbidities.

The major problem we have in the dialysis patient is that CRP is a moving target, with CRP levels varying considerably. We just completed a study in which we evaluated CRP levels every week for 3 months in 228 hemodialysis patients. CRP levels could vary 100-fold in each patient during this short time period.

So, it is extremely hard to interpret CRP levels in our patients. We need to follow longitudinal CRP escalations to get some kind of an impression.

Dr Hegazi: Do you think that nutritional intervention with an anti-inflammatory module can help us break the inflammatory cycle in these patients?

Dr Stenvinkel: I believe so. Some small studies, including the one I described, seem to suggest that it is indeed possible to decrease inflammation by lowering the proinflammatory status in these patients with nutritional intervention.

Based on CRP variability, we made power calculations. Because of the wide range of CRP in individual patients, we will need large studies to be able to show effects in randomized controlled trials, probably 300 to 400 patients in each group.

Dr Make: The hypothesis is not yet definitively proven. I am not even sure it is a reasonable hypothesis to make—that the inflammation of the lungs because of chronic obstructive pulmonary disease (COPD) spills over to the rest of the body and the rest of the organs. It is proposed, with little convincing data thus far, that cardiovascular disease, osteoporosis, depression, and many other disorders commonly seen in COPD are the result of inflammation that starts in the lungs.

If the hypothesis were true, I would agree with Dr Sartor. You could target the lungs, rather than target some other organ, to reduce the inflammation. However, targeting a specific organ such as the lungs assumes that we can do that—target
With regard to other complications, for example, macrovascular disease such as cardiovascular heart disease, stroke, or peripheral arterial disease, the only supplements accepted by the American Heart Association and American Diabetes Association are sterols and stanols—margarines and orange juice. Dietary supplements of stanols and sterols are accepted in that regard, and lipoic acid is accepted for diabetic neuropathy. I am not aware of any supplement for diabetic nephropathy, but I may be wrong about that. For heart failure, you have coenzyme Q. Studies and meta-analyses have shown a possible benefit with use of coenzyme-Q supplementation for patients with and without diabetes [Singh U et al. Nutr Rev. 2007;65(6 Pt 1):286-293].

Dr Hegazi: The second topic I want to discuss is the lack of a clear definition for sarcopenia, cachexia, and weight loss in diagnosing malnutrition. Which definition should we adhere to? Dr Jensen, you mentioned trying to define malnutrition in the setting of sarcopenia, and how a patient’s weight might not change, but he or she still might have sarcopenia. Can you elaborate on that?

Dr Jensen: I hope to spend more time talking about diagnosis of malnutrition during the wrap-up session tomorrow. I thought it was interesting that among our conference attendees we probably could have come up with at least four definitions for cachexia just in this room.

We actually did attempt to address some of these issues in a paper that came out in the Journal of Parenteral and Enteral Nutrition in December 2009 in which we attempted to redefine historic malnutrition terminology on the basis of a modern understanding of inflammation [Jensen GL et al. JPEN J Parenter Enteral Nutr. 2009;33:710-716]. We suggested that cachexia was erosion of body cell mass in the setting of chronic inflammation, that marasmus was pure starvation without inflammation, and that protein-calorie malnutrition was associated with more severe inflammation of acute disease or injury. People are so invested in the historic terminology and the baggage that went with it that it has proven difficult to move forward. This is part of the rationale behind our new etiology-driven definitions that encompass malnutrition in the setting of pure starvation without inflammation, malnutrition in the setting of chronic disease with mild to moderate inflammation, and malnutrition in the setting of acute disease or injury with severe inflammation.

The beauty of such a simple, etiology-driven construct is that it is very simple. It does not require a high level of clinical assessment skills and is very practical. A patient can fall into one of these categories and move to another. They can fall into two at the same time. If a patient is in chronic renal failure with chronic smoldering inflammation and has a new, acute superimposed infection, he or she has acute-on-
chronic inflammation. Another example would be end-stage COPD complicated by acute pneumonia. Iatrogenic malnutrition complicating chronic or acute disease will add a starvation component to inflammatory response.

This approach also easily accommodates sarcopenia and acute events superimposed on sarcopenia. Sarcopenia is likely a smoldering, inflammatory state, whether it is a sarcopenia of aging or obesity [Jensen GL. JPEN J Parenter Enteral Nutr. 2008;32:656-659; Jensen GL, Hsiao PY. Curr Opin Clin Nutr Metab Care. 2010;13:46-51], so we would generally place these entities in our category of malnutrition associated with chronic disease.

I think the sarcopenia construct is very useful, and so is that for cachexia. The problem is that the words carry such mixed meanings for so many people. In our suggested new approach, cachexia is abandoned in favor of malnutrition in the setting of chronic disease and sarcopenia is simply incorporated into whichever of our new diagnostic categories is appropriate to a given patient.

Dr Hegazi: I think the point is that we want to move it, as Dr Sartor said, from a philosophical point of view to something we can use as clinicians. First, is my patient malnourished? Is my patient sarcopenic or losing lean body mass? Is my patient inflamed? Is the inflammatory state acute or chronic?

By doing this, we can start building research that can meet these different layers of patients. Nutrition and inflammation are great discussion topics at this time, at this stage, and with this philosophical point of view, but we have to move on to something we can give the medical resident. We can say, for instance, “If you have a patient with this parameter and that parameter, this is a patient with acute sarcopenic inflammation whom you might target with certain nutritionals or drugs, in addition to antioxidants or anti-inflammatories.”

Dr Sartor: We have heard at least three different groups make the plea—the way to move forward is individualized, personalized medicine. We most likely are not going to come up with a formula that is going to work in every situation. It will take larger trials to ferret out which patient is in the appropriate group, but in the long run we are much better for targeting the correct therapy to the correct patient the first time, rather than making it the luck of the draw.

I am glad to hear you say you are approaching it with that mind-set, but I think this point needs to be emphasized. We heard it in the gut, we heard it in inflammatory response, and we certainly heard it in lungs and kidneys. Where we need to move forward is with individualized medicine.

The beauty of a malnourished patient is that you probably can measure some parameters that would actually give you guidance as to what you might need to replace.

Dr Hegazi: This approach of personalized medicine would certainly help determine who might need nutrition replacement and how nutrition intervention could result in successful clinical outcomes.

Dr Anne Coble Voss [Abbott Nutrition]: In the discussion earlier today, we were separating in some ways the concept of providing calories and protein, and providing the anti-inflammatory ingredients, some of the fatty acids, and the antioxidants.

One of the investigators who I work with often talks about this concept in patients who have severe weight loss, whether it is because of cancer, COPD, or other similar conditions that involve inflammation, saying that no spontaneous generation of matter exists. You can downregulate the inflammation, but you also have to provide the protein and calories for the patient to gain weight. I am interested in your views in that area.

Dr Make: A third component exists, which many of us have discussed during the past 3 days—simultaneous use of an exercise training program. In COPD, we are very concerned about providing therapy to increase patients’ weight without improving their muscles at the same time. If the ultimate goal is to improve functional capacity for daily activities in COPD patients, the optimal approach might include providing: 1) therapy to reduce inflammation, 2) therapy to increase weight, 3) adequate calories, and 4) adequate muscles by adding a simultaneous exercise program.

The prospect of developing a series of different kinds of products for different purposes is very reasonable with adequate research and development funding. Maybe you have different types of products for different types of patients based on their body mass index, muscle mass, and need for either weight gain or weight loss. I could see many different modules.

What if you have a pharmacist involved who mixes a physician-ordered module from larger bottles containing individual components, similar to compounding that was done years ago. For example, you could mix 20 mL of potion A and 15 mL of potion B. You could decide what was best for each patient and make the appropriate mixture.
Dr Jensen: The question is a good illustration of why it pays to not think about one size fits all. The acute critical care situation is a great example. There is likely a threshold of protein and calories that should be met to achieve optimal outcomes, but there is also a priority to deliver key micronutrients to preserve vital immune and wound-healing functions and to support organ system functions. Some critically ill patients are so proinflammatory that they will continue to lose muscle protein in the face of quite appropriate nutrition support, and there is little to be gained from excessive protein administration. On the other hand, in the chronic inflammatory conditions that we have spent much time talking about during the conference—lung disease, renal disease, obesity, etc—there is obviously a need for long-term, sound nutrition. In an interesting example, we have research with community-dwelling obese, older people that suggests that they are often, and not for want of food energy, in fact consuming such poor-quality diets that they have multiple micronutrient deficiencies.

Our goals should be to tailor nutritional interventions to the pathology, to the individual phenotype, and when the option becomes available, even to individual genotype.

Dr Sartor: In certain situations, we need to consider the formulation and how we are going to deliver it, as well. In the special situation of inflammatory bowel disease, 85% of Crohn’s patients lose weight. It is not malabsorption; it is usually fear of eating because of pain and/or diarrhea after meals. So, we need to create a vehicle to give calories, micronutrients, and protein, and maybe beneficial substrates for eicosanoids in a formulation that does not induce abdominal pain. This may be a little different from COPD.

I was struck with COPD, where decreased appetite was a poor prognostic sign. I know in cystic fibrosis that is a bad sign. Certainly in IBD our treatment is compromised in people who cannot eat. You need to think about exactly what volume and the way you put things together, and make sure that whatever you do does not induce diarrhea in the process, when you have gut inflammation as kind of a baseline.

Not always, but with statistically significant frequency, we can put people with active small bowel disease into remission. Yet the formulations are such that people are not willing to take them either because the diets taste terrible or some of the standard formulas might cause diarrhea. We have to resort to tube feedings, which most adults will not buy into, for both cosmetic and discomfort reasons. Children frequently agree to it if they have growth retardation, because we can guarantee them they are going to grow in the process.

I had a couple of patients whom I convinced to go on an exclusive oral diet supplement for 1 month—completely NPO (nothing by mouth) regarding other foods. Both did exceptionally well, but flared when they went back on regular diets.

The solution is to make the supplement palatable and without diarrhea side effects, and replace what the patient really needs, with the hometown pharmacist dialing in whatever is necessary, based on the analysis of the week. However, I am not sure this is practical.

Dr Hegazi: You can have the best science in a nutritional formula, but the patient has to like it first.

Dr Linke: We have just now talked about micronutrients and what people are missing, presumably some obvious things, such as fatty acids, tocoherol, and vitamin D. Given the anecdotal information that physicians still are not testing for vitamin D at a time when the lay press is full of this, what do you see in practice? What important insufficiencies exist in terms of getting a baseline on these major micronutrients? What would it take to get the rest of the community to do that?

Dr Szefler: I would say vitamin D is a good prototype for that, because we are taking more measures of vitamin D in the clinical setting now than ever before. In the allergy area, it started out in eczema. We monitor vitamin D routinely, not so much in asthma. In family practice now and in terms of risk for osteoporosis, it is almost always measured routinely and supplemented. It is kind of a test pilot for how these measures of potential nutritional deficiency will receive growing attention in clinical practice.

Dr Jensen: I would just add that in my clinic I frequently see referrals for patients with low 25-hydroxyvitamin D levels. Many of these referrals are driven by hematology-oncology practitioners who are ordering these levels because of all the information that is coming out about possible anti-inflammatory and potentially anticancer properties of vitamin D.

Vitamin D requirements remain a matter of considerable controversy. The Food and Nutrition Board of the Institute of Medicine is currently evaluating vitamin D requirements and the committee will release its finding soon.

Dr Jialal: I think in endocrinology, we are very interested in osteoporosis, as well as calcium and vitamin D, the first line before the bisphosphonates.

I would like to make two cautionary points. One, we have traveled down this road with beta-carotene and vitamin E. The epidemiology with vitamin D is very exciting.
I am not a vitamin D expert. However, in my view and from my reading of the literature, randomized clinical trials of benefits with vitamin D, other than for osteoporosis, are sadly lacking. We and others show that vitamin D is low in people with diabetes. We show it correlates with inflammatory markers such as toll-like receptors and CRP.

However, the big question is about giving vitamin D in sufficient doses to reduce parameters and reduce endpoints. Time will tell, but we do not have strong evidence in that regard. The measurement of vitamin D also is questionable. There is an immunoassay for measurements of vitamin D in addition to high-performance liquid chromatography (HPLC) and various other methods, but measurements are not standardized.

Dr Szefler: Getting back to your previous question, it would seem that a nutrition-based company should have a base that looks at normal development, plus disease and development. The different diseases have different components, for example, inflammation-based and nutritional deficiency.

You are trying to dampen the inflammation, plus restore the nutritional factor. I would think that when you get an element of disease, it is going to impact biologic pathways in different ways.

A nutrition-based company can move forward into the future by having subdivisions, similar to the way a hospital does, with certain groups focusing on certain diseases. Some commonality may exist in some of the diseases, but it is necessary to really understand them in depth and then educate clinicians and their patients. Otherwise, the physician is trying to serve as the common denominator to understand these various disease pathways and intervene appropriately. We are hoping for aids and organization of information in regard to a nutritional approach to chronic disease management.

I would think from your end, you kind of started out with infant formula, which is similar to human milk in some but not all ways. You were trying to mimic milk, but now you are trying in a way to look for the nutritional deficiencies and make sure you are restoring normal growth. However, you also are trying to play a role in dampening inflammation, which may play a part in early disease development. It is a challenge, but it is an opportunity to really move forward.

Dr Hegazi: I think we should share as a community, not just as an industry. I think as key opinion leaders and experts, we also should talk about how to transmit what we know about the nutritional knowledge that we have now that was not available 10 years ago beyond the group of experts who already have this knowledge. Recent literature, for instance, suggests the benefits of enteral over parenteral nutrition in patients with severe acute pancreatitis. However, only a few centers practice enteral nutrition. Instead, they use easy-to-administer total parenteral nutrition. Maybe we need to transmit this message beyond the experts’ circle and then we need to improve and refine our definitions and biomarkers.

Dr Stenvinkel: In the renal community we have learned that vitamin D deficiency is extremely common in renal patients. A randomized, controlled trial shows that we can reduce albuminuria as an add-on to renal antioxidant treatment in patients with chronic kidney disease. It is interesting that vitamin D analogues seem to be able to reduce albuminuria, which may be a risk factor for cardiovascular disease progression in renal patients.

Dr Hegazi: Other studies show that a 6-month intervention with vitamin D helps prevent albuminuria. Clinical trials are coming, so it is just a matter of wait and see. As for vitamin D, I think it is probably 5 years until we may say that it is not good for heart disease or something. It will take that amount of time until we can assure safety.

Dr Sartor: In order to really enthusiastically embrace measuring and replacing vitamin D, we need to see that it improves outcome. We know statistically vitamin D levels are low in IBD, but that does not necessarily mean that replacing it makes treatment better.

Dr Hegazi: In discussing the role of nutrition in IBD especially, we have to remain humble in the outcome we are looking for. If we say vitamin D replacement or supplementation will improve histological scores of colitis, maybe not. However, we may claim that vitamin D will relieve vitamin D deficiency.

Dr Sartor: If that impacts long-term osteoporosis, this is important. A reasonably large number of Crohn’s patients, particularly those with a subset of liver disease, have marked osteoporosis, particularly those on steroid therapies. We need to think about some beyond-the-disease activity, with implications for the whole body.

Dr Hegazi: Dr Lee, when you presented your studies of obesity, you always used the high-fat diet model. What defines a high-fat diet? What is the percent of calories, and what is the type of fat?

Dr Lee: Typically, several courses of variable high-fat diets are available. It depends on the company. What we are using is 60% of the high-fat diet, which means 60%
of the calories come from a high-fat diet. It turns out that the source of the fat also is important.

Some companies are using coconut oil and others use lard. Different sources of fat provide different types of lipids in animals, and certain lipids are more potent for inducing insulin resistance than others. In general, the high-fat diet prepared from lard increases many circulating proinflammatory mediators, but the increase is less when the diet is prepared from coconut oil.

Many labs are conducting experiments using different high-fat diets and, therefore, their results would be different. This is always a caveat in terms of animal models using high-fat diets. Diet-induced obesity (DIO) models in animals have their own problems. For example, in animal models, obesity is increased dramatically in a short time, while development of human obesity is a slower, more progressive process. So when we compare obesity-induced inflammation, we may not look at the same kind of inflammation. We do not always have a perfect animal model for human disease; however, I would say that the DIO model is the best we can come up with at this moment.

Dr Jialal: One statement confuses the situation. We thought that studies on the effects of vitamin E on heart disease were going to be a slam dunk, including the work we did in Dallas on the antioxidant effects. The data on heart disease are all over the place. At best, it is a null effect. Then a paper came out in the New England Journal of Medicine that shows a clear benefit of vitamin E, 800 units/day, against the disorder of oxidative stress and inflammation, showing that vitamin E prevents fibrosis in patients with early fatty liver disease [Sanyal AJ et al. N Engl J Med. 2010;362:1675-1685].

Dr Hegazi: I agree with you. That is what we are looking for—clinical interventions or trials. It is exactly what we highlight—the timing and duration of the study. We need to study the different effects of nutritional intervention depending on the progression of the disease process.

Dr Jialal: I refer you to the recent study I just cited that showed a clear benefit of vitamin E, a benefit that was superior to that of pioglitazone [Sanyal AJ et al. N Engl J Med. 2010;362:1675-1685].

Structured Panel Discussion

Nutritional Effects on Inflammation and Infectious Disease: Is There a Role for Specific Nutrients?

Melinda Beck, PhD

Interest in looking at host nutrition for the ability to modulate the inflammatory response is not a new idea. Numerous studies have shown that dietary long-chain polyunsaturated fatty acids (PUFAs) derived from fish oil have beneficial effects on chronic inflammatory and autoimmune disorders.1,2 Chronic diseases such as rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, cardiovascular disease, type 2 diabetes, and Alzheimer’s disease all benefit from a fish oil-enriched diet.

Studies with fish oil suggest that it acts to reduce inflammation by reducing T-cell proliferation, activation, and signaling, reducing natural killer activity and decreasing antigen presentation functions. Inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-2, IL-6, and interferon-gamma (IFN-γ) are all reduced when diets enriched with fish oil are fed. Although a decrease in inflammation is desirable under conditions of inflammatory disease, it is important to keep in mind that inflammation is a necessary component of the host response to infectious disease and may prove deleterious during an infectious process. Indeed, research in our laboratory has demonstrated that feeding fish oil during infection with influenza virus results in a poor pathological outcome and increased death in the group fed fish oil vs the group that was not fed fish oil.

Other nutrients that are important in a functioning immune response include micronutrients, such as vitamin E and selenium. Both vitamin E and selenium function as antioxidants, although by two very different mechanisms. Antioxidants are associated with providing cellular protection against inflammation. Increased oxidative stress is associated with chronic obstructive pulmonary disease (COPD), asthma, renal disease, Alzheimer’s disease, and other chronic diseases. Many studies in both humans and animal models have tested the effects of antioxidant nutrients on reducing inflammation. Specific antioxidant nutrients are important for reducing oxidant radicals and thereby reducing inflammation and limiting oxidative damage, but they also play important roles in viral adaption to the host. Our laboratory has found that deficiencies in specific micronutrients will result in viral mutations, using both animal models and a human population.
Dietary Fish Oil: Inflammation and Influenza Virus

Favorable effects of dietary fish oil stem from its ability to reduce excessive inflammation. A number of chronic diseases are related in part to uncontrolled inflammation, including cancer, obesity, Alzheimer’s disease, and cardiovascular disease. A number of studies in both humans and animal models have demonstrated that dietary PUFAs found in fish oils can modulate the progression and development of a number of chronic diseases. Recently reviewed by Wall et al, PUFAs are now used to treat patients with rheumatoid arthritis, inflammatory bowel disease, Alzheimer’s disease, and cardiovascular disease. Studies have had mixed results, with some studies showing a positive outcome and others showing no effects.

One overlooked aspect of using nutrition to reduce inflammation in order to treat a chronic disease is the effect on the immune response that is possibly necessary to fight infection. Inflammation is used by the host during an infectious disease process in order to fight infection. By dampening down inflammation in order to treat a chronic illness, the host may have less ability to fight infection and therefore become more susceptible to infections. To test this hypothesis, we utilized a well-established mouse model of influenza virus infection.

Although vaccination is available for influenza virus, influenza is continually a major cause of morbidity and mortality worldwide. Infection with influenza virus results in an acute and diffuse inflammation of the bronchoalveolar tract, from both an innate and adaptive immune response that is utilized to eliminate and prevent/limit viral spread. Inflammatory cytokines produced by cells of the immune response are critical to this response. What happens to this antiviral response if a diet containing fish oil is fed during an influenza infection?

We separated mice into two groups, feeding one a semipurified diet containing corn oil as a fat source and the second group a physiological level of a diet of fish oil—corn oil for 2 weeks. The amount of fish oil fed to the mice was comparable to that of humans who had a diet in which fish was consumed two times a week. Following 2 weeks on the diets, mice were infected intranasally with influenza virus A/Puerto Rico/8/34. Mice fed the diet containing fish oil were slow to recover lost weight (a marker for illness severity) and had a high mortality rate, compared with the control-fed mice (Fig 1). Although the lung pathology was improved in the mice fed fish oil (because of reduced inflammation), viral load was increased, demonstrating that inflammation under conditions of infection is a necessary response.5

Selenium Deficiency: Inflammation and Infection

Selenium (Se) is a trace mineral that is an essential component of a number of proteins, including glutathione peroxidase (GPX), glutathione reductase, and thioredoxin reductase. It is considered to play an essential role in antioxidant protection because of its incorporation as selenocysteine into antioxidant enzymes. Its central role in cellular antioxidant protection has led researchers to look at its effects on cancer, lung function, hypertension, coronary artery disease, diabetes, and aging. Studies have demonstrated that Se supplementation may benefit individuals with specific cancers, hypertension, and cardiovascular disease, although not all studies show an effect.

Reactant oxidant species (ROS) are highly reactive because of the presence of an unpaired valence shell electron. Often resulting as by-products of oxygen metabolism in the electron transport chain and by activity of cellular enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, ROS are capable of damaging lipid membranes, cellular proteins, and DNA. Although crucial for cell signaling, overproduction of ROS plays a role in aging, hypertension, atherosclerosis, diabetes, cancer, and neurodegenerative diseases. Most of these chronic diseases have an inflammatory component, and ROS are key components in the signaling processes leading to the release of inflammatory cytokines.
Se can lead to reduced inflammation because of its role as an essential component of antioxidant enzymes. Therefore, adequate Se nutriture is important for protection against excess ROS and subsequent inflammation. In addition, increased ROS and inflammation that occurs during Se deficiency can lead not only to problems with host cell function, but during a viral infection, can lead to changes in the viral genome. Using an animal model, we demonstrated that a deficiency in Se can lead to specific changes in the genome of coxsackievirus and influenza virus, causing a change in virulence from a mild pathogenicity to severe pathogenesis.6-8

As an example, mice were fed a diet either adequate or deficient in Se for 4 weeks. Following the 4-week feeding period, Se status was assessed by measuring the levels of GPX activity in serum, as a biomarker for Se. Mice fed the Se-deficient diet had 10-fold less GPX activity compared with mice fed an adequate diet. Therefore, these mice had impaired antioxidant function. Following the feeding period, mice in both groups were infected with influenza virus. Mice fed the Se-deficient diet were more susceptible to the effects of the Se-deficiency, with increased inflammation occurring in the lungs of Se-deficient mice (Fig 2).

Fig 2: Representative lung histopathology of a Se-adequate mouse (A) or Se-deficient mouse (B) inoculated with influenza A. Sections were stained with hematoxylin and eosin. X40.

Along with the increased inflammation and lung pathology, the Se-deficient mice had increased lung viral loads. In this case, the increased inflammation resulted in a less-efficient immune response against the viral pathogen. In further characterizing the immune response of the Se-deficient mice, we found that the Se-deficient mice produced more IL-10, IL-13, IL-4, and IL-5 and less IL-2 and IFN-γ compared with Se-adequate mice, suggesting a skewing toward a T helper 2 (Th2) rather than a Th1 response in the Se-deficient mice. In addition, Se-deficient mice produced increased levels of inflammatory chemokines, which likely contributed to the increased inflammation in the lungs of the deficient mice. Thus, Se helps to reduce inflammation, and the lack of Se can lead to dysregulated immune response.6

In addition to effects on the host, we also found that the virus itself was altered in the Se-deficient host. Sequencing of influenza viruses recovered from the Se-adequate mice compared with sequences of influenza virus recovered from the Se-deficient mice demonstrated specific nucleotide changes that occurred in the influenza virus that replicated in the Se-deficient host and were not represented in the virus that was recovered from the Se-adequate host. These mutations resulted in a change in virulence of the mutated virus, as passing the newly mutated virus back into Se-adequate hosts resulted in the Se-adequate host developing severe lung inflammation, similar to what was found in a Se-deficient host. Thus, the increased oxidative stress in the host cells may have facilitated the increased viral mutation rate in the Se-deficient host because of a lack of effective antioxidant protection.7

Human Studies

In the early 1990s, an epidemic of optic and peripheral neuropathy affected more than 50,000 individuals in Cuba. The disease was characterized by severe bilateral vision loss over the course of a few weeks, as well as burning of the hands and feet. Epidemiological data clearly associated the illness with host nutritional deficiencies, particularly of B vitamins, lycopene, vitamin E, and Se. The nutritional limitations followed the sudden collapse of trade relationships with Eastern Europe and the former Soviet Union in 1989, imposing acute shortages of foods and agricultural inputs, as well as fuel for food production, refrigeration, and transportation. A large hurricane and resultant crop failures exacerbated these problems. The illness was not identical with any known vitamin-deficiency syndrome and unexpectedly, coxsackievirus A9- and CA9-like viruses were isolated from cerebrospinal fluid of 86% of the neuropathy patients who were cultured.

Our laboratory fully sequenced CA9 viruses obtained prior to the nutritional limitations during the epidemic and then 7 years after the epidemic subsided because of the Cuban Ministry of Health distributing multivitamin supplements to the entire population. We found that a rapid and significant sequence divergence occurred between the strains isolated prior to the nutritional deficiencies and the strains recovered during the epidemic. The neuropathy-associated genotype persisted in circulating in Cuba
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7 years later, demonstrating its replacement of the previous strain. The extent of genomic divergence seen over a 2-year time period during the period of nutritional deficiency is unprecedented among enteroviruses.

It is clear that specific nutrients can influence host inflammation, either in a beneficial or deleterious manner, depending on the individual nutrients and the individual circumstances. Ongoing studies in our lab and in others are designed to determine the mechanisms behind nutrient modulation of inflammation, as well as their effects on both host responses and infectious pathogens.

References

Q & A

Q: What are your proposed mechanisms for this enhanced mutation rate? Is it that the virus hangs around longer in a place where it is replicated and therefore can mutate, or is it some feedback from the nutrient deprivation on the virus itself? Is a host problem a host milieu, or is it a direct effect on the virus?

Dr Beck: That is sort of what we are trying to focus on now. What we are looking at with the flu and coxsackieviruses are RNA viruses, so when they replicate and make a mistake in their genome, they do not have any enzyme to go back and fix it. They normally have a background level of mutation that is thought to have the ability to adapt for survival under different conditions.

When you sequence any RNA virus, what you actually are sequencing is the consensus sequence. RNA viruses exist as a cloud of viruses, called the quasispecies. Many more mutations exist in the quasispecies, but when sequencing, you only see the consensus sequencing. This is going to change with high-throughput deep sequencing, but for most studies of RNA viruses, the consensus sequence is what is reported.

What may happen is that under conditions of oxidative stress, the deficiency in vitamin E or a pro-oxidant stimulus, is that you have that virulent mutation already there in the quasispecies. Now you are selecting it, it outgrows, outcompetes, because it has a different condition that it is replicating in. It speeds up its rate, and you are pulling out something that already exists because you have changed the conditions.

The other possibility is that the oxidative stress actually is inducing changes in the genome of the RNA virus. The oxidative stress is damaging the viral RNA, giving you more chance to have more mutations.

What we are trying to do now is to use a high-throughput, deep sequencing, using Illumina sequencing technology, to do multiple sequencing in different organs over time to determine if we see the mutations suddenly appear, which would suggest that we are selecting for what is already there, or if we observe an accumulation of mutations that occurred over time.

When we did the initial sequencing, it was always at the time of peak pathology. So, we are sort of artificially selecting the most virulent genotype, but others may exist.
I do not think it is just totally selection, because occasionally you would see that virulent strain come up. If you pass a virus over and over again, you would think that eventually you would see that the virulent strain that was there would have a chance to outgrow. However, we never see that.

I think it is a combination of direct viral damage to the viral RNA, plus an enhanced selection process. I think the two are operating together, but we do not have the data yet.

Q: How much of a difference does the oil composition make? You sort of had a high and low fish-oil diet. Is there something intermediate? Also, what are the effects of the fish oil on changes in the virus?

Dr Beck: We did not look at that with the fish oil, so I do not know. You asked me about the amount of fish oil or the type? I collaborated with Philip Calder in England, who is an expert in fish oil studies, both in humans and animals, because I wanted him to give us what level we could use that was not pharmacological, but a physiological level. So, we got the numbers from humans, such as what you would expect if you ate two meals of fish during the week. Then we were going to try to match up those exactly—what the oils were in those fish—so we could go buy the individual oils. That became too expensive. We decided to just go with using the general fish oil, and then measure the specific oils in the fish-oil diet that we provided.

We have the data, but I do not have them here with me, to show the composition. It is pretty close to what we would have expected if we had initially added the individual oils. So, it is equivalent to, I think, two servings of fish a week (the amount that we added to the diets and fed to the mice).

I would like to go back and look at individual oils and say which one is important, because we just used the whole mix. Some are possibly more important than others for this effect. We do not know.

Q: Your fish oil experiment is really interesting. If we relate this back to individuals who consume a high amount of fish, such as in Japan, you would have high levels of fish-oil omega PUFAs in their blood. Do they have a different response to a viral infection? Are they more prone or susceptible?

Dr Beck: The only studies that were done to look at that, were looking at Inuits, and they have a really high tuberculosis (TB) rate. Nobody, as far as I know, has looked at influenza rates. The only infectious disease study with fish oil was the Inuits with the high TB rates, which may or may not relate to our study, but the proposition was that because of the high fish intake, it made them more susceptible to TB. However, not that many studies are done.
A Nutrigenomic Approach to Prevention of Inflammation

Henk F. J. Hendriks, PhD

Obesity has increased at an alarming rate over recent years and is now a worldwide public health problem. Proposals have recommended low-grade inflammatory status in people suffering overweight as one of the mediating processes in metabolic disease development. Several studies support a link between oxidative stress and inflammation in atherogenesis. Adipose tissue is crucial for the inflammatory status associated with obesity, primarily because of macrophage infiltration and subsequent secretion of both proinflammatory and anti-inflammatory adipokines. Reduced adiponectin levels and increased C-reactive protein (CRP) levels are associated with cardiovascular disease (CVD) and diabetes mellitus type 2. The established inflammatory marker CRP originates from the liver.

Reduction of the inflammatory status may prevent the occurrence of disorders and diseases related to overweight. The Mediterranean diet contains several of these compounds and is associated with a reduction of CVD and diabetes mellitus type 2. Expressed in nutrients, this means high contents of antioxidant polyphenols, vitamins, long-chain unsaturated fatty acids, and carotenoids. The present study’s aim is to investigate anti-inflammatory effects induced by nutritional intervention in overweight men with mildly increased CRP levels.

Low-grade inflammation, like many other effects, is difficult to assess in nutrition intervention studies. Such difficulty is caused by a number of factors. Usually, effects of nutrition are subtle and noticeable in the long term, rather than in the short term. In addition, effects are evaluated in a normal population, which is defined as apparently healthy, meaning that values of inflammatory biomarkers are in the normal range, so that changes in these values are hard to detect. Other confounding factors may include the interactions between nutrients and with other lifestyle factors. The relevant target tissue may further complicate evaluation because of its unavailability in human research.

The potential advantages of a new approach were investigated by applying multiple and complex biomarkers in a systems biology approach to evaluate the effects of a nutrient mix with the previously described physiological effects in inflammation. The effects were substantiated further by testing their efficacy on end points in an
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animal model and by projecting the effects in a “health space,” a new concept attempting to define health.

Potentially effective compounds were selected, aiming to cover a wide range of actions in reduction of inflammation. Polyphenols from green tea and red wine were selected. In addition, fish oil, vitamins E and C, and tomato juice were included in an anti-inflammatory dietary mix (AIDM). The effects of these dietary compounds were studied using a nutrigenomics approach.

Study Population and Methods
The study was a randomized, double-blind, placebo-controlled, crossover trial with four treatment periods of 5 weeks each. To demonstrate a difference of 1.4 mg/L for CRP, a sample size of 32 subjects was needed in order to detect a difference of 0.4 mg/L for adiponectin. The study started with 36 subjects, allowing for some dropout. The study is registered at clinicaltrials.gov as NCT00655798.

The men selected were generally healthy, having a body mass index (BMI) of 25.5-35.0 kg/m² and a low-grade inflammation as assessed by CRP of 1–10 mg/L. At week 4 of each treatment period, lymphocytes were isolated from blood. At week 5 of each treatment period, blood was taken for clinical chemistry and a range of inflammatory parameters and antioxidant markers, respectively.

Blood samples for the analysis of liquid chromatography-mass spectrometric (LC-MS) lipids and free fatty acids were taken after an overnight fast at the end of the 5-week dietary intervention. Blood samples for the analysis of LC-MS and protein profiling were taken before and after the high-fat load at regular intervals (time=0, 30, 60, 120, 180, 240, and 360 minutes).

The LC-MS methods used for analyzing plasma lipids and free fatty acids, as well as the gas chromatography-mass spectrometry (GC-MS) method used for analyzing a broad range of metabolites, were identical to the methods reported in Wopereis et al. Plasma samples were sent to RulesBasedMedicine Inc, Austin, TX, for measurement of expression levels of 124 proteins. RNA was extracted from lymphocytes and adipose tissue samples and hybridized on Affymetrix® Human GeneChip®. All data were analyzed for treatment differences using analysis of variance. Functional analysis of the data was performed in Ingenuity® Pathway Analysis (IPA®).

An established model for cholesterol-induced atherosclerosis in humans, APOE*3-Leiden transgenic mice, displaying a humanlike lipoprotein profile were fed a high-cholesterol diet, which induced atherosclerotic plaques that resemble human plaques in morphology and cellular composition, and AIDM.

Results
CRP and adiponectin were the main classical inflammation markers measured in this study. CRP levels did not change in response to the AIDM, whereas adiponectin levels increased significantly from 6.03±2.06 mg/L after placebo intervention to 6.48±2.57 mg/L after AIDM intervention (P<0.05). The effects of the AIDM were investigated in more detail by large-scale analysis of gene expression, proteins, and metabolites in blood, urine, and adipose tissue biopsies. The highest-scoring network in the analysis without the gene expression data illustrates effects of the AIDM on inflammation (immune response), oxidative stress (production of reactive oxygen species), and lipid metabolism (quantity of lipid). The network indicates a central role for transcription factor nuclear factor-kappa B (NF-kB) in the effects of AIDM. A more detailed biological interpretation of the data is described in Bakker et al.

APOE*3-Leiden mice, a model for hyperlipidemia and atherosclerosis, were used to study the effects of AIDM on end points such as atherosclerosis. Atherosclerosis development was studied in the aortic root, using histological evaluation and quantification. It was shown that mice that were induced to develop atherosclerosis and were given AIDM expressed dramatically less atherosclerosis in their aortic root (unpublished results).

The totality of all human data was integrated in a conceptual health space that depicted the subtle changes occurring in volunteers consuming AIDM. This multivariate model was built for the three processes, based on the significantly changed clinical chemistry, metabolites, and small protein data per process categorized as metabolic stress, inflammatory stress, and oxidative stress. The health space showed that each individual responded differently. Individuals would improve along one, two, or three of the defined axes of the health space, depending on their individual background.

Conclusion
In contrast to the accepted biomarkers, the application of nutrigenomics techniques for large-scale profiling of genes, proteins, and metabolites showed that the AIDM was able to influence processes of inflammation, oxidative stress, and metabolism.
Q: Can you tell us a little more about the supplement that was used?

Dr Hendriks: I have shown a little of the composition, but nothing of the concentration used. The choice of the component was based on literature data describing its influence on inflammatory pathways. The mix was composed in a way that would optimally affect inflammation based on theoretical grounds.

As far as the concentrations are concerned, we generally exceeded the regular recommended daily nutritional dose, which was below the tolerable levels indicated by the Dutch Health Council.

Q: I have a question about quality of life, because you were talking about health space. What was going on with these men in terms of what you measured with respect to psychological and social stress? How does that relate to dietary intake and some of those outcomes? Do you have information on that?

Dr Hendriks: No, unfortunately we do not have that information. I would find it really interesting to look at how a most stressful situation would have affected these outcomes.

In the original picture that I showed you, we had four areas of interest. Not all four of these four areas were specified in the health space. The fourth area was neurological stress. Maybe we should include in the health space an additional axis on neurological stress. However, we may have extreme difficulty with the evaluations, because stress and quality of life are not easy to evaluate. I think we need very structured questionnaires in that area and new ways of asking people about how they feel. We cannot just ask them if they are basically happy, which usually happens when people evaluate quality of life. I do not think that is really sufficient to get a full grasp of how they are doing in the short term.

Q: We talked about the need with the EFSA (European Food Safety Authority). Do you have any insight? Is this the type of data that you will need to get a health claim passed?

Dr Hendriks: That is difficult to say at this moment, because the EFSA probably will go for the classical way—when you have a biomarker that is established and validated in long-term studies. I think if we can show nice relationships between those pathway changes and the biomarker change in the long term, the EFSA would accept this data. I think it is just a matter of time.

Q: What is the cost comparison of doing this kind of analysis vs a high-sensitivity CRP?
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**Dr Hendriks**: The costs are enormous compared to a simple high-sensitivity C-reactive protein (hsCRP) analysis. I think this is more a methodology that you can use—at least that is what our aim is—to lead into new biomarkers that you can measure more easily. The glucose tolerance test started as a full curve. Today, a 2-hour blood sample after a glucose bolus is the routine. In that sense, I think that further development is needed in order to solve that issue.

On the other hand, you could say it gives you more insight. You will not look at only CRP, but you will have insight in various processes related to inflammation. In addition, you will obtain information on metabolic changes and get information on oxidative changes. In that context, we sometimes say that in case you do not yet know what claim you would like to go for, you may want to consider this kind of a technology. Perhaps it will help you to orient yourself toward a specific type of claim.

**Q**: As a follow-up, how predictive is this with this diet, if you had followed patients forward for extended periods of time? We have heard much about CRP. So, does the CRP go down with that diet with extended time, and is this predictive?

**Dr Hendriks**: Those are important questions that I cannot answer, because this study was basically the first of its kind. We should extend our research into this area to address all of these questions. Luckily, I think the Dutch government really wants us to go into this area more, because it may help solve some issues. For example, we all need to increase our consumption of vegetables. Epidemiological studies nicely show a positive correlation between high vegetable intake and improved health, whereas with intervention studies, it appears not always possible to show these beneficial health effects. So, we would like to and are moving into the area of studying and describing the health effects of vegetables. Through these types of studies, because of the short-term intervention study combined with the epidemiological long-term studies, we may have the ability to start addressing these questions.

**Q**: What about epigenetics? Have you thought about what to include in the studies?

**Dr Hendriks**: Epigenetics also is very relevant. The limitation in this study was that we could focus on the three levels presented. This is one of the first studies that looked at these three levels at the same time. Scientists usually study at the transcriptome level or at the protein level only. We should maybe work more hypothesis-driven in the near future, so we could address these hypotheses on various levels, such as epigenetics in combination with metabolomics. Focus will help in organizing the huge amounts of data involved in these techniques.
A Dietary Inflammatory Index to Predict Changes in Inflammatory Markers

James R. Hébert, MSPH, ScD

Chronic inflammation is associated with many chronic conditions, such as cancer, cardiovascular disease, obesity, and insulin resistance. Inflammation, resulting from a chronically poor diet, cigarette smoking, or obesity, is involved in the steps of atherosclerosis that lead to plaque rupture and thrombosis. The inflammatory microenvironment includes production of cytokines and chemokines, which also provides conditions ideal for tumor initiation, growth, and invasion.

The acute-phase protein, C-reactive protein (CRP), is produced in response to stimulation by interleukins (IL), such as IL-6. Although used as a marker of inflammation in conditions such as rheumatoid arthritis for many decades, the more recent development of a high-sensitivity C-reactive protein (hsCRP) assay permitted the detection of inflammation at the vascular level.

Many studies have shown that CRP is associated with a number of cardiovascular disease end points. In addition, CRP and inflammatory cytokines, such as IL-6 and tumor necrosis factor-alpha (TNF-α), are increased among obese individuals and positively correlated with diet-related factors, such as body mass index (BMI=weight [kg]/height [m]²). Studies also have found that higher levels of IL-6 among obese individuals are associated with insulin resistance. Ridker et al found that each component of the metabolic syndrome (obesity, hypertriglyceridemia, low high-density lipoprotein [HDL] cholesterol, hypertension, and abnormal glucose metabolism) is significantly associated with higher levels of hsCRP.

Diet plays a central role in the regulation of chronic inflammation. The Western-type diet, which is high in red meat, high-fat dairy products, refined grains, and simple carbohydrates, is associated with higher levels of CRP and IL-6. On the other hand, the Mediterranean diet, which is high in whole-grains, fish, fruit, and green vegetables, and associated with moderate alcohol and olive oil intake and low intake of red meat and butter, is associated with lower levels of inflammation. Because of their heavier reliance on plant-based components, it stands to reason that traditional diets, such as those found in Asia and Africa, also are anti-inflammatory. Diets high in fruit and vegetable intake are associated with lower levels of CRP. Also consistently shown is an association with lower levels of
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Inflammation and specific nutrients, such as omega-3 fatty acids, fiber, moderate alcohol intake, vitamin E, vitamin C, beta-carotene, and magnesium, were studied. We first performed a literature search in 2006 to determine if a diet-based inflammatory index already existed. Surprisingly, none had. With this in mind, the decision was to start developing one. The paper published in 2009 and briefly described here, is based on a search of the literature through 2007. A total of 919 qualifying articles were indexed, read, and scored. It is the first, and from what we can tell, the only attempt to date (most recent review on September 26, 2010) to describe the development and validation of such an index.

The overall goal of the original project was to define and then validate an index that assesses the inflammatory potential of the diet on a continuum from maximally anti-inflammatory to maximally proinflammatory.

One of three possible values was assigned to each article, based on the effect of the particular food or constituent on inflammation:

-1 if the effects were proinflammatory (significantly increased IL-1β, IL-6, TNF-α, or CRP, or decreased IL-4 or IL-10)
+1 if the effects were anti-inflammatory (significantly decreased IL-1β, IL-6, TNF-α, or CRP, or increased IL-4 or IL-10)
0 if the dietary variable produced no change in the inflammatory marker

In some instances in a single study, constituents were shown to decrease or increase both proinflammatory and anti-inflammatory markers. Other results have shown an increase in one proinflammatory marker, but a decrease in another. These are clear contradictions in the effect of a constituent on inflammation. To deal with this, the mean effect was taken. In some cases in a single study, a constituent had no effect on a number of inflammatory markers, but increased or decreased another. Scoring was based on the effect of the constituent on actual changes in inflammatory marker(s). For example, if a constituent did not affect levels of IL-6 or IL-1β, but significantly decreased CRP, the article was assigned +1.

A number of steps were taken to determine how to score the Inflammatory Index.

**First,** articles were weighted by study characteristics, weighted by the study type and design.

**Second,** using these weighted values, a score for each food and constituent was calculated. The following steps were used to calculate the score:

- **Step 1**—divide the weighted proinflammatory and anti-inflammatory articles by total weighted number of articles
- **Step 2**—subtract the proinflammatory fraction from the anti-inflammatory fraction (Fig)

![Fig. Example of procedure for weighting each food and constituent in the development of the Inflammatory Index.](image)

**Third,** scores were adjusted for each food and constituent by the total weighted number of articles. An arbitrary cut-off point of 100 was chosen to indicate an optimally robust pool of literature. This number was used to adjust scores by weighting the number of articles. All foods and constituents with a weighted number of articles ≥100 were assigned the full value of the score. Foods and constituents with a weighted number of articles <100 were adjusted.

**Fourth,** food-specific and constituent-specific scores were multiplied by the intake for each participant. After adjustment for the relative scale of the measure for each
food and constituent score, they were then summed to create the overall Inflammatory Index score for each participant.

**Fifth**, the Inflammatory Index score was rescaled by dividing by 100 in order to aid in interpreting results of the statistical analyses.

The Seasonal Variation of Cholesterol Levels Study (SEASONS), a prospective observational study of about 600 subjects, was used to test how well the Inflammatory Index scores for each participant predicted interval changes in hsCRP.2,3,5 In SEASONS, food and constituent intake data for each participant were obtained from the 24-hour dietary recall interviews (24HR). Three randomly selected days of 24HRs were collected at each quarter (including two weekdays and one weekend day) using the Nutrition Data System (NDS DOS Version 2.6, NDS 2.9) software.

The primary outcome variable for this analysis was the natural log of hsCRP. Values of hsCRP >10 mg/L were discarded, because these may have resulted from acute inflammation.3 The primary independent variable was the interval-specific score obtained from the Inflammatory Index. The original statistical model and subsequent models controlled for various possible confounders and effect modifiers. Effect modification was assessed by stratified analyses and by including the interaction term in the model.

Variables controlled in analyses were age, gender, race, body mass index (BMI), smoking status, physical activity, energy intake, mean hours of sleep, highest level (year) of education attained, employment status, marital status, total cholesterol (TC), HDL cholesterol, anti-inflammatory medication use, light season (ie, four seasons centered on the two solstices [summer and winter] and the two equinoxes [autumn and spring]), use of herbal supplements, and infection status (a dichotomous variable indicating whether the participant had a self-reported infection during the study quarter).

In addition to natural-log transforming the data, we computed the odds of elevated levels of hsCRP (>3 mg/L) among individuals (ie, the probability of having a high hsCRP). In order to do this, we dichotomized hsCRP to ≤3 mg/L and >3 mg/L, which were used to designate the two levels of the dependent variable in the model.3

**Results**

Results were consistent with the effect of a diet-derived estimate of inflammation on interval changes in hsCRP. Using the Inflammatory Index, it appeared that there was an effect of diet on hsCRP across natural-log transformed values (as it was not normally distributed), observed in SEASONS. However, a significant effect of increased inflammatory potential of the diet was reported on elevated hsCRP (ie, at levels >3 mg/L vs ≤3 mg/L).

While it was intended that the scoring system would offer off-the-shelf use, it is necessary to resolve several issues before recommending moving to this point:

- It was originally intended that the scoring remain free of arbitrary decisions regarding the inherent “weighting” that might come from standardizing to “real diets” as eaten by free-living humans, but it has become clear that just using “raw units” for the food constituents makes scaling very difficult. In the end, it was necessary to multiply or divide by an order or two of magnitude just to pull values in toward a “reasonable range.” In retrospect, these decisions taken as a whole are more arbitrary than standardizing to real, attainable intakes in human populations.
- Choosing one (or more) human population(s) as a standard is not trivial. However, if it is done transparently, then other choices could occur in the future as a sort of sensitivity analysis.
- Have a standard that represents a good range of exposure—perhaps combining is a good idea.
- Make the full constituent list available to those who can use it. However, few researchers, including epidemiologists with large data sets, will have access to 24HR-derived data. Food frequency questionnaires and other structured assessment instruments represent “truncated” lists. So, we need to develop algorithms to go from the very long “deluxe” listings of the 24HR to shorter ones provided by commonly used self-assessment instruments, such as food-frequency questionnaires.
- The literature review is now 2 years old. Even though nothing appears to have occurred in the interim that would lead to a qualitatively different view, this is an area of intensive research interest. So, the addition of several hundred more references would at least help refine the estimate.
- These refinements will help with prediction and for control of diet-related inflammation in a variety of other studies (eg, nutraceuticals), where inflammation-related end points are of interest.

These issues are part of the ongoing commitment to refining and improving the Dietary Inflammatory Index.
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Q & A

Q: What are your comments on SEASONS (the Seasonal Variation of Cholesterol Levels Study)?

Dr Hébert: It is a very interesting point. In the SEASONS Study, you see relatively large differences in terms of both seasonal intake of food and seasonal variation and BMI, and it was especially obvious in women. When we do the analyses, we use these very complicated models and include these sinusoidal curves, because it fits the data pretty well.

For this study, we account for season that way. What I have become convinced of over my career is that we do need to account for the chronobiological effects. So, it is partly season related. However, we also are diurnal animals, and depending on when you eat and when you exercise, a differential effect may occur.
In this study, we did a very careful measurement of the seasonal attributes, but not a very careful accounting for when the data were selected, even though every one of those 24-hour recalls was according to time assignments made by my group, then at the University of Massachusetts. So, we could go back and look at the time at which the interview was done, but what people are asked to recall is the previous day (ie, it is a single-calendar-day recall). However, subjects are not recalling very accurately the timing at which they ate.

Q: Yesterday I mentioned that the exacerbations seem to occur in the fall, particularly in children, and then it carries over to adults a little bit later in the fall. Was anything striking about the fall season?

Dr Hébert: The fall season is “the culprit” in all of this. Fall is when the spike increases. It goes through the Julian calendar to probably about January and then starts dropping off to summer lows. This is consistent with de Castro’s work [de Castro JM. Physiol Behav. 1991;50:243-248], who showed that people start eating more quickly in the fall. It is really the rapidity with which they eat that drives much of what is going on in terms of the dietary effects.

In addition, you also have all of this potential for infection that starts increasing at that time of year. People have seasonal effective disorder, so yes, what we observed was increases in the fall in this study, too.

Q: In terms of the parameter, is it more proinflammatory or anti-inflammatory?

Dr Hébert: I am inferring that it is more proinflammatory, but I am only inferring that, because I do not have the data in front of me.

Q: What about using urinary isoprostanes as a criterion measure, which is a pretty well-validated measure of oxidative stress, and relating that to your index? It already is used in field studies, but obviously somewhat challenging.

Discussion

but is there something different happening on the cellular level? Why does the viral infection increase mortality in your model?

Dr Beck: We do not work with bacteria, so I try to keep bacteria out of my lab. All of our studies are virus based. For our viral studies, what is nice is that we are just looking at RNA-based viruses. Because they cannot fix mistakes, it makes it kind of an easier experiment than looking at bacteria that have a larger genome and are more complicated.

I would like to do some bacterial studies. Nobody has really looked at this. The kind of things that we do evolve. I have not seen anybody do those types of studies with bacteria, so I do not know. The genome of a bacterium is more complicated than a viral genome. You might have the same effects on the immune response, but as far as changing the genome of a bacterium, that is a different story compared with a simpler viral genome.

Dr Make: We have a model, a smoking mouse, in which we then induce bacterial infections. Can you hypothesize what might occur based on your viral model and the effects of fatty acids on bacterial infection, a different kind of inflammatory response?

Dr Beck: It is hard to say. The Cuban studies looked at smoking, for example. Smoking was associated with nutritional deficiencies and the risk of developing neuropathy.

Smoking is definitely a pro-oxidant, and the kind of effect it has in dealing with the bacteria probably is similar to a viral response, but as far as affecting the individual bacterium, I just do not know. I have not done those bacterial studies.

Dr DeMichele: What about the age of the mice? What if we want to supplement fish oil in either children with chronic disease or in the elderly? Have you looked at the mouse at different ages?

Dr Beck: Yes, we did. Our mice were all young, but we did a collaborative study with Simin Meydani at Tufts University, where her graduate student used aging mice. Using the same model with coxsackievirus, she found genome changes in coxsackievirus replicating in the aging mice. Again, attributing that to the increased oxidative stress and the agent host, it is similar to what you see with nutritional stress. The same kind of thing holds true with the aging model—in those conditions you might need more oxidative protection as you get older.

Dr Pereira: Dr Beck, in your fish oil feeding studies, were the animals consuming the same amount of diet?

Dr Beck: That is a good question. We did not look at that.

Dr Pereira: That could make a difference. As they get sick, if one group starts eating less, the chance of mortality could increase.

Dr Beck: Right.

Dr Hegazi: Dr Hébert, I like the idea of the dietary index of inflammation. It is a novel idea to screen, and it is a great idea to integrate it, based on dietary recall in the patients whom we take care of. Should we include another layer, such as a lifestyle, exercise profile to this index? The Indian diet, for example, is the most anti-inflammatory, though prevalence of diabetes in India is one of the highest in the world.

Dr Hébert: I think it gets back to subsetting. It seems that Indians are particularly susceptible to the effects of a Western-type diet. It is really important to keep your “eye on that ball” (ie, that groups not only eat differently, but respond differently to the same diet), and the purpose of this is to get estimates of an individual’s intake. You have to remember, this is a distillation process—distilling a single (hopefully, single) index of diet from long lists of foods.

It starts with foods for a large number of people, but when we conduct our studies, typically we also measure physical activity, health-related quality of life, and all sorts of other things. It is part of a holistic way of looking at things and analyzing data, otherwise the utility is limited.

Dr Beck: I am not an epidemiologist, but I listen to people in my department who are epidemiologists. They talk about 24-hour recall and food frequency. They discuss how people lie. If they know you are going to watch them, people will change their diet, because they want to look better. When you went through all the studies, how do you deal with this?

Dr Hébert: My most widely cited paper is on response-set biases and dietary intake [Hébert JR et al. Int J Epidemiol.1995;24:389-398]. It does not have anything to do with cancer. Basically what we have shown are really interesting interactions by gender and education and response sets, which describe the kind of misrepresentation you suggested. The two most famous ones are social desirability and social approval. Highly educated women, no matter what their race, tend to
underreport according to social desirability—a demand characteristic of the test. This was not consistent with what I thought when we began this line of research. We have two ways to deal with this problem. Having measured these response sets, we can either use them to adjust the estimate of dietary intake or fit them in the statistical models. When we have done this, we find that it helps prediction of biological outcomes. In an intervention study that we did that led up to SEASONS (Seasonal Variation of Cholesterol Levels Study), we showed that we could predict serum lipid changes much better with this information in the model. We got within 10% of the equation and 15% of the Hegsted equation, when we fitted these parameters along with the dietary data. The other way to deal with this issue (eg, with 24-hour recalls) can drastically reduce bias. If you train interviewers well, they can pretty much remove the bias. Much of it has to do with the rapport that the interviewer develops with individuals. Most epidemiologists are completely clueless about this. They just think people are receptacles for information. They just are going to supply information, but that is not the way it works.

The problem really is worst on structured assessment instruments, such as the food-frequency questionnaires that are used in most large-scale epidemiologic studies.

Dr Beck: It seems like one person was saying that parents lie the most about their kids’ intake, because they do not want to say, “I took my kid to McDonald’s five times this week.”

Dr Hébert: Surrogate reporting is a huge problem. Again, if we are smart about understanding the functional anatomy of how that might work, we can measure and control for it. It is analogous to measuring something in the lab. You have to think through what the other parameters are.

Dr Jensen: One of the variables that you were talking about in terms of social desirability is that large people with an elevated body mass index (BMI) underreport their true food energy intakes and overreport their physical activities. An association with BMI also exists. Is the index solely based on foods and food components? Are you trying to record supplements as well? We do many multipass recalls, and supplement intake can be a tremendously challenging target.

Dr Hébert: The index focuses entirely on foods, but theoretically what you mention is an easy problem to solve. The problem, of course, is the reality of measuring exposure to these things. We essentially need to ask people to bring in their pill containers or to have them available when we interview them on the phone. Conceptually, it is no problem; it is just an add-in. But practically, we have a problem in dealing with it.
I will start by presenting additional information about a new approach to defining malnutrition, because that has been a relevant topic throughout the conference. Then I will try to unify the perspectives shared by participants throughout the conference.

We highlighted a couple of times that we have been working on a new consensus approach to etiology-driven definitions for malnutrition. The need to develop some common language was illustrated even by our conference over the last few days. Many of us used terms such as cachexia, wasting, and malnutrition, and it was not at all clear whether we were talking about the same things.

Almost 3 years ago, an international committee was convened by the American Society for Parenteral and Enteral Nutrition (ASPN) and the European Society for Parenteral and Enteral Nutrition (ESPEN) to examine this concern. Our members had broad international representation. After 3 years of little progress we had an epiphany: We turned the malnutrition syndromes around and based their definitions on the contributions of disease and inflammation etiology. This new construct has generated tremendous enthusiasm and interest among practitioners and investigators.

We held a series of meetings, exchanged many e-mails, and ultimately authored a paper that was recently co-published [Jensen GL et al. Clin Nutr. 2010;29:151-153; JPEN. 2010;34:156-159]. The proposal for new definitions was formally endorsed by ASPEN, ESPEN, and other nutrition societies.

One problem that has become clear is that many of the diagnostic criteria we historically have used to define malnutrition lack validity. Yet numerous textbooks and many hospital coders still equate reduced albumin or prealbumin with malnutrition. Unfortunately the presence of any active inflammatory state or disease will render these proteins unreliable indicators of nutritional status.

Our approaches to the diagnosis of malnutrition have suffered poor specificity, poor sensitivity, and limited interobserver reliability. The only genuinely valid indicators in routine clinical use for the diagnosis of malnutrition are nonvolitional weight loss and underweight status. We lack sound and practical laboratory indicators of malnutrition or inflammation. The other problems with the historic definitions for malnutrition syndromes such as marasmus, kwashiorkor, protein-calorie malnutrition, and...
Cachexia are overlapping definitions and frequent misdiagnosis. In modern health care, patients are frequently assigned to the diagnoses of protein-calorie malnutrition or kwashiorkor solely on the basis of reduced visceral proteins, when they may not be malnourished in any way.

Widespread confusion about this issue exists in our health care facilities, universities, and research groups. The bottom line is that the historic definitions we use lack an appreciation for a modern understanding of inflammatory response. According to the article cited previously, “The pathophysiology of malnutrition that is associated with disease or injury invariably consists of a combination of varying degrees of undernutrition or overnutrition and acute or chronic inflammation, leading to altered body composition and diminished biological function” [Jensen GL et al. Clin Nutr. 2010;29:151-153; JPEN. 2010;34:156-159]. A key observation is that when disease or injury is present, we are often dealing with inflammatory response. Of course, the response can vary in duration and severity, which I will touch on in a moment.

Ultimately this malnutrition must be defined in the way it affects body composition and function. This proposed etiology-driven construct easily accommodates severe undernutrition and severe overnutrition—obesity—and it can also accommodate conditions such as sarcopenia and sarcopenic obesity.

This approach may not seem like a particularly new insight, but for much of the general nutrition and medical world, I think that it is. We characterize disease-related malnutrition as the point at which the severity or persistence of inflammation results in a decrease in lean body mass associated with functional impairment [Jensen GL et al. Clin Nutr. 2010;29:151-153; JPEN. 2010;34:156-159]. So we are talking about potentially measurable outcomes.

Why is this clinically useful? First, if inflammation is absent, such as with a pure eating disorder or inadequate dietary intake in someone who is very depressed, then even advanced malnutrition because of starvation is readily treatable. We have to resuscitate such patients cautiously, but we can bring them back from the brink of death. If inflammation is present, however, it may limit the effectiveness of our nutrition interventions, and the associated malnutrition can compromise a favorable response to medical treatments and therapies.

It is important to clarify the severity and duration of inflammation, and we are trying to develop approaches to do this. Is it mild to moderate or severe? Is it transient or sustained? These factors have relevance to the chronic disease issues that we have discussed during this conference.

In the case of acute disease-related malnutrition with severe inflammation such as in multi-trauma, a serious burn, or overwhelming sepsis, the priority must be to provide nutrients to support patients’ vital organ system functions while other acute medical interventions are delivered. The bottom line is that this is a self-limiting process. Patients are either going to get better with the treatment they receive or they will suffer adverse outcomes. One cannot live in a highly proinflammatory, severely inflamed state. We try to support the vital functions, wound healing, and immune status of these patients as best we can. Unfortunately, with the interventions currently available to us, we cannot fully prevent loss of muscle protein in an active severe inflammatory state.

On the other hand, much of the focus of this conference has been chronic disease-related malnutrition. In this scenario, inflammation is of mild to moderate degree but sustained. Examples include rheumatoid arthritis, chronic infections, and organ failure syndromes. Although these are chronic states, acute superimposed inflammatory stressors also can exist. A patient with advanced lung disease, for instance, can suffer acute pneumonia. We need to recognize both the chronic and acute components because malnutrition will be exacerbated. With chronic disease-related malnutrition, a positive response to nutrition intervention is ultimately going to require successful treatment of the underlying disease or condition. End-stage renal disease is a good example because when the goals are accrual of body cell mass and improved function, the patient will require a kidney transplant before we see dramatic improvements. Nonetheless, the patient with end-stage renal disease will clearly warrant appropriate medical nutrition therapy while we are trying to address the underlying chronic medical condition.

We have arrived at three simple etiology-driven definitions [Jensen GL et al. Clin Nutr. 2010;29:151-153; JPEN. 2010;34:156-159]:

- Pure starvation-related malnutrition—no inflammation. Examples: eating disorders, depression, and esophageal stricture
- Chronic disease-related malnutrition—chronic inflammation of mild to moderate degree. Examples: organ failures, pancreatic cancer, rheumatoid arthritis, and sarcopenic obesity
- Acute disease or injury-related malnutrition—severe acute inflammation. Examples: burns, trauma, overwhelming infection, and closed-head injury

Not only can patients suffer an acute inflammatory event superimposed on pure starvation or chronic disease-related malnutrition that places them in more than one of these states at a time, but they also can move from one category to another. The attractiveness of the proposed approach is its simplicity and flexibility. Assigning patients to these categories does require clinical judgment and should help to
We have heard about a variety of exciting opportunities for intervention during the conference. I will attempt to summarize below some of the central themes and questions that came out of this conference.

First, I hope it is clear that inflammation is a unifying theme in nutrition and medicine. It also is clear that inflammation is much more complex than we ever imagined. It is like so many other things in medicine: We start thinking that this is simple—give patients an aspirin and hope that the problem will go away. But it is not so simple. It also became evident in our conference discussions that the timing of interventions is critical. And when we intervene, whom do we target and what dose do we use? We have much to learn about these important issues.

Second, we have the question of preventive intervention vs treatment of active inflammation. It seems a bit depressing to listen to all the presentations that describe limited efficacy of various interventions, but in most of the cases the patients had well-established inflammation. Early recognition of the potential for robust inflammatory response might lay the foundation for preventive measures. In distinction, if we can blunt well-established inflammation with nutrition intervention, it would help us to preserve vital host functions until we can address the underlying medical condition.

Is it possible that we can target specific detrimental aspects of inflammation? We previously heard about giving fish oil to infected mice. Although their lung inflammation decreased they died. Obviously we have desirable, physiologic aspects to an inflammatory response. For example, we need to have the ability to mount a white blood cell count and to mount a fever. We are just beginning to scrape the surface, targeting interventions with some degree of specificity.

Can we distinguish physiologic from pathologic inflammation? It is obvious when the patient is going into shock or dying that this is pathologic inflammation, but we need to learn to recognize the potential for this concern earlier than we are able to now. Can we tailor interventions to genotype and phenotype? Some individuals are clearly predisposed to more robust and dangerous inflammatory response.

Finally, some of the other key issues that were discussed during this conference are listed below:

- Need to develop better indicators of both nutritional and inflammatory status, including better tests and provocative challenge tests
- Need for a common language of malnutrition and inflammation across disciplines
Fanjiang

Structured Panel Discussion

Leader: Gary Fanjiang, MD, MBA, MS

Dr Fanjiang: Here at Abbott Nutrition, we are true believers in the science of nutrition. As Dr Miller mentioned previously, we have been involved in nutrition science since the early 1900s and have conducted more than 500 clinical studies since the 1960s. This year alone, we are doing more than 30 clinical studies around the world, in addition to hundreds of animal studies and treatment work.

During this conference, we have had the pleasure of hearing some cutting-edge and very thought-provoking work on inflammation and nutrition, but I cannot help but to think back to my days in medical school, where I probably had only about 2 hours of training on nutrition. It took a fellowship for me to dive deeply into the science of nutrition.

So before we leave, I want to pose a question to the panel. How can we translate the cutting-edge science that we heard here into practical applications, whether it is nutritional products or clinical science? What would increase our credibility—educational programs or scientific grants? What can we do as a nutrition organization to support the translation of this inflammation nutrition science into clinical real-world practice?

Dr Hébert: One of the talks I give focuses on type 3 error, and type 3 error does not have a statistical test. Basically, it results from asking the wrong question. You could have a study in which you have a good type 1 error rate (ie, low probability of incorrectly accepting that an effect exists) and type 2 error rate (ie, high probability of detecting a true effect) and still ask the wrong question or conduct the study in a way that the relevant question is not answered.

Much of what most people in my business, epidemiology, do is to go after populations that are easy to study, rather than the ones that either need it the most or would derive the most benefit, or parenthetically, in which we would learn the most scientifically. We do it because academic advancement is predicated on making rapid progress in accumulating the currency of the realm, which are publications and grants.

We need to figure out how to connect with high-risk populations, such as African Americans in the rural Southeast, who are in bad shape in many ways. We need to figure out a way to engage those populations. Sometimes that means going outside the conventional medical systems to do it, by going straight to the community and creating a dialogue where people can become interested in doing this research and where you can engage them in community-based participatory research.

This forum resulted in a provocative conference that should help to guide future directions. Having professionals from different disciplines come together to tackle difficult problems is very helpful. In our own disciplines we tend to have tunnel vision but when we come together innovation often results.

• Value of Mediterranean and other potentially anti-inflammatory diets, functional foods and bioactive food components, and nutritional supplements such as omega-3 fatty acids, antioxidants, and vitamin D
• Importance of patient selection, dosing, and timing
• Need for outcome measures and identification of outcome measures that are comparable between studies

Closing Thoughts and Future Directions
Structured Panel Discussion

We used a good amount of jargon in this meeting, but much less than what you would normally hear in our subspecialty groups. I think it is because we come from many different disciplines. If we could do a little bit of work up front in translating what we spoke about, the rural South Carolinians with whom I work would understand what the probes are and how they might become part of the solution. If you put it to them right, some members of the community would want to become engaged.

Dr Szefler: As I said earlier, there is a window of opportunity to do something different, at least in the area of asthma—opportunity to really understand the driving factors in the disease, how to modify them, and shift to prevention. We are always looking for improvement and advancement in the field, and we are going to have a gap in terms of the introduction of new medications for several years.

I think if you convince the specialists how to pay attention to the benefits of a nutritional approach to disease prevention, then secondarily, the primary care physicians will pay attention to this type of approach.

Pediatricians are geared toward growth and development, but are focused on normal growth and development. I thought that some of the models offered in this conference started to point to the concept of preventing disease through a nutritional approach. We think about disease, its impact, and relieving the disease, but not necessarily about the big picture in terms of growth and development.

We often criticize the additional burden related to electronic medical records. However, we may see benefits in organizing the process of long-term follow-up. Those are opportunities.

You are correct, Dr Fanjiang. Our base in nutrition knowledge in medical school was called biochemistry. I am not sure how much nutrition-based sections in medical schools have improved, but this addition to a core curriculum would provide a way to encourage a stronger nutritional approach to disease management.

Dr Fanjiang: In the United States health care system, we focus on acute care and acute medicine. During the average 5- to 10-minute visit with a primary care physician, the physician probably deals with a list of 10 morbidities that a patient has, so nutrition ends up falling to the bottom of that list. It is certainly easier for a physician to prescribe a quick-fix pill than to go over the intricacies of nutrition management. That is something that keeps me up at night.

As a company, what are some of the things we can do to help shift that paradigm and bring out the robust science behind nutrition and the validity of it?

Dr Szefler: In terms of health care providers, academic centers are focused on critically ill patients. However, certain health care systems, Kaiser-Permanente for example, are thinking about prevention and actually have that kind of language in their communications about diet. You could use this level of dialogue as a template, and have physicians and other health care providers communicate the key messages to their patients. This is a window of opportunity, because health care systems now are concentrating on prevention to reduce the costs related to poor outcomes.

Dr Jensen: The state of medical education in the United States today is no better than it was 20 years ago. Depending on the definition used, only 10%–20% of American medical schools have an identifiable, dedicated nutrition curriculum. Nutrition is often hidden in biochemistry and physiology.

Practically speaking, physicians are under more time pressure than ever—shorter patient visits and more pressure to generate revenues. I wonder whether an important opportunity exists for us to target physician extenders, including dietitians, nurses, pharmacists, and others. We could give them additional nutrition resources and tools and empower them to work with physicians as part of a multidisciplinary nutrition health care team. They can help us bring modern medical nutrition therapy to the ambulatory patient and to the bedside.

Certainly a cadre of true physician nutrition specialists does exist, but it is small in number and more appropriately serves large regional referral bases.

Dr Sartor: Dr Jensen, I am delighted to hear your emphasis on prevention and treatment with two elements of prevention, preventing onset and preventing relapse once inflammation is initiated.

I think you got 2 more hours of nutrition education than I recall getting in my medical school career. I think the number of physicians who are delivering nutritional care who are in practice far outstrip those in academic medical school. Clearly, we need to teach nutrition to medical students well, but we have to engage the interests of the practitioners—both primary care specialists and physician extenders. We need evidence-based nutrition information. A criticism of nutrition is that it is scientifically fuzzy. We talk about nutrition being good for you, but how is it good for you? What element of nutrition? We need to identify the evidence-based areas that have strong support and then educate physicians with review articles, which probably nobody in practice reads, as well as in presentations at annual meetings, which people do attend. Webinars are becoming a good vehicle. If the evidence-based literature is not very robust, we as a group need to do studies to increase the robustness of the evidence.
Structured Panel Discussion

We need to have a message. Nutrition may be good but how am I going to translate that to my clinical care? If I should measure vitamin D, what is the evidence that vitamin D actually improves health? We now measure cholesterol because we are convinced that a lipid-lowering intervention has a long-term impact.

As a large nutrition-based company, Abbott could make an impact. What do we know that is well documented? What do we think we know that is not well documented? What do we suggest may be important in affecting outcomes, but we do not have a clue if it really does? Answering these questions will require some funds, but I think it would have a long-term benefit if we did it.

**Dr Fanjiang:** As a nutrition company, we are stuck in a quandary. On one side of the spectrum, we sometimes are viewed as a dietary supplement company, and dietary supplements are sometimes considered pseudoscience. On the other side of the spectrum, large pharmaceutical companies spend hundreds of millions of dollars for clinical trials that enroll thousands of patients. When we approach health care professionals with our nutrition trials, which are somewhat smaller in scale, they often are gauged against those pharmaceutical trials and we are asked why we do not have thousands of patients in our trials. “You have only 50 patients or 100 patients or 200 patients? That is not as robust as this 1000-patient trial,” even though both hit statistical significance. Even though we are a large nutrition company, the reality is that we probably are never going to spend $100 million or $300 million on one large clinical trial. Those are some of the struggles that we are trying to deal with. Much of the struggle is translating the basic science or the cutting-edge pilot clinical science into real-world clinical products.

**Dr Sartor:** I would not discount the really well-done, focused, high-information small study. I showed you a probiotic study that had 40 patients [Gionchetti P et al. *Gastroenterology*. 2000;119:305-309]. It had a dynamite difference in the outcome that gave credibility to a whole field. I am not saying that is the optimal study design, but small studies can have an impact if they have a powerful message, and people do believe the results.

**Dr Beno:** We have discussed a variety of disease states here. How influential are advocacy groups for these disease states? Should we target them to increase our education of the public?

**Dr Sartor:** I am the chief medical adviser of the Crohn’s & Colitis Foundation of America (CCFA). We had a nutrition-oriented webinar that had the highest number of listeners of any that we have done to date. It had more than 3000 participants. I think you can engage the American Lung Association, National Kidney Foundation, and CCFA, and perhaps nutrition-oriented agencies, which I do not know much about. There are many organ-specific, disease-oriented areas, and the beauty is that these associations know how to do things pretty cheaply. They already have a broad membership, which may provide an effective, low-budget approach that is very well received and tax deductible.

**Dr Hegazi:** I want to add to Dr Sartor’s point that focused studies were effective enough to make a difference in the probiotics field. This is where we have to start this—to better define the outcome that we think the nutritionals will show as an effect. I remember the sentinel study of VSL#3 probiotics in pouchitis patients because the outcome was well defined and a well-defined biomarker was tested for effectiveness of the intervention [Gionchetti P et al. *Gastroenterology*. 2003;124:1202-1209]. As we discussed, better definition of the effects of nutrition in the setting of acute inflammation or chronic inflammation would definitely help.

**Dr Make:** You have a series of issues to address and are limited by the available resources. However, you might consider a medical education model. Several decades ago, the National Heart, Lung, and Blood Institute implemented the Pulmonary Academic Award to fund physicians in medical schools to develop and deliver pulmonary curricula. That program lasted about 15 years, and I believe that almost every medical school eventually received a Pulmonary Academic Award. Participants met regularly and shared experiences. That funding mechanism then was successfully used by branches of the National Institutes of Health (NIH) as well.

You might piggyback such an effort on the obesity issue. When you talk about malnutrition, look at a much broader perspective of topics in the public realm, public interest, and public press every day and try to build on that. I do not know which NIH branch is the most relevant to obesity, but I think that all branches would have some interest and may combine resources into one program. You might consider an initiative through patient organizations or the public and Congress to develop nutrition education in medical schools through something similar to the Pulmonary Academic Award system.

Even if you did not proceed at the national level with NIH support, you might consider a similar initiative through the Abbott Nutrition Health Institute to fund clinical educators at different medical schools and bringing them together to share their curricula. They could then develop and provide a free curriculum that others could adapt for their own use. You also might consider a consortium of pharmaceutical companies to assist you in providing that funding.
Structured Panel Discussion

You can influence medical education in many ways. For example, who do you want to identify as the recipient of the education—practitioners or medical students? My bias is to start in medical schools and work your way up to practitioners. I think it is more difficult to change the behavior of health care practitioners. Once practitioners develop an approach, it is harder to get them to change, compared to reaching students early in their education.

Dr Madsen: For the last 10 years, I was the nutrition coordinator for undergraduate medical curriculum at the University of Alberta in Edmonton, Alberta. In Canada, we have a large amount of nutrition in our curriculum, but 10 years ago the students did not really care. What I have noticed lately is that the students themselves are driving the change. They are asking for more nutrition. They want the knowledge, and the well-established practitioners are not interested in giving it to them. You need to focus on the generation coming up now. If you can give them what they want, then they are going to take it into practice with them.

Dr Hébert: The big winners in the current health care reform in the United States are the federally qualified health centers. They have a population of health care providers that is maybe of interest here. Many physicians who are practicing in the periphery may look at these educational opportunities to do business in different ways in a very positive light.

While you are at it, you also could think about places such as India, where the health care delivery system is as messed up as it is here. I know you are doing trials there for various, probably pretty good, reasons. At the same time, it is really interesting to think creatively about how you can do this intercultural work.

Dr Hendriks: In Europe, legislation has changed quite dramatically because of the European Food Safety Authority, which is acting severely on product claims. So, evidence-based nutrition claims will become the standard.

We may go even further than involving just health care professionals. You may know about the initiative in France that is fighting obesity in children. Everybody in the community is involved in the fight, including the mayor and role-model sports figures. This seems to help people acquire a healthy lifestyle. This is desirable and helps to prevent long-term disease.