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NCP: Nutrition in Clinical Practice

Nutrition in Clinical Practice

NCP

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THE AMERICAN SOCIETY FOR PARENTERAL & ENTERAL NUTRITION

Inflammation

Glucose Control and the Inflammatory Response

Obesity, Inflammation and the Potential Application of Pharmaconutrition

Inflammatory Mediators and Home Parenteral Nutrition

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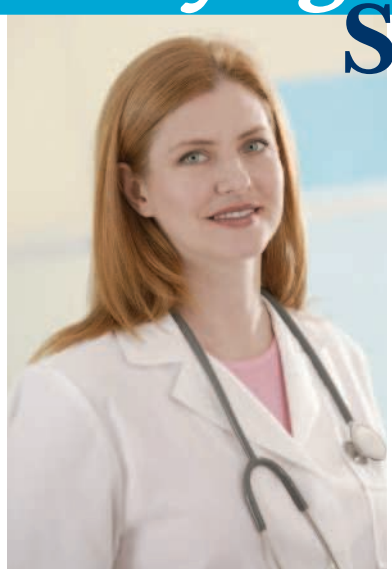

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Coming in

JPEN

Field Testing of the 2006 Growth Charts From Birth to 2 Years: Assessment of Hospital Under- and Over-Nutrition Rates and the Usefulness of BMI
A Nash et al

Iatrogenic Malnutrition in Neonatal Intensive Care Units: Urgent Need to Modify Practice
A Grover et al

Sesame Oil Attenuates Hepatic Lipid Peroxidation by Inhibiting Nitric Oxide and Superoxide Anion Generation in Septic Rats
D-Z Hsu et al

Effects of Oral Nutritional Supplements in Normal or Mild Undernourished Geriatric Patients After Surgery for Hip Fracture: a Randomized Clinical Trial
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The Role of Selenium in Chronic Disease
M G Boosalis

Nutrition Focused Evaluation and Management of Dysnatremias
S J Whitmire

Diagnosis and Treatment of Simple Acid-Base Disorders
P Ayers et al

Iron Deficiency Anemia
S F Clark

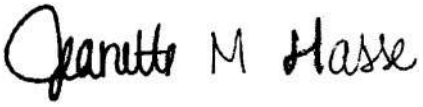
Health Benefits of Magnesium
C M Champagne

Editor's Note

We often think of inflammation as occurring as a result of an acute injury. However, we learn in this issue that inflammation is associated with chronic diseases. The issue begins as Dr Gordon Jensen defines the realm of inflammation as "an expanding universe" and highlights implications of nutrition therapy on inflammatory conditions. Next, Dr Collier and colleagues expound on the link between hyperglycemia and inflammation. This thorough article reviews mechanisms of stress hyperglycemia, relationships between inflammation and elevated glucose levels, methods of glucose control, and outcomes associated with stringent glucose control. In a similar fashion, Dr Cave et al review, in detail, proposed mechanisms of obesity-induced inflammation, as well as the role of adipokines and cytokines in the inflammatory process. The authors then evaluate the potential role of ω-3 fatty acids, soy protein, leucine, arginine, betaine, S-adenosylmethionine, carnitine, magnesium, zinc, and α-lipoic acid as pharmaconutrients. As DeLegge and Smoke discuss, neurodegeneration can also be considered an inflammatory condition. The authors review data suggesting that nutrition interventions could potentially reduce inflammation associated with neurodegeneration. Parenteral nutrition may also cause inflammation. Drs Hise, Compher, and Brown review the literature with regards to the etiology of inflammation associated with long-term use of parenteral nutrition. Clark and Mullin follow with a review on complementary approaches to immunomodulation. They review the immunodulatory roles of resveratrol, green tea, curcumin, boswellia, fish oil, vitamin D, and probiotics. Rounding out the themed articles is a paper by Liepa, Sengupta, and Karsies. They focus on polycystic ovary syndrome—the etiology, prevalence, and treatment including proposed nutrition interventions.

The next article by Overholser and Sowinski is a follow-up article on biostatistics. Part 1 of this article in the December 2007 issue addressed descriptive statistics; this article focuses on inferential statistics.

There is a trio of unsolicited papers in this issue of *NCP*. Keswani, Neven, and Semrad report the results of their investigation to determine if undiagnosed celiac disease was present in a small cohort of patients with short bowel syndrome. In the next article, Roberts and Lyman evaluate rates of microbial contamination in enteral feeding sets used at home. They conclude that in their population, a majority (77%) of their patients did not have significant contamination of feeding sets even after 48 hours. Scolapio and colleagues then summarize effective change in knowledge and practice among attendees of a continuing education course. A book review by Harper finishes this issue of *NCP*.



Jeanette M. Hasse, PhD, RD, FADA, CNSD
Editor-in-Chief

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Cover Art
NCP's theme this month is Inflammation. The flames on the cover art remind readers that specific nutrients can incite or help extinguish inflammatory conditions.

Invited Commentary

Inflammation: An Expanding Universe

Gordon L. Jensen, MD, PhD
Department of Nutrition Sciences, The Pennsylvania State University, University Park, Pennsylvania

On the occasion of my Presidential Address for the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) at Clinical Nutrition Week on February 14, 2006, I presented a provocative examination of future opportunities in clinical nutrition by exploring the key role of inflammation at the interface of nutrition and medicine.[1] This vision of the future has met with a highly receptive audience of nutrition practitioners throughout the world. Inflammation has been a prime focus of recent sessions at the A.S.P.E.N. Clinical Nutrition Week, the American Society for Nutrition meeting at Experimental Biology, the American Dietetic Association's Food and Nutrition Conference Expo, and the European Society for Parenteral and Enteral Nutrition's Congress. The list of inflammatory clinical conditions with nutrition implications continues to grow. This issue of *Nutrition in Clinical Practice* touches on some of these conditions, with critical implications for nutrition intervention and management.

Glycemic Control

Hyperglycemia appears to be a cytokine-mediated indicator of active inflammatory response. Indeed, acute onset of hyperglycemia is often a harbinger of brewing infection or other inflammatory event. Nutrition support interventions have the potential to foster poor glycemic control and to fuel inflammatory pathways. Although studies[2–4] have been mixed in findings, there may be opportunity to secure enhanced clinical outcomes with improved glycemic control. It is possible that insulin therapy is actually an anti-inflammatory intervention.

Neurodegeneration

There has been growing interest in the role of inflammation in a host of neurodegenerative diseases. Alzheimer's and Parkinson's diseases have attracted particular attention. Oxidative stress has been implicated as a potential causal factor in epidemiologic studies finding that consumption of diets rich in antioxidant and anti-inflammatory agents may lower the risk of developing these age-related neurodegenerative diseases. Trials are under way, testing nutrition interventions as both preventive and therapeutic measures.[5–7]

Home Parenteral Nutrition (PN)

Chronic use of home PN may well be associated with a smoldering low level inflammatory state,[8,9] but this is difficult to fully discern in view of underlying potential contributory factors that include chronic medical conditions, subclinical infections, associated hepatic dysfunction, and poor glycemic control. Nonetheless, the possibility that PN may itself fuel inflammatory pathways and contribute to immune suppression lends priority to the push to develop novel PN formulations that will not have these undesirable effects and to the application of clinical practice guidelines that promote the transition of patients receiving PN to enteral nutrition at first opportunity.

What Do We Really Mean?

There is a pressing need to understand malnutrition syndromes in light of our current understanding of inflammatory response. It is now evident that much of what has historically been designated protein-calorie malnutrition in acute and chronic care settings is often at least partially a manifestation of inflammatory response that results in an altered metabolic state.[1,10,11] Indeed, a call for the development of new consensus definitions for malnutrition syndromes has arisen at recent international meetings. It will extremely helpful if we can all speak a common nutrition language. Clinical nutrition will involve so much more than protein and calories. Modulation of inflammation with specific nutrients and functional foods offers the opportunity for nutrition practitioners to be part of the future medical team that brings highly individualized patient care to the bedside. This vision can guide an exciting research agenda, with both basic and translational portfolios.

Correspondence: Gordon L. Jensen, MD, PhD, Department of Nutrition Sciences, The Pennsylvania State University, 126 Henderson South, University Park, PA 16802.

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Invited Review

Glucose Control and the Inflammatory Response

Bryan Collier, DO; Lesly A. Dossett, MD; Addison K. May, MD; and Jose J. Diaz, MD
Division of Trauma and Surgical Critical Care, Department of Surgery, Vanderbilt University Medical Center, Nashville, Tennessee

ABSTRACT: Though first introduced more than 130 years ago, the concept of stress diabetes or stress hyperglycemia has gained tremendous attention in recent years in view of the landmark article by van den Berghe and colleagues in 2001. As opposed to earlier work that suggested that hyperglycemia in the acute clinical setting may be beneficial, it now appears that lower glucose levels are associated with improved outcomes. The mechanisms behind the improved outcomes are numerous and seem to be tied to the inflammatory process. Both lower glucose values and insulin therapy seem to be anti-inflammatory, whereas hyperglycemia increases the proinflammatory process and negatively affects the innate immune system. Despite the numerous approaches to achieve normoglycemia described in the literature, only modest success has been achieved. Understanding the pathophysiology driving stress hyperglycemia—the stress response and modulation of the inflammatory process—seems to be the key to improving the care of the most critically ill and injured patients.

During the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) Clinical Nutrition Week 2006, Jensen's[1] presidential address focused on inflammation as the key interface between medicine and nutrition. The focus on inflammation has occurred in other clinical sciences, as evidenced by an explosion of inflammation-related research. A PubMed search using the search term *inflammation* produced >255,000 documents (in July 2007), and a similar search of the internet search engine Google returned more than 26 million websites. These totals represent an astounding increase of 3 to 6-fold, respectively, in the last 3 years alone.[1] Though classic chronic inflammatory conditions such as obesity, arthritis, asthma, and cardiovascular disease are well described, it is the acute stress response and associated cytokine-driven pathophysiology that have gained the most attention in recent years. The current focus on glucose control in the critically ill patient has been equally intense, with countless manuscripts, practice management guidelines, protocols, and cost-saving analyses having been published in the last decade. Concurrent with the explosion of research on these 2 separate but very important clinical topics has been the focus on the interface between inflammation and glucose control.

The earliest mechanistic links between inflammation and hyperglycemia were in the chronic diabetic population. A growing body of literature now supports the concept of type 2 diabetes as a chronic inflammatory condition associated with both obesity and the metabolic syndrome, with both causative and correlative links to hyperglycemia. Since a landmark study by van den Berghe et al[2] in 2001, intensive insulin therapy and glucose control in the critical care setting have become a focus. Whether acute or chronic, inflammation in severe, sustained, or repeated bouts is clinically detrimental. In the chronic setting, inflammation leads to diabetes, cardiovascular disease, and the metabolic syndrome. In the setting of acute inflammation, as in the critically ill patient, similar clinical syndromes are observed: stress hyperglycemia, hemodynamic instability, and other metabolic derangements such as hyperlipidemia and hypercortisolemia. Attenuation of this sequela of acute inflammation through nutrition and metabolic support is paramount and can be considered one of the key components of modern-day critical care. This review will (1) present a historical perspective of the relationship between inflammation and glucose control, (2) review the basic mechanisms of stress hyperglycemia, (3) outline our current ability to control blood glucose, and (4) discuss some of the current controversies of intensive insulin therapy as they relate to the inflammatory response.

Historical Perspective

The earliest contributions to the literature highlighting the link between hyperglycemia and stress

Correspondence: Bryan R. Collier, DO, Vanderbilt University Medical Center, Department of Surgery, Division of Trauma, 1211 21st Avenue, 404 Medical Arts Building, Nashville, TN 37212. Electronic mail may be sent to bryan.collier@vanderbilt.edu.

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are credited to Bernard[3] and Cuthbertson.[4] In 1878, Bernard described hyperglycemia during hemorrhagic shock, establishing the concept of *stress diabetes* or *stress-induced hyperglycemia.* In the 1930s, Cuthbertson described a biphasic immune, inflammatory, and metabolic response, which he termed the ebb-flow phenomenon. The first phase (shock) seems to be aimed at immediate survival, characterized by vasoconstriction and the conservation of both water and salt. The second phase, *the ebb*, appears with concurrent decreases in energy expenditure. This response occurs at the systemic and local levels, and the extent largely depends on the severity of injury. Secondarily, a hypermetabolic phase marked by hyperglycemia and glycosuria ensues—*the flow*—which only disappears during the later phases of recovery. In this phase, the body increases the available metabolic substrates of glucose, amino acids, and free fatty acids for use by vital organs such as the central nervous system, adrenal medulla, heart, and immune system. The hypermetabolism results in increased activity in key reparative cells such as leukocytes. Because hyperglycemia occurs as a natural response to injury or illness, a survival advantage could be speculated.

Beginning in the early 1960s, the concept of hyperglycemia as a natural and beneficial response would be challenged. Infusing glucose together with insulin and potassium (GIK) was introduced as a therapy to protect the ischemic myocardium after acute myocardial infarction.[5] In 1970, Opie[6] suggested that this cocktail would reduce the morbidity and mortality of an acute myocardial infarction by promoting glycolysis for the generation of adenosine triphosphate (ATP). By encouraging the insulin-mediated use of glucose as fuel substrate instead of free fatty acids, oxygen consumption is decreased, thus restoring the supply and demand axis, which is critical during cardiac ischemia. The improvement of cardiac function associated with this therapy suggested that providing glucose and insulin was more protective for the myocardium than allowing the inherent hyperglycemia to ensue. Despite the improvement in these secondary outcomes, overall survival was not improved, thus dampening the enthusiasm for this therapy.

During the Vietnam conflict and the study of its casualties, hyperglycemia was noted to correlate with injury severity,[7] but the metabolic benefits of hyperglycemia continued to be questioned. In animal models of hemorrhagic shock during this era, the administration of a hypertonic glucose solution increased cardiac output and improved subsequent survival.[8,9] This again gave credence to the idea that hyperglycemia in the acute setting was an appropriate adaptation and a natural survival mechanism, and suggested that there should be no attempt to control blood glucose during acute illness.

As the 20th century neared its end, the idea of hyperglycemia as adaptive and protective was once again challenged. In the Diabetes Insulin-Glucose in Acute Myocardium Infarction (DIGAMI) trial, 19 Swedish hospitals evaluated 620 patients who were randomized to insulin therapy *vs* no insulin to reduce hyperglycemia (goal of <200 mg/dL) after myocardial infarction. Insulin therapy resulted in a reduction of inpatient and 1-year mortality of >50%, an observation that was most profound in diabetic patients who had not previously required insulin and were considered to be at low cardiovascular risk.[10] A follow-up study, the DIGAMI-2 trial, did not corroborate these outcomes; however, the goal of very strict glucose control was not achieved. A *post hoc* epidemiologic analysis of these patients enrolled in DIGAMI-2 demonstrated that hyperglycemia was a strong independent predictor of long-term mortality.[11] These studies suggested that insulin therapy to treat hyperglycemia after an acute event could have long-term benefits.

After the DIGAMI trials, data supporting glycemic control in the acute care setting continued to emerge. In a large prospective evaluation of 2467 cardiac surgery patients with diabetes, Furnary and colleagues[12] found that glucose control (150–200 mg/dL) *via* an insulin infusion diminished deep sternal wound infections by 66%. Two years later, a landmark trial by van den Berghe et al[2] changed the culture of blood glucose control in the critical care setting. In a randomized trial of 1548 patients in a surgical intensive care unit (ICU), the majority being cardiac surgery patients, intensive insulin therapy (target blood glucose, 80–110 mg/dL) improved outcomes compared with those with conventional therapy (target blood glucose, <180 mg/dL). One-year mortality was decreased from 8.0% to 4.6% in the intensive insulin therapy arm. This effect was most attributable to patients requiring >5 days in the ICU secondary to multiorgan failure in the face of a proven septic focus. In addition to a 1-year survival advantage, intensive insulin therapy reduced in-hospital mortality by 34%, bloodstream infections by 46%, blood component transfusions by 50%, acute renal failure requiring dialysis or hemofiltration by 41%, and critical illness polyneuropathy by 44%. These outcomes followed a fall in inflammatory markers such as C-reactive protein (CRP).[13] A subsequent trial in medical ICU patients resulted in similar decreases in acute renal failure, ventilator dependence, and length of stay.[14] Mortality was diminished from 52.5% to 43.0% for those medical patients who required 3 or more days of intensive care. There was no difference in mortality for patients requiring <3 days of ICU care, although the study was not powered to detect this difference. Further analysis of these data demonstrated an overall total treatment cost savings in favor of intensive insulin therapy.[15]

In response to these landmark articles, intensive insulin therapy was rapidly and widely adopted in acute care settings across the globe. Not surprisingly, as the van den Berghe et al[2] principles have been adopted on a large scale, a number of criticisms

and controversies have arisen. Intensive insulin therapy has been linked to markedly increased rates of hypoglycemia,[16–19] and some have reported adverse outcomes in patients with hypoglycemia. Two large multi-institutional trials—the Glucocontrol Trial (Belgium; presented at the European Society of Intensive Care Medicine [ESICM] meeting; October 2006; Barcelona, Spain) and the Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis Trial (VISEP; Germany)—were recently stopped for safety concerns based on increased incidence of hypoglycemia and associated increased mortality.[20] As safety and efficacy concerns regarding intensive insulin therapy mount, research has begun to focus on the underlying pathophysiology of stress hyperglycemia—the stress and inflammatory response—in an attempt to better understand the underlying pathophysiology and perhaps identify more specific targets of therapy.

Table 1 *Mechanisms of stress hyperglycemia and the contributing hormones or factors*

Mechanism	Hormone or factor
Increased glycogenolysis	Glucagon Epinephrine
Increased gluconeogenesis	Epinephrine Glucagon Norepinephrine Corticosteroids Growth hormone
Increased peripheral insulin resistance (skeletal muscle)	Epinephrine Glucocorticoids Growth hormone TNF-α
Increased hepatic insulin resistance	TNF-α
Suppression of insulin secretion	Epinephrine
Increased lipolysis (free fatty acid substrate)	Epinephrine Norepinephrine Glucocorticoids Growth hormone

TNF-α, tumor necrosis factor-α.

Mechanisms of Hyperglycemia During States of Stress and Inflammation

The inflammatory and stress response to critical illness or injury is a predictable, well-orchestrated set of events. The hypothalamic-pituitary axis (HPA), proinflammatory cytokines, and the noradrenergic system work collectively and synergistically to induce hyperglycemia. Because stress hyperglycemia results directly from the presence of excessive amounts of counterregulatory hormones (primarily epinephrine, norepinephrine, glucagon, glucocorticoids, and growth hormone) and cytokines (primarily tumor necrosis factor-α [TNF-α], interleukin-1 [IL-1], and IL-6), the more severe the illness or injury, the greater the degree of hyperglycemia (Table 1). This neuroendocrine response results in activation of the HPA and the adrenergic system. Corticotropin releasing hormone (CRH) is released from the hypothalamus; CRH subsequently stimulates adrenocorticotropic hormone (ACTH) release from the pituitary gland, causing glucocorticoid release by the adrenal cortex. In contrast to type 1 diabetes, which represents a deficiency of insulin secretion, and type 2 diabetes, which is primarily characterized by peripheral insulin resistance, stress hyperglycemia represents a complex neuroendocrine response to stress and inflammation characterized by excessive gluconeogenesis and glycogenolysis, relative insulin deficiency, and impaired glucose use (Figure 1). It is the disruption of this normally homeostatic relationship that leads to elevated serum glucose concentrations.

Excessive Gluconeogenesis and Glycogenolysis

During episodes of stress and inflammation, glycogenolysis and gluconeogenesis are triggered by a complex neuroendocrine reaction involving counterregulatory hormones and cytokines (Table 1). Both epinephrine and norepinephrine stimulate hepatic glycogenolysis and gluconeogenesis, and norepinephrine has the added effect of increasing the supply of glycerol to the liver *via* lipolysis.[21,22] Epinephrine also stimulates glycogenolysis in skeletal muscles, which enhances the production of glucose-6-phosphate from glycogen. In some situations of stress and inflammation, glucagon is the primary stimulant of excess glucose production.[23]

The normal response to excessive gluconeogenesis and ensuing hyperglycemia is the secretion of insulin and inhibition of the secretion of glucagon, effects that will diminish the degree of hyperglycemia. In situations of stress hyperglycemia, catecholamines (whether endogenous or exogenous) interfere with this normal feedback mechanism, further exacerbating elevated serum glucose.[24] Additionally, whereas in normal volunteers an acute elevation of glucagon leads only to a transient increase in glucose, in burn patients, glucagon remains elevated for weeks, with its effect maintained in stimulating hepatic gluconeogenesis. This dichotomy suggests that the effect of glucagon is dependent on synergism from other counterregulatory hormones.

Although pyruvate is the principal substrate for gluconeogenesis, in the setting of reduced caloric intake and persistent metabolic demands, noncarbohydrate substrates are used (Figure 1). The hyperlactatemia, which commonly accompanies severe inflammation, usually reflects increased glycolysis in peripheral tissues and possibly the inhibition of pyruvate dehydrogenase.[25] This lactate is transported to the liver, where it is converted to pyruvate *via* the Cori cycle, which is then used as a substrate for glucose production. Glucose can also be produced *via* the glucose-alanine cycle when alanine is released *via* muscle catabolism. Finally, hormone-stimulated lipolysis results in the release of glycerol,

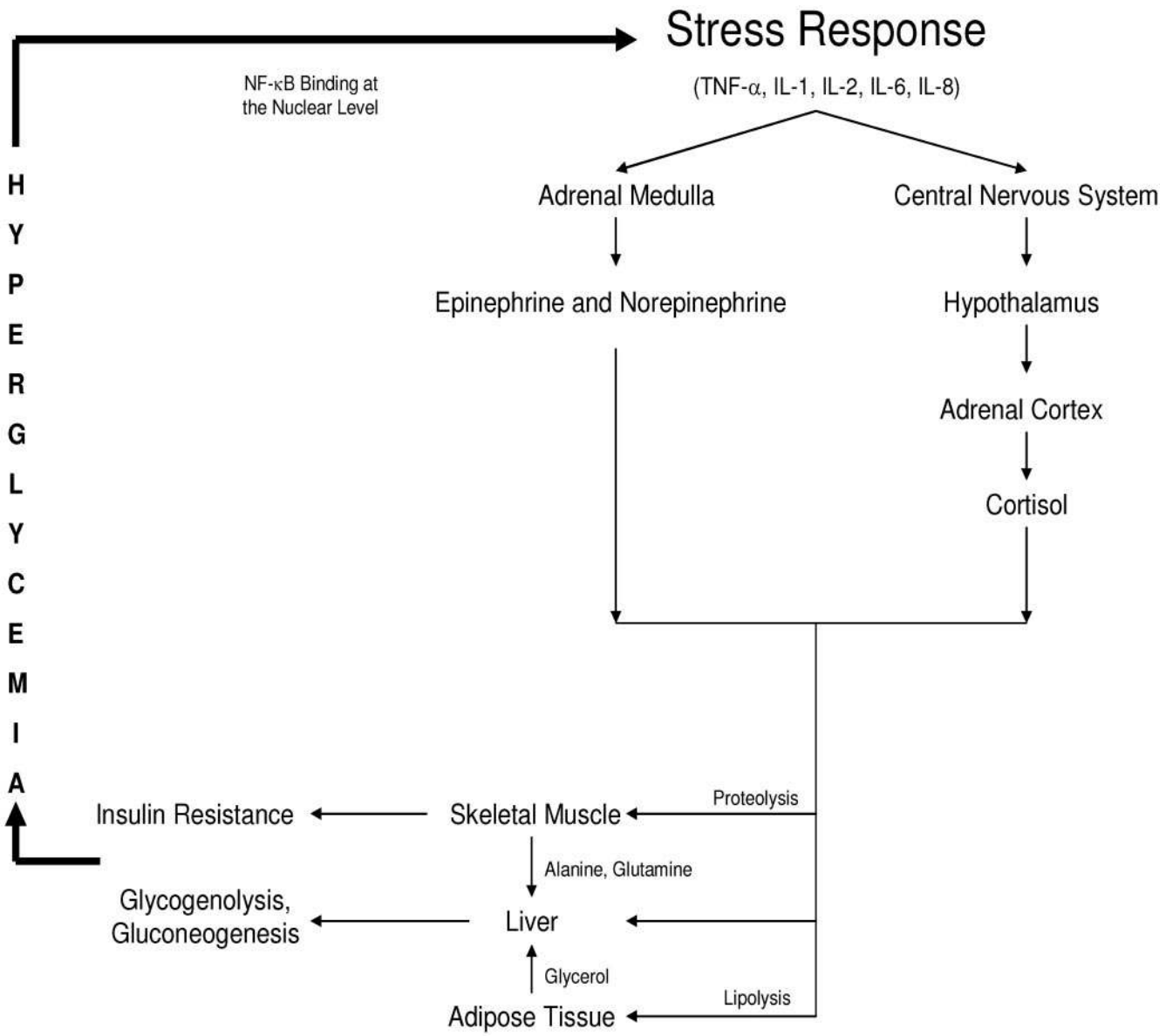

Figure 1. Mechanisms of hyperglycemia during states of stress and inflammation. IL, interleukin; NFκB, nuclear factor-κB; TNF-α, tumor necrosis factor-α.

which provides new carbon skeletons for glucose production. In hypermetabolic states, glycerol generated from lipolysis can account for up to 20% of glucose production.[26]

Although excess glucose production plays little to no role in chronic diabetes, a number of human and animal studies underscore its importance in the evolution of stress hyperglycemia. Jeevanandam et al[27] used stable isotopes to track glucose fluxes in critically ill trauma victims and demonstrated that postinjury hyperglycemia is mainly caused by the increased hepatic output of glucose, more so than impaired tissue glucose extraction. In this study of hypermetabolic patients compared with healthy controls, the mean rate of hepatic glucose production was 30% higher in the stressed patients than in the healthy controls, whereas the absolute rates of glucose clearance and oxidation were similar. This increase in hepatic gluconeogenesis has been confirmed in animal studies. McGuinness and colleagues[28] demonstrated that infusion of cortisol, glucagons, and catecholamines in conscious dogs significantly enhanced net hepatic glucose production, but peripheral glucose clearance was not altered. This occurred despite a 3-fold increase in plasma insulin, which under normal conditions would inhibit hepatic gluconeogenesis. This hepatic production of glucose continues despite hyperglycemia and abundant insulin, suggesting hepatic insulin resistance.[29] Together, these findings suggest that hepatic

insulin resistance and the resulting excessive gluconeogenesis are the driving forces of stress hyperglycemia.

Impaired Glucose Use

Although the increase in hepatic gluconeogenesis seems to be the driving force behind stress hyperglycemia, there is also impaired glucose use in peripheral tissues. Although total body glucose uptake is increased,[30] insulin-independent tissues such as brain cells and blood cells account for the majority of the uptake. Although the insulin-*independent* transporters—glucose transporters (GLUTs) GLUT-1, GLUT-2, and GLUT-3—remain active in settings of stress, they cannot match the concurrent increase in hepatic glucose production.[31] In insulin-*dependent* tissues, such as skeletal and cardiac muscle, as well as adipocytes, insulin is required to stimulate the GLUT-4, which leads to glucose crossing the cell membrane for metabolism. Activation of the GLUT-4 transporter is impaired in sepsis and critical illness through the phosphorylation of various molecules along the insulin-signaling pathway.[32] Glucocorticoids and epinephrine are known to impair GLUT-4 translocation from its internal membrane stores to the plasma membrane,[33] with epinephrine having an added effect of inhibiting insulin binding.[34] Despite reduced uptake, once inside the cell, the oxidation of glucose by glycolysis and the tricarboxylic acid cycle seems to be maintained. Rates of glucose oxidation have been shown to be entirely normal in children with burn injury.[35]

Inflammatory mediators, specifically, the cytokines TNF-α, IL-1, IL-6, and CRP, also induce peripheral insulin resistance. TNF-α was first linked to hyperglycemia in 1994. Hotamisligil et al[36] demonstrated that TNF-α mediates insulin resistance in animal models of obesity. Locally elevated TNF-α directly interferes with insulin signal transduction, creating a local desensitization to insulin. Neutralization of TNF-α with soluble TNF-α receptor restores insulin sensitivity in animal models, but reversal of insulin resistance by anti-TNF-α has not been reproduced in humans.[36] Other cytokines have also been linked to the development of diabetes in a correlative way. In healthy women, elevated IL-6 and CRP predict the development of type 2 diabetes.[37] From these observations, Pickup and colleagues[38] describe type 2 diabetes as a disease of the immune system, particularly the acute-phase response, evidenced by the finding that elevations in CRP strongly correlate with diabetes.[39]

The Vicious Cycle

In both the chronic and acute settings, the mechanisms by which inflammation induces hyperglycemia are becoming more clear, but equally clear is the reverse—the mechanisms by which hyperglycemia induces inflammation. These mechanisms likely underlie the adverse outcomes associated with elevated blood glucose. Hyperglycemia acts to induce a proinflammatory state, which includes both cellular inflammation and oxidative stress. Inflammation at the cellular level can be described as an increase in the proinflammatory transcription factor nuclear factor κ-B (NFκB) in the nucleus. The activation of NFκB induces the transcription of proinflammatory cytokines (TNF-α, IL-1, IL-2, IL-6, IL-8, etc), adhesion molecules, and other genes that regulate transcription, apoptosis, and cell proliferation. Oxidative stress is an increase in reactive oxidative species (ROS), which disrupts the normal balance between the generation and detoxification of ROS.[40] Glucose acts to induce increased intranuclear NFκB binding[41]; it also increases activator protein-1 and early growth response (EGR)-1. All 3 are key proinflammatory transcription factors suppressed by insulin.[42] At the cellular level, hyperglycemia can induce cellular inflammation at surprisingly low levels of plasma glucose. As little as a 75-g glucose load given orally to normal subjects results in profound oxidative stress and inflammatory changes at the cellular and molecular levels.[43] This effect can occur even without an increase in plasma glucose, and in spite of endogenous insulin.[41] Sustained elevations in glucose concentrations are likewise proinflammatory.

Healthy volunteers have an increase in the proinflammatory cytokines IL-6, TNF-α, and IL-18 when the plasma glucose level is acutely raised by glucose infusion while endogenous insulin secretion is blocked with octreotide. Patients with hyperglycemia on admission to the ICU had increased levels of IL-6 and IL-10. Glucose also induces an increase in ROS and reduces the availability of nitric oxide. Together, this creates a milieu of oxidative stress, inflammation, vascular constriction, and platelet hyperaggregability. This vicious cycle is propagated by decreasing insulin release by β-cells, an effect that is blunted by blocking the inflammatory cascade.[44]

Hyperglycemia also has specific effects on the host defense. Acute, short-term hyperglycemia affects all major components of innate immunity.[45] Neutrophil activity is reduced, leading to decreased chemotaxis, decreased phagocytosis, decreased bacterial killing, and overproduction of free radicals.[46] In addition to changes in cellular function, other components of the innate immune response contribute to the proinflammatory state in hyperglycemia. *In vitro*, human monocytes show a glucose-dependent increase in TNF-α and IL-6 production.[47–49] So although hyperglycemia is overall proinflammatory, it has specific effects that blunt the innate immune system; this likely contributes to the adverse outcomes observed in clinical studies, particularly those demonstrating increased infectious complications.

Stress Hyperglycemia: Maladaptation or Appropriate Adaptation?

Hyperglycemia in the acute phase is a physiologic response, and many believe the attempt to completely normalize glucose levels during this natural adaptive process may be counterproductive. With a more strict definition (blood glucose >110 mg/dL), hyperglycemia has been observed to be as high as 78%–98%, though most studies are small and carried out on specific populations.[2,50,51] Historically, stress hyperglycemia has been interpreted as an adaptive stress response that is important for survival. The overall increase in glucose turnover and the fact that hyperglycemia persisted despite abundant insulin were considered to suggest that moderately elevated blood glucose levels during critical illness would be beneficial. Moderate hyperglycemia (blood glucose 160–200 mg/dL) was recommended as a way to maximize cellular glucose uptake while avoiding hyperosmolarity.[52] This moderate hyperglycemia could be viewed as a buffer against hypoglycemia-induced brain damage.

Though the relatively high incidence of hyperglycemia could suggest an appropriate adaptation for survival, the negative consequences of high glucose levels suggest otherwise. Hyperglycemia among critically ill medical,[14] surgical,[2] cardiothoracic,[2,53] and trauma[50,51,54,55] patients is associated with a worse outcome.[50,56,57] Specifically, it has been associated with conditions such as infections, longer ventilator stays, acute renal failure, and polyneuropathy that lead to further inflammation on the systemic level. In addition, as hyperglycemia is controlled to blood glucose <150 mg/dL or the tightest of control 80–110 mg/dL, outcomes are further improved.[58] This would suggest that hyperglycemia is maladaptive.

Another possibility is that hyperglycemia does not play a causal role *per se* but that it is only a surrogate of severity of illness and is neither protective nor detrimental. In 1971, McNamara et al[7] demonstrated a correlation between lactate levels and hyperglycemia. This concept was recently revisited by Duane et al.[59] Through prospective analysis of 226 patients, these investigators demonstrated a linear relationship between both the injury severity score and serum lactate. Also, at least in trauma patients, early or admission glucose predicts infection rates, length of stays, and mortality.[50,51,55,60] In addition, persistent or repeated bouts of hyperglycemia, despite intensive insulin therapy, were also found to predict ventilator days, infections, length of stay, and mortality.[50,54]

In primarily observational studies, it is difficult to establish a causal role of hyperglycemia because it parallels known predictors of adverse outcomes. Although the randomized interventional trials do suggest a maladaptive response, achieving strict normoglycemia has been impossible on a population scale,[61] which limits the ability to clearly establish a causative role.

Insulin Therapy: Metabolic Effects

A growing consensus of critical care physicians supports glycemic control in the setting of stress and acute inflammation. This consensus includes critical care societies and organizations that now view the treatment of hyperglycemia as a critical component of the care of the acutely ill and inflamed patient. As one example, in 2004 the Surviving Sepsis Campaign Guidelines for Management of Severe Sepsis and Septic Shock included glucose control in its multifaceted approach to the care of the critically ill patient.[62] As the evidence for improved outcomes with intensive insulin therapy mounts, there remains uncertainty regarding the mechanisms by which insulin therapy provides outcome benefits—is it the glucose lowering effect *per se* or some other benefit of insulin therapy that leads to improved outcomes?

Insulin therapy acts to lower blood glucose by overwhelming the peripheral insulin resistance present in the skeletal muscle of the stressed patient. The peripheral uptake of glucose by skeletal muscle is improved as levels of messenger ribonucleic acid (mRNA) of GLUT-4 and hexokinase II are increased with insulin therapy.[63] Conversely, hepatic insulin resistance does not seem to be overcome by insulin therapy as the rate-limiting enzymes of gluconeogenesis (phosphoenolpyruvate carboxykinase) and glycogen synthesis (glucokinase) are unaffected by insulin therapy. Although strict glycemic control with intensive insulin therapy seems to prevent or reverse hepatocyte mitochondrial ultrastructure changes associated with hyperglycemia, analysis of liver biopsies obtained immediately after death from nonsurvivors in the Leuven study showed the classic insulin-regulated metabolic hepatic pathways do not respond to insulin.[63,64]

Many believe it is the anti-inflammatory properties of insulin that account for the beneficial effects of insulin therapy, regardless of the degree of glucose control.[13] Both CRP and mannose-binding lectin levels are lowered by the use of an insulin infusion intended to lower glucose.[2,13,63] Both of these findings seem to be independent of the presence of infection, an observation that has been confirmed in animal models of critical illness.[65] Whether insulin exerts its anti-inflammatory effects directly at the level of NFκB pathways or by lowering glucose to subsequently decrease systemic inflammation has not been elucidated. Whatever the mechanism, the result is a potent and comprehensive anti-inflammatory and antioxidant effect. The action is rapid (observed within 2 hours), and the magnitude of 2 units/h of insulin is similar to the effects of 100 mg of hydrocortisone given IV.[66]

During critical illness, once a caloric deficit of 6000–10,000 (25–30 kcal/kg/d over 1 week) is reached, mortality rates doubles.[67,68] Thereby, any adjunct to care of the critically ill preventing severe protein-calorie malnutrition would improve clinical outcome. Another possible mechanism by which insulin improves outcomes is by blunting the catabolic response to stress and inflammation. Hyperglycemia, blood glucose >200 mg/dL, alone seems to be associated with protein catabolism, illustrated by increased rate of muscle wasting.[69] The binding of insulin to its receptor normally suppresses proteolysis and activates protein synthesis. In humans, insulin therapy improves the protein content of skeletal muscle,[64] and in animals, insulin therapy has been demonstrated to prevent weight loss.[70] In burn patients, euglycemic hyperinsulinemia has been shown to improve both upper- and lower-extremity lean body mass as glucose levels were maintained between 100 and 140 mg/dL.[71] The Leuven study also observed less polyneuropathy of critical illness with intensive insulin therapy, a potential surrogate of weakness and muscle wasting seen in the ICU.[2] The exact mechanism for this effect is unknown because multiple anabolic factors, including growth hormone, are altered by intensive insulin therapy.[63] Whether hyperglycemia and insulin therapy or the resolution of the underlying systemic inflammatory response is responsible for the reversal of catabolism is unclear. In either case, improved outcomes are noted.

Deranged metabolism in the critical care unit is not merely represented by hyperglycemia. Triglyceride levels are also disturbed. In addition, there are increases in very-low-density lipoprotein (VLDL) and decreases in high-density lipoprotein (HDL) cholesterol levels.[72] This abnormal lipid profile found in critically ill patients has been shown to be proinflammatory.[73] However, as insulin therapy improves hyperglycemia, lipid profiles also improve. One component of the Leuven study examined whether this lowering of hypertriglyceridemia would affect outcome. Their multivariate logistic regression analysis demonstrated that this phenomenon explained a significant part of the beneficial effect on mortality and organ failure and seemed to be more important than the degree of glycemic control or insulin dose.[63]

In an attempt to determine whether it is the glucose-lowering effect or one or more of the other therapeutic effects of insulin that account for outcome benefits, *post hoc* multivariate logistic regression was performed from the Leuven study. In this analysis, both high insulin dosing and hyperglycemia independently predicted ICU mortality, suggesting that the glucose-lowering effects are the key mechanism of action.[58] There was also a lower risk of death as blood glucose levels were lowered from 200 mg/dL to 150 mg/dL, and then again to levels <110 mg/dL. Another large prospective trial by Finney and colleagues[74] demonstrated that control of glucose levels over absolute exogenous insulin levels accounts for the mortality improvement associated with intensive insulin therapy.[58] These concepts were recently supported in an animal model of prolonged and critical illness. The Leuven group demonstrated that avoiding hyperglycemia avoids excess mortality, endothelial and leukocyte dysfunction, and liver and kidney injury. In this model, insulin also had marginal positive effects on cardiac contractility and leukocyte dysfunction.[75] Although the actions of intensive insulin therapy are clearly complex, and the exact mechanism(s) by which benefit occurs is uncertain, both human and animal data are mounting in support of glycemic control in the critical care unit using insulin therapy.

Glucose Control: Easier Said Than Done?

If one accepts stress hyperglycemia as a maladaptive and detrimental response to stress and inflammation and wishes to achieve normoglycemia *via* intensive insulin therapy, the question arises: can we do it? Strategies to achieve normoglycemia in the critical care setting include the addition of insulin to the formula for parenteral nutrition (PN), scheduled subcutaneous insulin, IV insulin infusions titrated according to blood glucose response, and combinations of these strategies. Although all of these strategies have indications for use, the focus of blood glucose control has been on intensive insulin therapy using IV infusions. Before the van den Berghe et al[2] work, these other methods of glucose control—including subcutaneous sliding scale insulin—had achieved inadequate blood glucose control and worse clinical outcomes. The van den Berghe et al work hinges on the finding that *normoglycemia* (80–110 mg/dL) is the goal and that anything short of this range is associated with worsened outcomes. In practice, normoglycemia has only been consistently achieved with IV insulin infusions. In these studies, Mebis et al[76] reported 70% success in achieving target daily mean blood glucose, but this degree of control has not been duplicated.

In our own experience, precise blood glucose control in the ICU is impossible. We use a complex computer-based protocol designed to achieve normoglycemia (blood glucose between 80 and 110 mg/dL) with an IV insulin infusion. Trained nursing staff performs blood glucose measurements at 2-hour intervals and uses standard measures to prevent, detect, and treat hypoglycemia. We have published results of our ability to achieve normoglycemia as defined by the proportion of measurements within the therapeutic range.[77] Although mean (116 mg/dL) and median (117 mg/dL) blood glucose values (n = 53,031 data points) approach the euglycemic target, examining the densely collected raw data illustrates the enormous variability in not only the blood glucose values but also the timing of blood glucose measurements (Figure 2).[78]

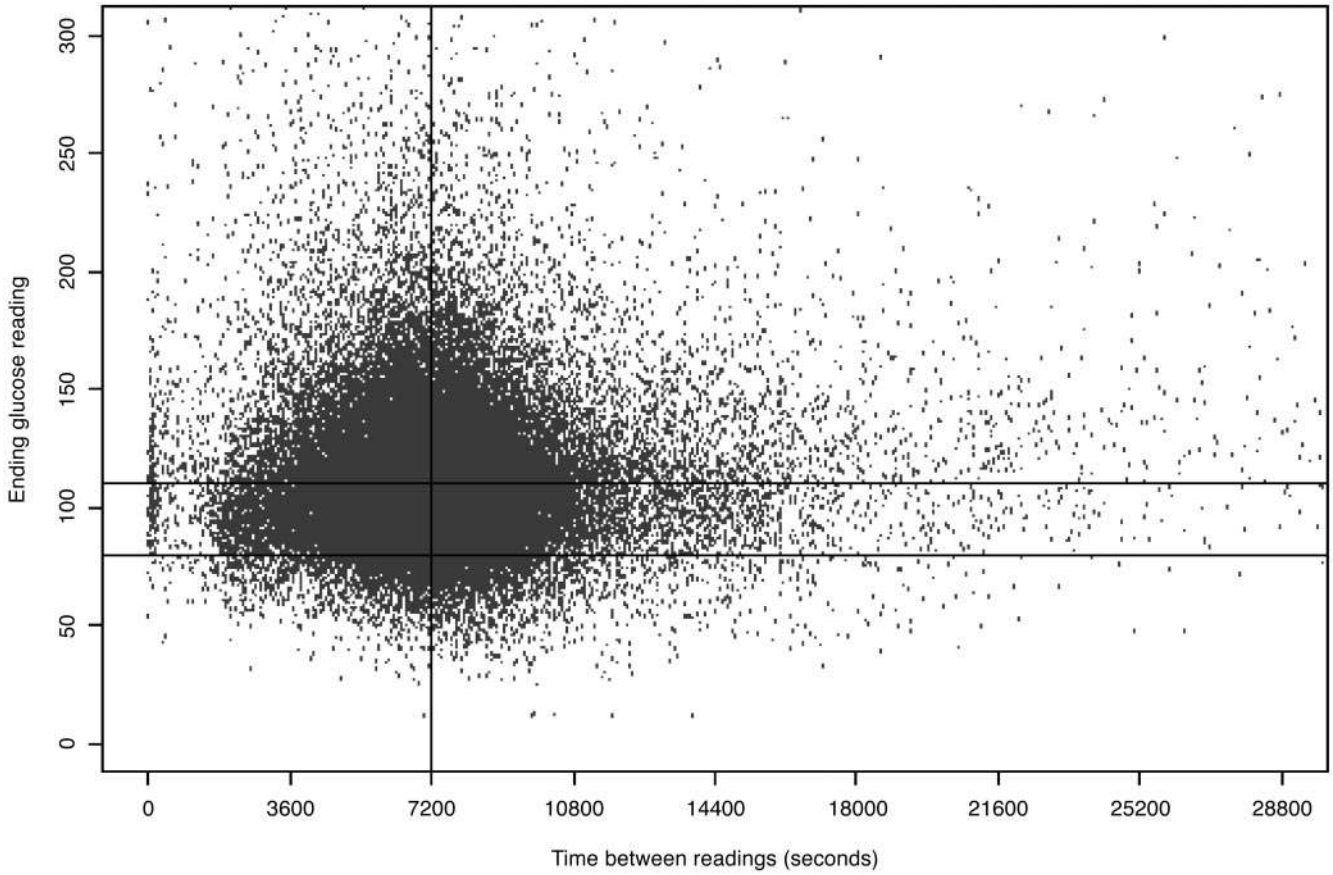

Figure 2. Blood glucose variability in the intensive care unit. Scatterplot of blood glucose (mg/dL) measurements (n = 53,031) as a function of the timing between measurements (every 2 hours per protocol) from critically ill surgical patients treated with intensive insulin therapy. This plot illustrates the wide variability in both blood glucose and the timing between measurements.

The variability in blood glucose reflects the overall difficulty in the implementation of tight glycemic control in the ICU. Despite sophisticated informatics, nursing care recognized as excellent by the American Nurses Credentialing Center (ANCC; http://nursecredentialing.org/magnet), and standard patient care protocols, the proportion of values within range (46%) is marginal. A number of factors likely contribute to this difficulty; perhaps most important is the lack of personalized care. Intensive insulin therapy largely continues to be a one-size-fits-all algorithm. However, acute care illnesses and inflammatory states of patients differ, as do their preexisting conditions that predispose them to stress hyperglycemia and the degree of insulin resistance.

An understanding of the pathophysiology of stress hyperglycemia sheds light as to why precise blood glucose control is difficult, if not impossible, with insulin therapy alone. Although the current literature raises concerns regarding the safety and efficacy of intensive insulin therapy—particularly in inexperienced hands—it is important to keep in mind these underlying mechanisms and direct therapies to controlling the patient's global inflammatory state. In this regard, therapies intended to achieve appropriate resuscitation, source control (well-timed operative intervention, appropriate antibiotics), and nutrition support will, at least indirectly, lessen the driving force of stress hyperglycemia (Figure 3).

Glucose Control in Light of Acute Inflammation: Current Controversies

Is Normoglycemia Necessary for All Patients?

Although others had suggested improved survival with treatment of hyperglycemia, van den Berghe and colleagues[2,14,15,58] described the need for normoglycemia—blood glucose 80–110 mg/dL—to obtain optimal survival benefit. In a *post hoc* analysis of the Leuven study, an upper target of 150 mg/dL could not be safely substituted for 110 mg/dL. Despite the reduction in mortality seemingly associated with strict blood glucose control, many question whether normoglycemia is necessary for all patient groups. Laird et al[51] evaluated 516 trauma patients and found blood glucose >200 mg/dL to be an independent predictor of infection and mortality, but no

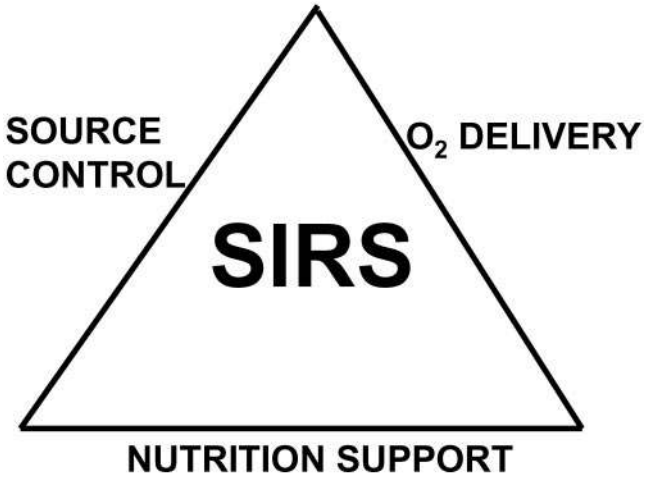

Figure 3. Key components in the treatment of the systemic inflammatory response syndrome (SIRS). Effective treatment of SIRS requires a focus on 3 strategies: the delivery of oxygen (O_2) to vital tissues, source control (control of bleeding, necrotic and infected tissues), and provision of nutrition support. Control of the patient's global inflammatory response is crucial for achieving normoglycemia.

such relationship existed for cutoffs at lower levels of blood glucose control such as 150 mg/dL or 110 mg/dL. We have demonstrated worse outcomes when 1 or more blood glucose levels exceed 150 mg/dL.[50] However, those patients with hyperglycemia greater 150 mg/dL not only had worse outcomes but also they could not achieve glucose control, nor did the insulin protocol to lower glucose improve outcomes when compared with those controlled without protocol. In a similar population, Bochicchio et al[54] demonstrated worse outcomes only when the glucose level exceeded 139 mg/dL. Most recently, Reed and colleagues[79] demonstrated a mean blood glucose of 125 mg/dL and associated lower mortality *via* the implementation of a fully integrated glucose protocol. Similar to other trauma literature, the glucose values could not be lowered to the goal obtained in the Leuven study. However, outcomes including infection, ventilator days, and mortality improved with the implementation of a protocol. Determining the optimal range for therapy—one that maximizes benefit while minimizing adverse events—will require carefully controlled prospective studies. Until these data become available, one should aim for achieving strict glucose control with a protocol that has acceptable rates of hypoglycemia.

It is unclear at what blood glucose cutoff the resulting proinflammatory cascade and disruption of the innate immune system occur. Hoedemaekers et al[80] randomized patients to strict (blood glucose 80–100 mg/dL) or conventional (blood glucose <200 mg/dL) insulin therapy after cardiac bypass and did not demonstrate a difference in circulating proinflammatory cytokines between the groups, although the study was small (n = 10 in each group) and likely underpowered.

Are Other Measures of Glucose Control Important?

In the chronic diabetic patient, hemoglobin A_1C is considered the gold standard for blood glucose control, but a growing body of evidence suggests that blood glucose variability is also important.[81] In these patients, measures of blood glucose variability have been linked to outcomes (eg, development of retinopathy), and the combination of blood glucose variability and hemoglobin A_1C is considered to be a more reliable indicator of blood glucose control in regard to complications.[81] These findings are thought to be due to the increased oxidative stress that results from rapid fluctuations of blood glucose because rapidly fluctuating blood glucose is more detrimental than sustained hyperglycemia.[82] Egi et al[83] recently reported the importance of blood glucose variability in a large cohort of critically ill patients. These investigations concluded that blood glucose variability, as measured by standard deviation, was an important determinant of outcome. The major limitation of this study was that patients were not receiving a standardized insulin protocol and there were no data on insulin dosing. As noted by Ouattara et al,[84] it is uncertain whether or not these results apply to patients managed with IV insulin. Vogelzang and colleagues[85] evaluated a similar measure, the hyperglycemic index (HGI), a measure of abnormal glucose levels divided by the length of stay. This seemed to be a slightly better predictor of mortality compared with other glucose indices such as admission or mean glucose values. HGI was further evaluated in trauma patients and was found to more accurately predict mortality than in nontrauma patients.[86]

Whether blood glucose and insulin dosing variability is simply a reflection of the magnitude of a patient's inflammatory response or somehow causes adverse outcomes is unknown. Varying degrees of insulin resistance related to a patient's global inflammatory state or differences in patient genotype or phenotype could account for these differences. What is uncertain, though, is whether or not a patient's variability in glucose control and insulin dosing during the course of a critical illness could pathophysiologically contribute to ongoing inflammation and adverse outcomes. Although establishing a causal role of blood glucose variability would be exceedingly difficult, several known biologic mechanisms could support this hypothesis. Apoptosis is markedly increased in human umbilical vein endothelial cells exposed to periodic exaggerations of hyperglycemia.[87] Quagliaro et al[88] investigated the differential effect of variable glucose concentrations *vs* stable high glucose levels on hyperglycemia-induced reactive oxygen species generation. Recent evidence in the chronic diabetic population indicates that rapid fluctuations in blood glucose are associated with increased oxidative stress.[89] Others have shown that cytokine production from fibroblasts is increased in cells exposed to fluctuating levels of

glucose *vs* high glucose levels alone.[90] These data suggest that variability in glucose could be more deleterious to cells than constant high blood glucose, although constant levels of near-normal blood glucose would be least damaging of all.

If indeed glucose variability is an important cause of oxidative stress, ongoing inflammation, and adverse outcomes in the critically ill population, insulin protocols should be adapted to minimize extreme glucose excursions when possible. Prevention and timely treatment of complications in the ICU will also help to avoid a second-hit phenomenon that could contribute to fluctuations in blood glucose after appropriate control had been previously achieved. Although measures of glucose variability are intriguing, they are dependent on retrospective analysis and real-time use, and prospective outcome prediction is not possible.

Potential Harm of Hypoglycemia

In 2005, 33% of fatal medical errors were reportedly related to insulin therapy,[91] and this is the biggest fear of physicians and nurses implementing intensive insulin therapy. The degree to which they fear this complication can introduce psychological barriers to aggressive insulin therapy. This fear is stimulated by the fact that the symptoms of hypoglycemia are not easily recognized in ICU patients and the fact that severe or prolonged hypoglycemia can cause convulsions, coma, irreversible brain damage, cardiac arrhythmias, and death.[14,16,17,91,92]

The frequency of hypoglycemia (typically defined as blood glucose <40 mg/dL) varies, depending on the target range and the overall aggressiveness of the protocol, with the highest incidence associated with the most aggressive regimens. In the Leuven studies, the rate of hypoglycemia was significantly higher in the aggressive *vs* conservative therapy arms, but there was no detected difference in either short- or long-term outcomes. The increased incidence of hypoglycemia led to the interruption of 2 large multicenter prospective trials in Europe, and in at least 1 study, hypoglycemia was linked with increased mortality despite similar illness severity scores.[20]

Retrospective reviews have been useful in identifying risk factors for hypoglycemic events. They include sepsis, diabetes, requirement for inotropic support, and continuous venovenous hemofiltration (CVVH)[18,19] What is uncertain is the sequela of these events. van den Berghe et al[93] performed a pooled analysis of the 2748 patients enrolled in both the surgical and medical randomized controlled trials to assess the clinical consequences of hypoglycemic episodes. Although intensive insulin therapy was associated with an increased incidence of hypoglycemia (12%) *vs* conventional therapy (4%), there was no increase in either short-term or long-term mortality. Vriesendorp et al[18] addressed the same issue in a nested case-control study comparing 156 consecutive patients with hypoglycemia during intensive insulin therapy in their mixed ICU with closely matched control patients at risk of hypoglycemia. Although 3 patients had possible hypoglycemia-induced coma or general seizures, there was no difference in mortality between the groups.

Before intensive insulin therapy is widely adopted, we need to understand both the short- and long-term consequences of hypoglycemia and balance this risk with the need to achieve normoglycemia. If hypoglycemic events are indeed associated with adverse outcomes, and these events occur more frequently with the most aggressive glucose targets, a careful look at the goals of intensive insulin therapy is warranted.

Summary

Almost a century ago, insulin was discovered as a glucose-lowering endogenous hormone. Since then, the provision of insulin has been used to treat hyperglycemia in both the acute and chronic setting. The pendulum has swung back and forth, attempting to understand hyperglycemia in the acute care setting as friend or foe. There appears to be good clinical and animal research to suggest that insulin provision or lower glucose levels are anti-inflammatory. In addition, if glucose levels can be lowered safely in the acute critical care setting, there is reduced morbidity and mortality. Despite many unanswered questions, glycemic control *via* intensive insulin therapy seems to be an adjunct to overall source control by limiting the inflammatory cascade. Normoglycemia is a target, but it may only represent a surrogate of decreased inflammation, reflective of the improved patient care found within the modern ICU.

In the last 10 years, the discipline of critical care medicine has prospered, with several large randomized trials demonstrating an improvement in outcomes.[2,94–98] These points of care include early antibiotic and resuscitation provision, low tidal volume administration, lower transfusion threshold, appropriate administration of corticosteroids and activated protein C, and tight glucose control, and share a common theme—they address the inflammatory cascade directly or indirectly. Thereby, the systemic inflammatory process during the secondary insult is controlled. In the future, investigations will focus on antisepsis drugs, antioxidants, and other adjuncts similar to insulin provision that focus on this anti-inflammatory source control at the cellular and molecular level. Modulating inflammation will not only be the focus of research in the years to come but will likely be the key component to improve survival in the critically ill population.

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Invited Review

Obesity, Inflammation, and the Potential Application of Pharmaconutrition

Matt C. Cave, MD*; Ryan T. Hurt, MD*†; Thomas H. Frazier, MD*; Paul J. Matheson, PhD‡; Richard N. Garrison, MD‡‖; Craig J. McClain, MD*§‖; and Stephen A. McClave, MD*

**Departments of Medicine, †Physiology and Biophysics, ‡Surgery, and §Pharmacology and Toxicology, University of Louisville, Louisville, Kentucky; and the ‖Louisville Veterans Affairs Medical Center, Louisville, Kentucky*

ABSTRACT: Obesity is an emerging problem worldwide. Hospitalized obese patients often have a worse outcome than patients of normal weight, particularly in the setting of trauma and critical care. Obesity creates a low-grade systemic inflammatory response syndrome (SIRS) that is similar (but on a much smaller scale) to gram-negative sepsis. This process involves up-regulation of systemic immunity, is characterized clinically by insulin resistance and the metabolic syndrome, and puts the patient at increased risk for organ failure, infectious morbidity, and mortality. Through lipotoxicity and cytokine dysregulation, obesity may act to prime the immune system, predisposing to an exaggerated subsequent immune response when a second clinical insult occurs (such as trauma, burns, or myocardial infarction).

Specialized nutrition therapy for such patients currently consists of a hypocaloric, high-protein diet. However, this approach does not address the putative pathophysiologic mechanisms of inflammation and altered metabolism associated with obesity. A number of dietary agents such as arginine, fish oil, and carnitine may correct these problems at the molecular level. Pharmaconutrition formulas may provide exciting innovations for the nutrition therapy of the obese patient.

Obesity, defined as body mass index (BMI) >30 kg/m^2, increased from 15% to 33% of the adult population in the United States between 1980 and 2004.[1,2] Perhaps even more alarming is the increase in overweight children (by age- and height-specific guidelines) from 6% to 19% over the same time period.[1,3] Numerous conditions complicate obesity, such as type 2 diabetes, hypertension, hypercholesterolemia, hypertriglyceridemia, and nonalcoholic fatty liver disease (NAFLD). These comorbidities significantly increase the cost and complexity of patient care.[1] The direct medical costs of obesity in the United States are difficult to determine.[1] Finkelstein et al[4] estimated U.S. medical costs for obesity at more than $92 billion dollars per year in 2002. Obesity-associated pathologic conditions are the probable cause of the increase in mortality observed in obese individuals, rather than obesity *per se*. As a direct result of the various obesity-associated conditions, these patients present challenging and complex issues in medical and surgical intensive care units (ICUs). In this review, we examine the impact of obesity on patient outcome in the ICU, the current standard of nutrition therapy for obese patients, and the role that chronic inflammation plays in obesity and its associated comorbidities. According to current information available in the literature, potential benefits exist for the treatment of obese patients with immune-enhancing diets, or pharmaconutrition, which could potentially modulate the chronic inflammatory state and lessen the severity of the comorbid conditions seen in this disease process.

Correspondence: Stephen A. McClave, MD, Department of Medicine, Division of Gastroenterology/Hepatology, University of Louisville School of Medicine, 500 S. Jackson Street, University of Louisville, Louisville, KY 40292. Electronic mail may be sent to samcclave@louisville.edu.

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Impact of Obesity on Patient Outcome in Trauma and Critical Illness

In the obese critically ill patient, multiple associated comorbidities, such as diabetes, may increase risk in the ICU. With few exceptions,[5] most reports in the literature indicate that obese patients have a more adverse outcome in a medical ICU compared with nonobese patients. Obese critically ill patients tend to have a higher simplified acute physiologic score (SAP), are more likely to have depressed left ventricular function, and are at greater risk for multiple-organ failure (MOF) than the nonobese.[6,7] Obese patients have been shown to have longer duration of mechanical ventilation (7.7 *vs* 4.6 days) and increased ICU length of stay (9.3 *vs* 5.8 days) compared with nonobese patients.[6,8] Obese patients also had higher rates of ICU-acquired infections,

sepsis, and ventilator-associated pneumonia (VAP) than their nonobese counterparts.[9] Not surprisingly, several studies have shown overall mortality to be significantly higher in obese (30%–32%) *vs* nonobese (17%–18%) ICU patients.[6,7,9]

Similarly, the data suggesting more adverse outcome are even more compelling in obese trauma patients. In blunt (nonpenetrating) trauma, obesity has been shown to increase pulmonary infections, ICU length of stay (LOS), and mortality. Choban and colleagues[10] reported a direct correlation between BMI and mortality. The mortality rate was 42% in obese trauma patients (BMI $\geq$31 kg/m^2) compared with 8% in those patients who were overweight (BMI 27–31 kg/m^2) and 5% in those who had normal weight (BMI $\leq$27 kg/m^2).[10] After controlling for factors such as age, diabetes, chronic obstructive pulmonary disease (COPD), and injury severity score (ISS), obese patients were shown to be 7.1 times more likely to die compared with nonobese patients.[11] Obesity has been shown to affect the incidence of MOF in blunt force trauma patients as well.[12] Obese trauma patients had a higher rate of MOF (37% *vs* 22%) than nonobese control patients.[12] Obesity was independently associated with MOF when controlling for variables such as age and ISS.[12] Additional findings from these trauma studies suggest that obese individuals have increased hospital LOS and infectious morbidity (bacteremia, urosepsis, and VAP) compared with nonobese controls.[11,12]

Of interest is the observation that nutrition therapy, a critical component of ICU care, has not been independently investigated in any of these aforementioned ICU or trauma studies. In fact, specific evidence gleaned from the evaluation of enteral nutrition therapy in obese ICU patients is limited to a handful of inadequately controlled studies.

Nutrition Therapy and Obesity

The current gold standard of nutrition therapy for obese ICU patients includes enteral tube feedings that are high in protein and low in total calories.[13] Previous studies evaluating parenteral feeding in obesity showed benefit from hypocaloric or hypoenergetic diets.[14,15] These diets contained nutrient mixtures characterized by a low calorie-to-nitrogen ratio, with an exaggerated provision of protein (up to 2 g per kg of ideal body weight per day). In 2002, Dickerson et al[16] reported a study that evaluated hypocaloric enteral tube feeding in critically ill obese patients. Results showed improved outcome from this hypocaloric diet, with decreased ICU LOS and a trend toward reduced duration of mechanical ventilation compared with patients given a "eucaloric" diet.[16] Combined, these studies suggest that critically ill obese patients given a hypocaloric diet either enterally or parenterally could achieve nitrogen balance despite ongoing weight loss. Furthermore, Choban and colleagues[17] examined a combined database of hospitalized obese patients stratified by BMI. They found that higher BMI correlated with more negative nitrogen balance, hyperglycemia, and even mortality at a given level of dietary protein intake. Therefore, as the degree of obesity (BMI) increases, the dietary protein intake required to achieve nitrogen balance also increases (>2.5 g/kg ideal body weight [IBW]/d). Although these findings are a step in the right direction, the strategy of providing a high-protein hypocaloric diet for these patients does not address the key underlying pathologic process of obesity: chronic inflammation. Inflammation generated by obesity itself may be the link to the adverse health consequences that place these patients at increased risk in the ICU.

Mechanisms of Obesity-Induced Inflammation and Organ Dysfunction

Obesity causes inflammation and organ dysfunction through a variety of mechanisms that are becoming increasingly well defined. Understanding these mechanisms is important in order to identify molecular targets for nutrition intervention. Mechanistically, lipotoxicity is a critically important emerging concept. During lipotoxicity, a key factor of obesity-associated inflammation is adipokine dysregulation.

Lipotoxicity

A critical emerging concept is that of "good fat *vs* bad fat."[18] There is great variation between individuals in their ability to safely manage caloric excess. Not all obese patients develop the metabolic syndrome or other obesity-associated diseases. Some patients may gain weight but remain relatively healthy, whereas others lose weight but deteriorate from an overall health standpoint. For example, in a rodent model of obesity, weight loss with the popular dietary supplement conjugated linoleic acid (CLA) produces lipodystrophy, insulin resistance, and NAFLD.[19] Ironically, these rodents have shown progression from a state of being relatively "fat and healthy" to a state of being "skinny and unhealthy." Lipid management at the cellular level influences the degree to which disease processes and comorbidities develop in obesity. Adipogenesis may actually be a protective adaptation against caloric excess.

When caloric intake exceeds energy expenditure, excess calories will be stored as lipids. Both the location and type of lipid accumulation determine the presence or absence of obesity-related disease. All tissues have an inherent ability to store lipid. However, different tissues vary greatly in the quantity of lipid they can safely store. For example, adipose tissue has a much greater innate lipid storage capacity than other "ectopic" tissues, such as liver or muscle. When lipid accumulation exceeds this innate storage capacity, cellular and ultimately

organ dysfunction may ensue. This phenomenon has been called *lipotoxicity*. Experimentally, tissue-specific disease has been demonstrated to occur in the presence of excess lipid accumulation in the following organs: liver (NAFLD), pancreas (diabetes), muscle (insulin resistance), and heart (diabetic cardiomyopathy).[20] Furthermore, there seems to be an organ-specific hierarchy for safe lipid storage. Peripheral (subcutaneous) adipose tissue is preferable to central (visceral) adipose tissue, which, in turn, is preferable to ectopic tissues such as the liver. However, it is becoming increasingly clear that even adipose tissue may be subject to lipotoxicity, with manifestations of adipokine dysregulation.[21]

Although the amount and location of lipid storage are critical, perhaps the most important determinants of lipotoxicity are the specific type of lipid stored and how it is stored. Saturated free long-chain fatty acids (LCFA) seem to be the most plausible inducers of lipotoxicity, whereas monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) may be protective.[22–25] Furthermore, esterification of LCFA into triglycerides appears to be a protective "sink," or less toxic storage form for LCFA.[23,26] In an animal model of NAFLD, disruption of triglyceride synthesis resulted in much more severe hepatic inflammation and fibrosis due to increased intracellular free fatty acids (FFAs).[26]

There are a variety of intracellular and extracellular mechanisms responsible for lipotoxicity. These include adipokine dysregulation, apoptotic cell death ("lipoapoptosis"), oxidative stress, unfolded protein response (endoplasmic reticulum stress), mitochondrial dysfunction, endothelial dysfunction, and alterations in the trans-methylation or trans-sulfuration pathways.[27,28] Intriguingly, many of these mechanisms are amenable to specialized nutrition therapy, apart from traditional weight loss diets. Each mechanism may have some degree of tissue specificity, as evidenced by the fact that an effective treatment for one organ may be potentially deleterious to another. For example, peroxisome-proliferator-activated receptor-γ (PPAR-γ) agonists (such as the oral hypoglycemic agent rosiglitazone) promote adipocyte proliferation and insulin sensitivity, while down-regulating the inflammatory response through inhibition of nuclear factor κ-B (NFκB) activation.[29] Likewise, vitamin E may be protective against oxidative stress in lipotoxicity. Although these characteristics would make these agents appropriate for the treatment of NAFLD,[30] their clinical use is thwarted by reports of toxicity on another organ system (increased rate of cardiac events for both PPAR-γ agonists and vitamin E).[31,32]

Similar to lipotoxicity, deleterious metabolic changes may be caused by excess dietary intake of simple carbohydrates. Reports have shown that NAFLD patients with significant inflammation on liver biopsy report higher carbohydrate and simple sugar consumption than those without inflammation.[33,34] In fact, high-carbohydrate diets are used in rodent models to generate NAFLD. Although the term *glucotoxicity* has been used to describe this phenomenon (implying a generalized effect from all carbohydrates), the most plausible culprit is the specific carbohydrate, high-fructose corn syrup. The obesity epidemic (with associated NAFLD) in the United States that has occurred over the past 4 decades parallels the increased consumption of high-fructose corn syrup (used as a sweetener, replacing pure cane sugar).[35] Different dietary simple sugars may promote different patterns of fat distribution in obesity. In a mouse model, fructose promoted the development of hepatic fatty infiltration, whereas glucose promoted peripheral adiposity with relative sparing of the liver.[36]

The concepts of lipotoxicity and glucotoxicity have important implications in the generation of "good fat *vs* bad fat." Available data suggest that in the optimal situation, excess dietary calories would be stored as PUFA esterified into triglycerides in subcutaneous adipose tissue ("good fat"). In the worst-case scenario, excess calories would be deposited in the form of saturated free LCFA in more vital, nonadipose organs such as the heart and liver ("bad fat").

Adipokines

The most important mechanism of inflammation in lipotoxicity associated with obesity is adipokine dysregulation (Figure 1). This topic deserves special attention because restoration of these pathways is central to immunonutrition in obesity. Adipokines are a group of soluble molecules that are largely secreted by white adipose tissue (WAT).[37] A wide array of metabolically active molecules is produced by adipocytes and a group of cells associated with WAT known as stroma vascular fraction (SVF) cells (Table 1; ie, preadipocytes, fibroblasts, endothelial cells, histiocytes, and macrophages).[38] This "cocktail" of inflammatory factors includes a complex mixture of cytokines, factors of the Complement cascade, and chemoattractant molecules (Table 1). In addition, increased levels of acute-phase proteins such as haptoglobin, C-reactive protein (CRP), interleukin IL-6, and serum amyloid A protein have been shown to correlate with increasing degrees of obesity. The increased expression or release of these mediators seen in obesity is ameliorated by weight loss.[38,39]

Tumor Necrosis Factor α (TNF-α). TNF-α is a critical proinflammatory cytokine associated with both the inflammation and insulin resistance seen in obesity. Release of TNF-α is tightly linked to increased circulating levels of FFAs, which, in turn, have been associated with increasing obesity, insulin resistance, and lipotoxicity.[40] TNF-α is both a cause and an effect of increased circulating FFAs in obesity. Infusion of TNF-α has been shown to increase the amount of FFAs in circulation and worsen insulin resistance.[41–43] Levels of TNF-α in

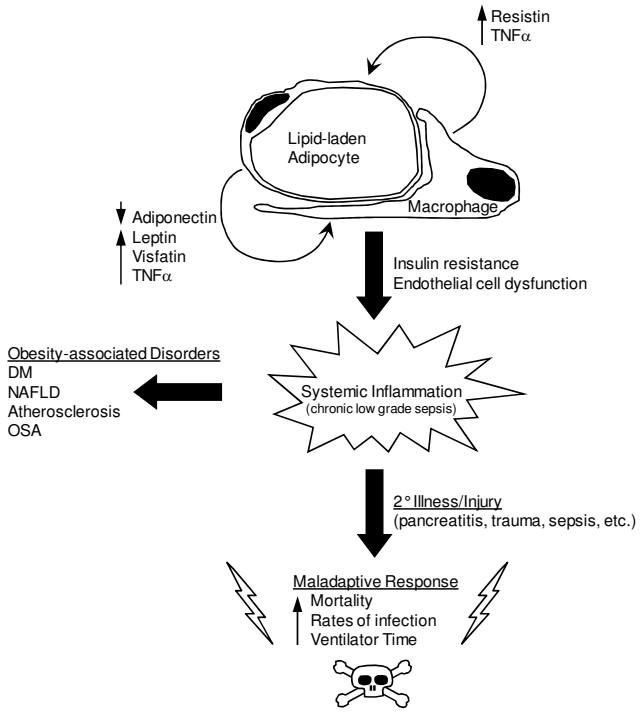

Figure 1. Obesity-associated inflammation acts clinically as a chronic, low-grade SIRS response. This figure depicts the pathophysiological progression and underlying mechanisms that lead to insulin resistance, endothelial cell dysfunction (vasoconstriction), and release of cytokines. With the underlying systemic inflammation, obese patients exposed to secondary illness or injury may experience exaggerated infection, duration of mechanical ventilation, and mortality.
DM, diabetes mellitus; NAFLD, nonalcoholic fatty liver disease; OSA, obstructive sleep apnea; SIRS, systemic inflammatory response syndrome; TNFα, tumor-necrosis factor-α.

Table 1 *Metabolically active molecules produced by adipocytes and stroma vascular fraction (SVF) cells of white adipose tissue (WAT)*

Cytokines
Tumor necrosis factor-α (TNF-α)
Adiponectin
Leptin
Resistin
Transforming growth factor-β (TGF-β)
Interferon-γ (IFN-γ)
Interleukins (IL-1, IL-6, IL-8, IL-10)
Factors of the Complement cascade
Plasminogen activation inhibitor-1 (PAI-1)
Fibrinogen
Angiopoietin-related proteins
Complement factor-3
Chemoattractant molecules
Monocyte chemotactic protein-1 (MCP-1)
Macrophage inflammatory protein-1α (MIP-1α)

adipose tissue are positively correlated with the degree of obesity, as are levels of circulating FFAs.[25,44,45]

Shi et al[25] demonstrated that certain FFAs induce production of TNF-α from cultured macrophages *via* Toll-like receptor 4 (TLR4) and NFκB activation. Particularly robust production of TNF-α can be induced by the long-chain saturated fatty acids myristic, palmitic, and stearic acid. This inflammatory effect induced by FFAs is similar to that effect observed for endotoxin or lipopolysaccharide (LPS) although to a lesser extent, which also acts through binding of TLR4 and subsequent activation of NFκB. These saturated FFAs are structurally similar to the lipid-A moiety of LPS, and it is probably this similarity that allows them to bind and activate TLR4. These data may indicate that the immune system cannot fully differentiate certain circulating FFAs from endotoxin or LPS. Therefore, to the immune system, obesity may be indistinguishable from chronic, low-grade, gram-negative, bacterial sepsis. The key effector of this interaction appears to be TNF-α, although other proinflammatory cytokines under NFκB regulation are likely involved as well. The increased TNF-α expression caused by FFAs can be almost completely blocked by docosahexaenoic acid (DHA), which is a component of both fish oil and many pharmaconutrition formulas.[25] This information implies that fish oil may be an effective anti-TNF-α agent in obesity.

Adiponectin. Adiponectin is a key mediator of obesity-associated insulin resistance and tissue inflammation. Considered a peripheral long-acting adipokine released from adipose tissue, adiponectin acts primarily by reducing inflammation and improving insulin sensitivity. Adiponectin exists in multiple isoforms with varying functions.[37] Two receptors have been identified: adiponectin cellular receptor 1 (ADIPOR1) expressed widely throughout WAT and ADIPOR2 expressed mainly in the liver.[37,39,46] In contrast to other adipokines, adiponectin is markedly reduced in individuals with visceral adiposity when compared with their lean counterparts.[47] A number of factors have been shown to regulate the production of adiponectin by WAT, including TNF-α, IL-6, and PPAR-γ.[48–50]

Adiponectin exerts its anti-inflammatory effect through opposition to TNF-α.[51] Adiponectin attenuates the macrophage response to TLR4 through the activation of ADIPOR1.[52] In this way, adiponectin suppresses TLR4-induced NFκB activation and suppresses the production of interferon-γ generated by LPS.[53] By inhibiting the expression of adhesion molecules induced by TNF-α, adiponectin attenuates macrophage adherence, phagocytic capacity, and transmigration.[54] In addition, adiponectin induces the production of other anti-inflammatory mediators (such as IL-10 and IL-1 receptor antagonist) by macrophages, monocytes, and dendritic cells. These molecular effects of adiponectin are illustrated in Figure 2 and Table 2.

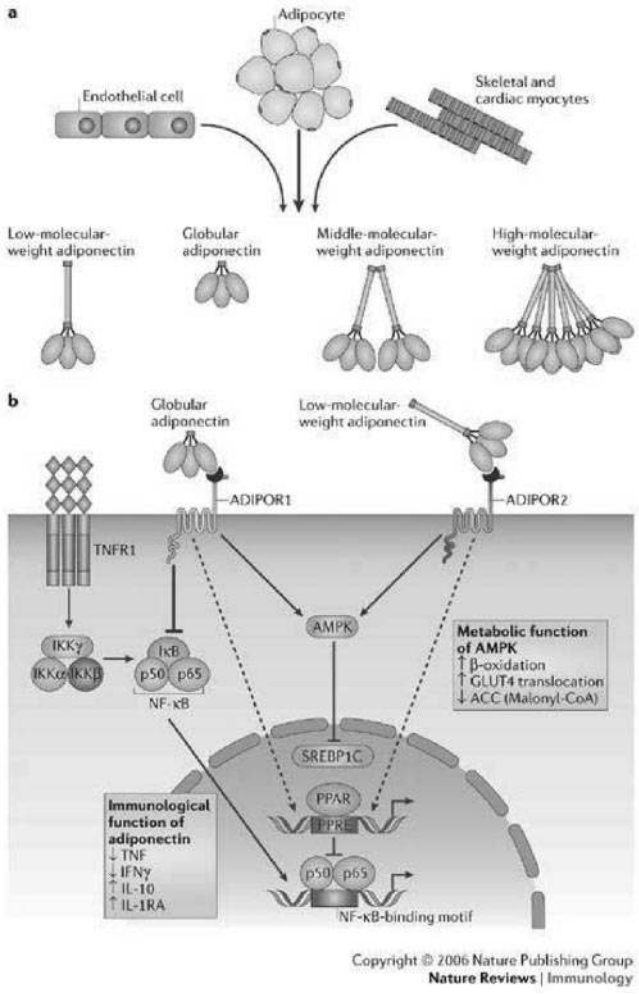

Figure 2. A, Adiponectin is produced mainly by adipocytes, but other cell types such as myocytes (skeletal and cardiac) and endothelial cells can also produce this adipokine. B, Adiponectin interacts with 2 ADIPOR receptors (1 and 2) to stimulate the activation of PPAR, AMP-K, and p38 MAP-K. Adiponectin regulates the expression of several pro- and anti-inflammatory cytokines. Its main anti-inflammatory function might be related to its capacity to suppress TNF and IFN-γ and to induce IL-10 and IL-1 RA. Activation of PPAR exerts anti-inflammatory effects.
ADIPOR, adiponectin cellular receptor; AMP-K, AMP-activated protein kinase; GLUT, glucose transporter; IFN, interferon; IL, interleukin; IL-1 RA, interleukin-1 receptor antagonist; NFκB, nuclear factor κB; MAP-K, mitogen-activated protein kinase; PPAR, peroxisome-proliferator-activated receptor; PPRE, peroxisome-proliferator response element.
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Finally, reduced circulating levels of adiponectin are a key component of obesity-induced insulin resistance and dyslipidemia.[53] Treatment of obese animals with adiponectin has been shown to attenuate serum hyperglycemia, reduce levels of FFAs, and improve insulin sensitivity.[55] Adiponectin directly stimulates β-oxidation of fat in hepatocytes and down-regulates the major transcription factor involved with lipid synthesis (sterol-regulatory-element-binding protein 1C).[53] The role of adiponectin in improving insulin sensitivity appears to be mediated through phosphorylation of adenosine 5′ monophosphate-activated protein kinase (AMPK) and its subsequent activation in the liver.[56] Through this same mechanism (AMPK activation), adiponectin has been shown to protect the myocardium from ischemia-induced apoptosis.[57]

Leptin. Leptin, an adipokine produced and secreted by subcutaneous WAT, modulates food intake and energy balance by controlling appetite.[58] Leptin regulates neuroendocrine function, energy homeostasis, hematopoiesis, and angiogenesis. Structurally, leptin is similar to other pro-inflammatory cytokines such as IL-6 and IL-12. Serum levels of leptin are proportional to overall adipose mass.[58] High serum leptin and, more importantly, leptin resistance (implied by the failure of leptin to induce satiety) is observed in diet-induced obese rats.[47]

In addition to controlling appetite, leptin also plays a role in both innate and adaptive inflammatory responses.[58] Leptin has been shown to increase the production of pro-inflammatory cytokines from macrophages (TNF-α, IL-6, and IL-12) and hepatic stellate cells (MCP-1).[59,60] The inflammatory response to leptin is, in part, mediated by the activation of NFκB and the subsequent production of TNF-α (an effect which is attenuated by release of adiponectin).[60,61] Leptin causes proliferation of macrophages and leads to the activation, chemoattraction, and cytotoxicity of both neutrophils and natural killer cells.[62] These processes result in production of reactive oxygen species, raising the overall level of oxidative stress.[62] These effects of leptin are illustrated in Figure 3A and Table 2.

Resistin. Resistin is a polypeptide adipokine produced by numerous tissues, including adipocytes, muscle, pancreatic tissues, and mononuclear cells.[51] Expression of this adipokine is increased in response to IL-6, IL-1, TNF-α, and LPS.[41,63] Adiponectin and PPAR-γ agonists have the opposite effect, decreasing synthesis and release of resistin.[41] Increased resistin levels are associated with NFκB activation and the subsequent expression of numerous proinflammatory cytokines, including TNF-α, IL-1β, IL-12, and IL-6.[64] Resistin has been implicated in the pathogenesis of type 2 diabetes mellitus, as studies have suggested a relation between increasing resistin levels and insulin receptor insensitivity.[65] Finally, the effect of resistin on the microvasculature opposes that of adiponectin, such that resistin induces endothelial adhesion molecules, promoting injury to the vascular endothelium and increasing risk for atherosclerosis.[66] These effects of resistin are illustrated in Figure 3B and Table 2.

Table 2 *Adipokines and inflammation*[51]

	Overall action	Role within the innate immunity	Associated diseases
Adiponectin	Anti-inflammatory	↑ IL-10, IL-1RA ↓ NFκB-mediated endothelial adhesion molecule expression and cytokine release, phagocytosis, IL-6, TNF-α, and INF-γ,	DM, OSA, NAFLD, ASH, CAD, RA, cancer, IBS
Resistin	Proinflammatory	↑ VCAM1, ICAM1, IL-6, TNF-α, IL-1β, NFκB-mediated endothelial adhesion molecule expression and cytokine release	DM, OSA, NAFLD, CKD, CAD, RA
Leptin	Proinflammatory	↑ TNF-α, IL-6, IL-12, neutrophil activation, ROS release and chemotaxis, NK-cell function, macrophage activation, and cytokine release	OSA, NAFLD, asthma, cancer
Visfatin	Proinflammatory	↑ IL-6, IL-8; ↓ apoptosis of neutrophils	Sepsis, acute lung injury, DM

ASH, alcoholic steatohepatitis; CAD, coronary artery disease; CKD, chronic kidney disease; DM, diabetes mellitus; IBS, irritable bowel syndrome; ICAM, intercellular adhesion molecule; IFN-γ, interferon- γ; IL, interleukin; IL-1RA, IL-1 receptor antagonist; NAFLD, nonalcoholic liver disease; NFκB, nuclear factor κB; NK, natural killer; OSA, obstructive sleep apnea; RA, rheumatoid arthritis; ROS, reactive oxygen species; TNF-α, tumor-necrosis factor-α; VCAM, vascular cell-adhesion molecule.

Emerging Adipokines

Several new adipokines are now under investigation. Much like adiponectin, visceral adipose tissue-derived serine protease inhibitor (VASPIN) suppresses the production of leptin, TNF-α, and resistin and thus helps improve insulin sensitivity.[67] Serum retinol-binding protein 4 (RBP4), released from adipose tissue, which lacks a specific glucose transporter, has the opposite effect, inducing insulin resistance and increasing risk for clinical diabetes mellitus.[68] Finally, visfatin is a recently identified adipokine that decreases insulin resistance but has been linked in the past to several inflammatory disease states, such as acute lung injury (Table 2).[69,70]

Pharmaconutrition

Delivery of enteral nutrition in the ICU has been shown to improve patient outcome. When used in the appropriate trauma, burn, or critically ill patient, formulas containing specific immune-modulating agents have been shown to have an increased benefit to that seen from standard formulas alone.[71] In 2001, Heyland et al[71] reviewed 22 randomized trials comparing pharmaconutrition (or previously termed *immunonutrition*) formulas to standard enteral diets. Use of the pharmaconutrition formulas decreased rates of infection, hospital LOS, and duration of mechanical ventilation compared with use of standard formulas.[71] The benefits of pharmaconutrition involve down-regulation of the proinflammatory response in patients who already have exaggerated inflammation due to trauma, sepsis, or other critical illnesses.[71,72] Because obesity induces a chronic, low-grade proinflammatory state, use of pharmaconutrition agents in obese patients may help down-regulate obesity-induced inflammation and improve metabolism.[73] While there are many immunonutrients that might be potentially useful in obesity, Table 3 lists 11 of these.

ω-3 PUFAs

The fat composition of an ingested diet, in turn, determines the fatty acid composition of membrane phospholipids in cells such as white blood cells, endothelial cells, and tissue target cells. These fatty acids are broken down by specific phospholipases to produce prostaglandins, leukotrienes, thromboxanes, and other lipid-derived mediators during stress, such as trauma or infection. Diets rich in ω-3 PUFAs alter the prostaglandin and leukotriene profiles that are created during stress in a manner that reduces the host inflammatory response. Likewise, ω-3 PUFAs, specifically the eicosapentaenoic acid (EPA) and DHA components, reduce the inflammatory response through numerous distinct mechanisms. Studies have shown that ω-3 PUFAs down-regulate LPS-induced NFκB, which, in turn, decreases activation and release of TNF-α.[74,75] In a specific animal model (db/db diabetic obese mice), ω-3 PUFAs were shown to inhibit infiltration of macrophages into WAT, thereby reducing the degree of inflammation subsequently induced within that tissue.[76] Not surprisingly, diets rich in ω-3 PUFAs have been shown to improve inflammatory symptoms in other disease processes such as arthritis and ulcerative colitis.[77,78]

In addition to their impact on systemic inflammation, ω-3 PUFAs have a favorable effect on metabolism. PUFAs nonselectively activate PPAR-γ and PPAR-α, 2 agents that have been shown in an animal model to increase both basal and postprandial glucose-induced insulin production from pancreatic islet cells. ω-3 PUFAs have been shown to

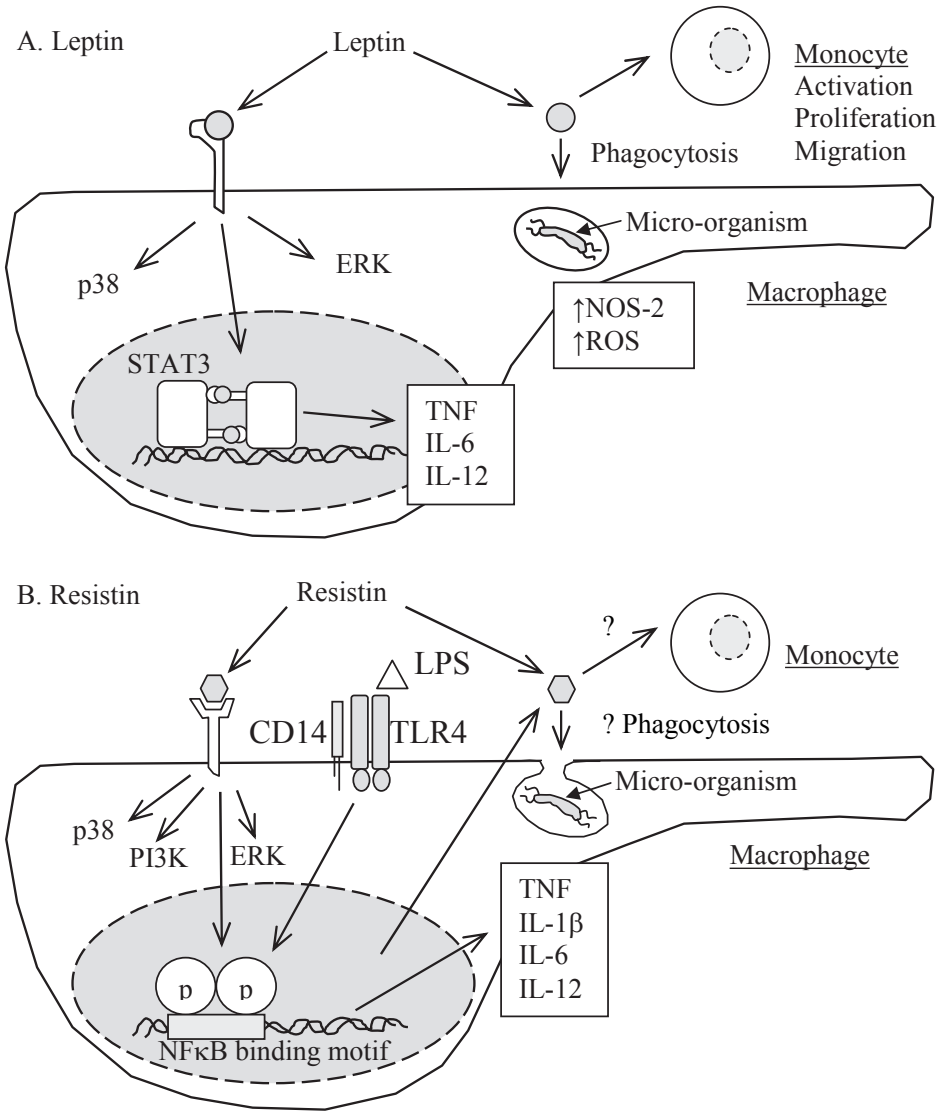

Figure 3. A, Leptin signals increase proinflammatory cytokines (TNF, IL-6, and IL-12), induce nitric oxide synthase-2 (NOS-2 or inducible NOS), increase reactive oxygen species (ROS), and activate monocytes. B, Resistin signals increase proinflammatory cytokine production (TNF, IL-1, IL-6, and IL-12), but its role in monocyte activation is unclear. Whereas adiponectin can be considered an anti-inflammatory strategy of the "adipose organ," leptin and resistin have dominant proinflammatory features.
IκB, inhibitor of NFκB; LPS, lipopolysaccharide; PPAR, peroxisome-proliferator-activated receptor; TLR4, Toll-like receptor 4.
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decrease serum triglyceride levels and may favorably alter adiponectin levels.[79]

As a result of the decreased inflammation, reduced triglycerides, and increased adiponectin, it is not surprising that ω-3 PUFAs have been shown to ameliorate the pathophysiology of NAFLD. In a prospective randomized trial, patients with NAFLD who received 1 g/d of ω-3 PUFA for 12 months had significantly decreased serum levels of alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase, and triglycerides compared with controls who took a placebo.[80] Circulating arachidonate levels and the serum ratio of ω-6/ω-3 fatty acids were reduced in study patients compared with con-

Table 3 *Immunonutrients for obesity*

Category	Immunonutrient
ω -3 Polyunsaturated fatty acids	Eicosapentaenoic acid (EPA) Docosahexaenoic acid (DHA)
Protein and amino acids	Soy protein L-Leucine L-Arginine Betaine *S*-Adenosylmethionine (SAMe) L-Carnitine
Minerals	Magnesium Zinc
Antioxidants	α-Lipoic acid

trols. Liver ultrasonography performed after treatment showed significant improvement in hepatic echo-texture and increased Doppler perfusion, suggesting improvement in liver blood flow in response to ω-3 PUFA supplementation.[80] NAFLD has been associated with decreased liver perfusion, which may place patients at increased risk during trauma or critical illness.[81–83] In an animal model, diets supplemented with ω-3 PUFAs, RNA fragments, and arginine were found to increase distal small intestinal blood flow when compared with isocaloric, isonitrogenous control diets (without the immune-modulating agents).[84–86]

The appropriate dosage of ω-3 PUFA supplementation to treat the metabolic syndrome has not been determined. A valuable reference point for clinicians might be the Food and Drug Administration (FDA)-approved doses recommended for treatment of hypertriglyceridemia, which are 1860 mg/d of EPA and 1500 mg/d of DHA.

Soy Protein

Soy provides a high-quality vegetable protein source that may help reduce risk of lipotoxicity and the metabolic syndrome.[87] In rat pancreatic islet cells, soy protein has been shown to decrease high-fat diet-induced hyperinsulinemia and raise glucagon levels (when compared with casein diets).[87,88] In adipocytes of leptin-resistant rats, soy protein may increase PPAR-γ expression, resulting in adipocyte hyperplasia (but not hypertrophy).[89] In various animal models, soy protein has been shown to be associated with increased serum adiponectin, decreased plasminogen activator inhibitor, and improved fatty liver (*via* decreased expression of the lipogenic transcription factor sterol regulatory element-binding protein [SREBP]-1, and increased lipid oxidation due to activation of PPAR-α).[89] Soy protein may also promote cholesterol uptake by the liver, thus lowering serum cholesterol.[87] The beneficial effects of soy protein in obesity and NAFLD are illustrated in Figure 4.

Although clinical studies in obese humans are limited, available data suggest that soy protein may have a role in the treatment of obesity and dyslipidemia.[90,91] In an outpatient weight loss study, daily provision of 90 g of soy protein in meal replacement shakes resulted in a 2.5-fold greater weight loss than a control diet.[92] Isoflavones (available in most but not all soy preparations) have estrogenic activities and seem to mediate many of the beneficial effects of soy seen in obesity.[90] Because of their estrogenic effect, these compounds may be contraindicated in certain conditions such as breast cancer. Although rare in adults, soy protein allergy occurs in 3%–4% of infants and children,[90] an incidence that compares favorably to the allergy profile of cow's milk (25% in infants and children).[90]

L-Leucine

Sarcopenia is a syndrome of muscle wasting normally seen as a consequence of a prolonged medical illness such as cancer or COPD. By inducing inflammation, obesity may play an important role in the development of age-related sarcopenia.[93] Thus, decreasing total body mass while maintaining or increasing skeletal muscle lean body mass is an important consideration when treating obesity, and the metabolic syndrome and may be achieved by providing a high-protein, low-carbohydrate regimen.[94] Dietary amino acids are known to stimulate protein synthesis, but this is not simply due to the provision of increased exogenous substrate.[95] Rather, the anabolic effects of dietary protein seem to be mediated almost entirely by the molecular signaling of the single amino acid, leucine.[95] Leucine activates the initiation phase of protein translation at several levels.[94,95] Clinical studies indicate that an intake of as little as 2.5 g of leucine acutely stimulates muscle protein synthesis. When given chronically with meals as part of a protein-rich weight-loss diet, leucine causes proportionally greater loss of body fat, with relative sparing of lean body tissue.[94] Through a hypothalamic "fuel sensor" mechanism that regulates hunger and satiety, leucine may provide additional benefit as an anorexic agent in the treatment of obesity.[96]

The combination of leucine and soy protein may be a particularly potent anabolic combination. Although not studied in obesity, soy protein supplemented with leucine enhanced whole-body protein synthesis in patients with COPD.[97] When used pharmacologically, it is important to obtain adequate "drug levels" of leucine. It seems that peak levels are critical, and leucine should be given in boluses of at least 2.5 g, which can be repeated several times daily to obtain a total daily dose of 6–8 g.[94]

L-Arginine

Arginine is a nonessential amino acid under normal physiologic conditions, which becomes essential under conditions of increased stress, including sep-

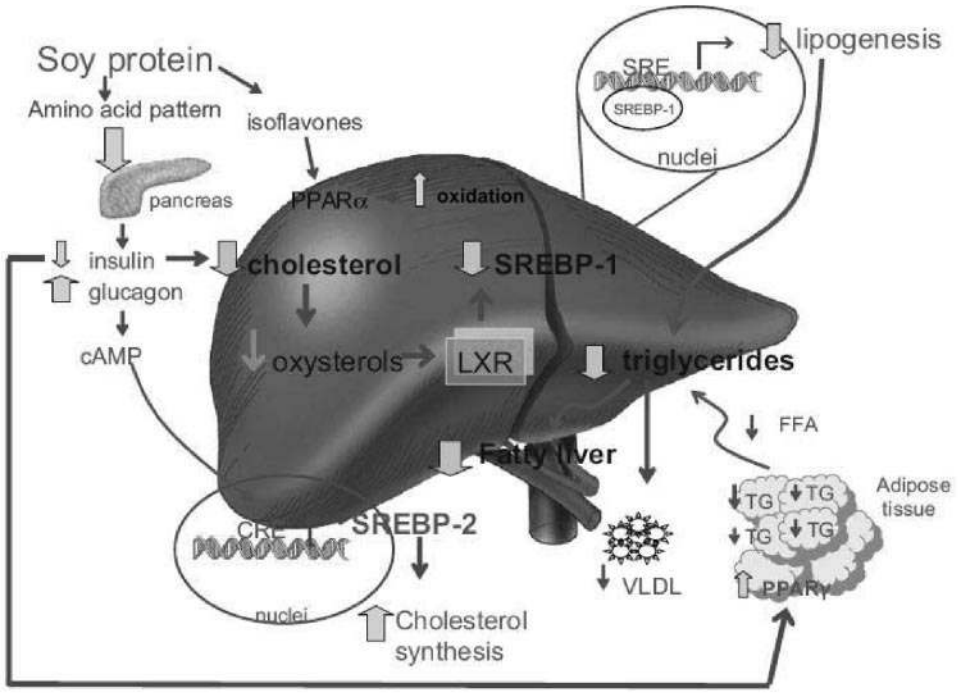

Figure 4. Proposed molecular mechanism of soy protein action on lipid metabolism to prevent the accumulation of triglycerides and cholesterol in liver that involves down-regulation of sterol regulatory element-binding protein (SREBP) -1 and up-regulation of SREBP-2. cAMP, cyclic adenosine monophosphate; CRE, FFA, free fatty acids; LXR, liver × receptor; PPAR-γ, peroxisome-proliferator-activated receptor; TG, triglycerides; VLDL, very-low-density lipoprotein. Reprinted from Torres N, Tovar AR. The role of dietary protein on lipotoxicity. *Nutr Rev.* 2007;65:S64–S68, with permission from International Life Sciences Institute Press.[87]

sis.[98,99] Arginine is metabolized through variable pathways, including inducible nitric oxide synthase (NOS) and arginase I and II, all of which are up-regulated during inflammation.[78] Arginine is involved in regulation of vascular tone, modulation of white blood cell function, and control of wound healing.[100] Dietary arginine supplementation promotes wound healing by enhancing protein synthesis of proline and hydroxyproline (*via* ornithine). Arginine acts as a secretogogue by stimulating release of insulin and insulin-like growth factor (IGF-1).[101] Depressed T-cell-mediated immunity, seen commonly after major surgery or trauma, can be ameliorated by arginine supplementation.[102]

Asymmetric dimethylarginine (ADMA) is a metabolic byproduct of cytoplasmic protein processing. Because it is structurally similar to L-arginine, ADMA appears to interfere with NOS function.[103] ADMA is elevated in the blood of patients with cardiovascular risk factors such as hyperlipidemia, hypertension, obstructive sleep apnea, diabetes, homocysteinemia, and obesity.[104,105] Elevated ADMA concentrations in obese, insulin-resistant women can be modulated by weight loss.[106,107] These elevated levels of ADMA associated with obesity might explain the endothelial dysfunction seen in the metabolic syndrome. The imbalance of ADMA and arginine often seen with obesity is illustrated in Figure 5.

In patients with elevated ADMA, L-arginine supplementation may provide beneficial effects by competing with circulating ADMA levels for normal regulatory processes. Patients with normal ADMA levels seem to be unaffected by arginine supplementation.[104,105] In a rat model of NAFLD,[108] L-arginine supplementation enhanced the hepatic microcirculation and directly increased blood flow through the hepatic artery and portal vein.[108] These effects on hepatic blood flow were reversed when nitric oxide was blocked by giving L-NAME, an agent that is similar in structure to ADMA.[108] These data suggest that L-arginine supplementation may increase hepatic perfusion and thus could possibly reverse the hepatic endothelial dysfunction that occurs in NAFLD.

Arginine is extensively metabolized, and there have been recent concerns that this phenomenon may hamper its usefulness, especially at lower doses. Commercial enteral formulas supplemented with arginine provide approximately 12.5 g of arginine per 1000 kcal, which should deliver a reasonable dose for most patients. An alternative strategy is the administration of the amino acid, citrulline, which is a prodrug that is converted into arginine. A recent study in human volunteers showed that orally administered citrulline improved the arginine to ADMA ratio in a dose-dependent fashion, with the greatest effects occurring at 3 g twice daily.[109] As

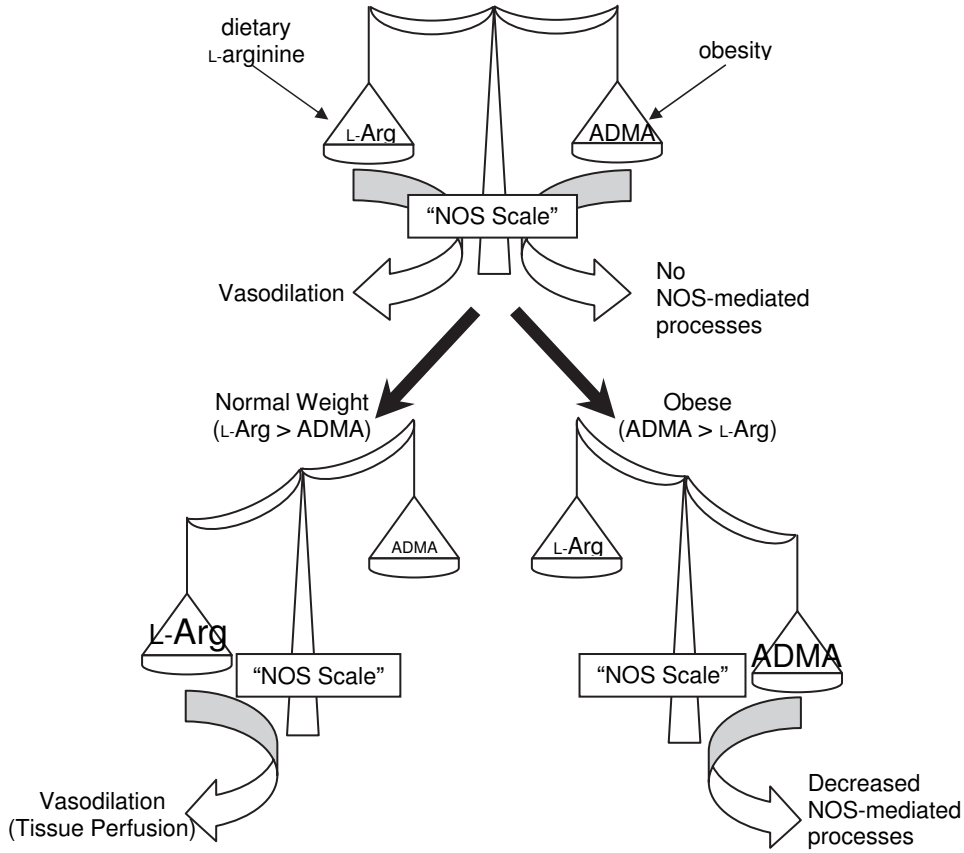

Figure 5. Asymmetric dimethylarginine (ADMA) competitively inhibits nitric oxide synthase (NOS) function. Obesity increases ADMA leading to decreased eNOS activity, decreased nitric oxide–induced vasodilation, and impaired tissue perfusion. In normal conditions, L-arginine (L-Arg) vastly outnumbers ADMA to allow NOS-mediated activity to occur. However, obesity alters the ADMA:L-Arg ratio to favor ADMA such that NOS-mediated processes are lost, including loss of vasodilation leading to impaired tissue perfusion.

arginine increases nitric oxide levels, it is theoretically contraindicated in patients with systemic septic shock and hypotension.[110]

Betaine and S-Adenosylmethionine

Trans-methylation and trans-sulfuration pathways are affected by obesity and NAFLD (Figure 6). Homocysteine, a sulfur-containing intermediate, may become elevated with insulin resistance and the metabolic syndrome.[111,112] Hyperhomocystinemia has been associated with a variety of adverse effects, including endothelial dysfunction, decreased ADMA catabolism, impaired methylation, and oxidative stress. These changes lead to an increased risk of cardiovascular disease and possibly nonalcoholic steatohepatitis (NASH).[113,114]

Betaine (trimethylglycine), originally discovered in the juice of sugar beets, serves as a methyl donor and functions to protect cells from osmotic stress. Betaine reduces circulating levels of homocysteine by facilitating its conversion back to methionine, which also decreases *S*-adenosyl homocysteine (SAH).[115] In a rat model, decreased betaine levels were observed in the setting of fatty liver.[116] Betaine has been successfully used in a pilot study in NASH patients.[117]

S-adenosylmethionine (SAMe) is the major methyl group donor in humans but has several other actions independent of methyl donation, which may also be important.[118] SAMe is formed from methionine and ATP in a reaction catalyzed by methionine adenosyl transferase (MAT; Figure 6). After methyl donation, SAMe is converted to SAH (Figure 6). Oxidative stress in NAFLD may lead to hepatic SAMe depletion through a variety of mechanisms, including decreased MAT activity.[28] A vicious cycle may ensue because SAMe deficiency can promote oxidative stress through reduced glutathione production.[28] In a MAT knockout mouse model, SAMe deficiency may promote steatohepatitis.[119] An increased SAH:SAMe ratio, which is a hallmark of many forms of liver disease such as NAFLD, may also occur in diabetes and diabetic nephropathy.[28,120] SAH, which may be converted to homocysteine, is a toxic metabolite that sensitizes the liver to TNF-α induced hepatotoxicity.[121] In a multicenter clinical trial, SAMe improved clinical outcome in alcoholic liver disease, a disease process that is very

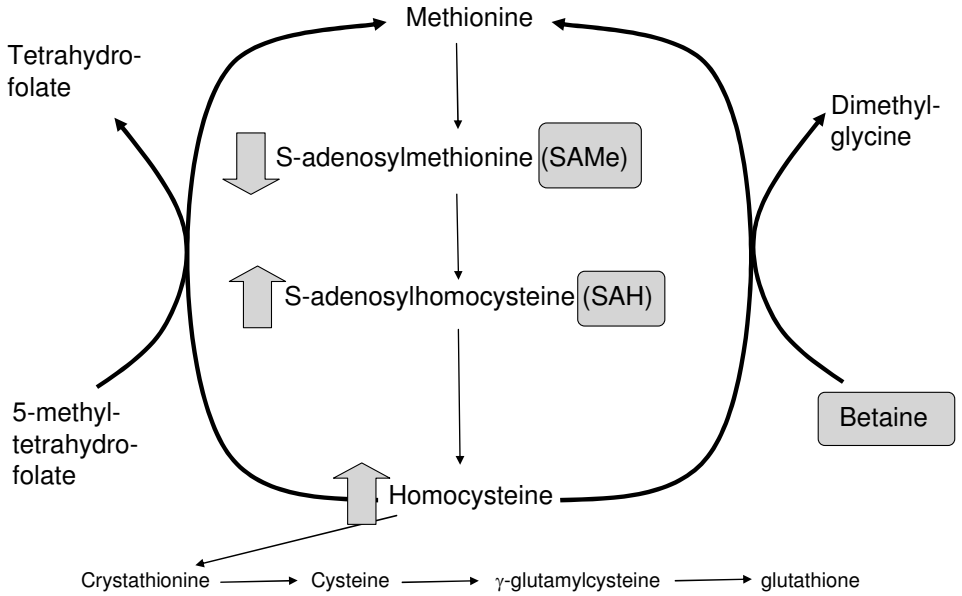

Figure 6. Diagram of hepatic methionine metabolism. Obesity impairs conversion of methionine to *S*-adenosylmethione (SAMe) *via* decreased hepatic methionine adenosyltransferase (MAT1A) activity. Low SAMe may predispose to increased endotoxin stimulated TNF production. In contrast, many forms of fatty liver increase levels of both *S*-adenosylhomocysteine (SAH) and homocysteine. Increased homocysteine has been implicated in the etiology of fatty liver, and increased SAH sensitizes to TNF hepatotoxicity. Homocysteine and SAH can be removed either by giving betaine to regenerate methionine or through 5-methyltetrahydrofolate metabolism. Polymorphisms in the 5-methyltetrahydrofolate reductase gene can impair this pathway and potentially exacerbate fatty liver. Further, the ratio of SAMe:SAH is critical in controlling methyltransferase reactions. Thus, this metabolic pathway seems to be vital in the genesis of many types of liver disease, and there are molecular targets, such as SAMe and betaine, that may provide novel therapeutic interventions. These potential interventions may have great relevance to prevention/treatment of toxin-induced NASH. Rerinted from *The Journal of Nutritional Biochemistry*, Vol. 18; Cave M, Deaciuc I, Mendez C; Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition; pp. 184–195; Copyright © 2007, with permission from Elsevier.

similar to NAFLD.[122] In LPS-stimulated monocytes, SAMe has also been shown to down-regulate the proinflammatory cytokine TNF-α while up-regulating the anti-inflammatory cytokine interleukin 10.[123] According to these results, SAMe would be an appropriate agent to treat NAFLD. SAMe is also an emerging treatment for depression and osteoarthritis, both of which are associated with obesity and the metabolic syndrome.[124,125]

Insufficient clinical data exist to make firm dosage recommendations for betaine and SAMe in obesity and the metabolic syndrome. However, the dosages likely reflect those used for liver disease. In these studies, betaine was given at a dose of 10 g twice daily, and SAMe was given at a dose of 1.2 g, typically in 3 divided doses of 400 mg each.[117,122]

L-Carnitine

In humans, 75% of carnitine comes from dietary sources, the remainder being synthesized in the liver, kidney, and brain after methylation of lysine.[126] Some evidence suggests that carnitine biosynthesis is impaired in the setting of SAMe deficiency. The overwhelming majority (99%) of carnitine is intracellular. Carnitine is concentrated in skeletal and cardiac muscle, where it supports mitochondrial β-oxidation of fatty acids (Figure 7). Carnitine influences carbohydrate metabolism by modulating the ratio acyl-CoA:CoA (Figure 7).[126,127] Carnitine insufficiency is likely when the serum ratio of conjugated to free carnitine is >0.4.[126]

In an animal model of carnitine deficiency (the juvenile visceral steatosis mouse), lipotoxic cardiomyopathy and NAFLD readily occur with hepatic accumulation of the long-chain saturated fatty acids palmitate and stearate.[128,129] Likewise, carnitine deficiency has been implicated in fatty liver associated with parenteral nutrition (PN).[130,131] Carnitine deficiency has been associated with many other medical diseases, including cirrhosis, chronic kidney disease, valproic acid therapy, Alzheimer's disease, and heart failure.[126] Muscle carnitine levels have been shown to decline with aging.[132] Patients with type 2 diabetes (particularly those who are insulin dependent or have complications of their disease process) seem to be at increased risk for carnitine deficiency.[133,134]

Evidence is mounting that carnitine supplementation may be beneficial in obesity, insulin resistance, and the metabolic syndrome. Older studies indicated that neither oral nor IV carnitine supplementation altered carnitine levels in skeletal mus-

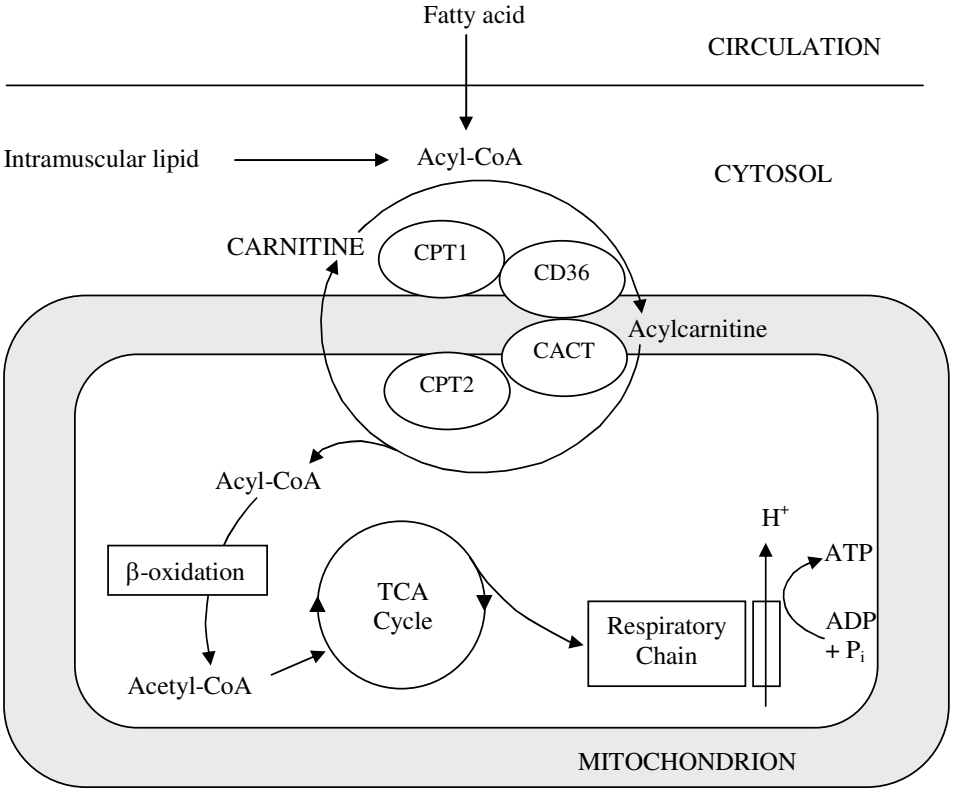

Figure 7. This is a schematic diagram of the metabolic role of carnitine in skeletal muscle to introduce fatty acid derivatives into the mitochondria for β-oxidation. The carnitine-acylcarnitine cycle is required for fatty acid translocation (acyl group) into the mitochondrial matrix for conversion to ATP.
ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; CAT, carnitine acetyltransferase; CACT, carnitine acylcarnitine translocase; CPT, carnitine palmitoyltransferase; CD36, fatty acid translocase; TCA, tricarboxylic acid cycle.
Adapted from Stephens FB, Constantin-Teodosiu D, Greenhaff PL. New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. *J Physiol.* 2007;581(Pt 2):431–444,[127] with permission from Blackwell Publishing.

cle.[127] However, it now seems that carnitine transport into skeletal muscle after oral feeding does occur in the setting of hyperinsulinemia, high-carbohydrate diet, or insulin infusion.[127] Carnitine, in turn, exerts a biologically active effect, shifting fuel use in skeletal muscle from carbohydrate to fatty acid oxidation.[127] Because hyperinsulinemia is common in the metabolic syndrome, it is plausible that in these patients skeletal muscle would avidly take up orally administered carnitine, resulting in increased fatty acid oxidation. In animal models, at least, such is the case. In spontaneously hypertensive rats, carnitine has been shown to attenuate lipid peroxidation and increase antioxidant defenses.[135] In obese rats with insulin resistance, carnitine supplementation improved glucose tolerance and increased total energy expenditure.[136] Carnitine supplementation data from human clinical studies are limited. However, carnitine has been shown to attenuate endothelial dysfunction caused by elevation of FFAs.[137] Furthermore, acetyl-carnitine has been shown to be an effective treatment for diabetic neuropathy at doses ranging from 1.5 to 3 g/d.[138]

In documented cases of carnitine deficiency, the FDA has approved an oral replacement dose ranging from 1.98 to 2.97 g/d. The optimal dose for supplementation in obesity and the metabolic syndrome is unknown, but a range of 1.5–3.0 g/d is reasonable until more data become available. Carnitine may have several gastrointestinal side effects, including abdominal pain, vomiting, and diarrhea. Caution is advised if there is an underlying seizure disorder.

Magnesium

After potassium, magnesium is the most abundant intracellular cation,[139] having numerous roles in health and disease. Magnesium is a cofactor for more than 300 enzymes involved in bioenergetics, protein phosphorylation, glutathione production, and synthesis of cyclic adenosine monophosphate (cAMP).[140] Magnesium availability affects the structure and function of nucleic acids, cell membranes, and ion channels.[140]

Because the magnesium content of food is greatly reduced by processing, it is estimated that 75% of

Americans do not meet the recommended daily allowance (420 mg for men, 320 mg for women).[140] Magnesium is stored intracellularly, and as such, serum magnesium concentration does not necessarily reflect whole body magnesium stores (serum levels can be normal in clinically deficient states).[139]

Strong epidemiologic and mechanistic data support a role for magnesium deficiency in the genesis of insulin resistance and the metabolic syndrome. Magnesium deficiency contributes to the development of the metabolic syndrome (and, conversely, may also be caused by the metabolic syndrome). Multiple studies have associated magnesium deficiency with obesity, diabetes, diabetic vascular complications, dyslipidemia, hypertension, NASH, insulin resistance, and the metabolic syndrome.[139,141] The mechanism of this effect in obese humans is multifactorial and involves reduced tyrosine kinase activity at the insulin receptor, modulation of intracellular calcium activity, and increased circulating TNF-α levels (Figure 8).[142] Magnesium deficiency is most commonly precipitated by the combination of suboptimal dietary consumption and increased renal losses.[139] Large, long-term, population-based studies have shown that increased dietary magnesium consumption is protective against the development of diabetes and the metabolic syndrome.[143–145] The insulin-sensitizing drugs metformin and pioglitazone may exert their clinical effects, in part, by favorably modulating magnesium levels.[146,147] Small clinical studies have shown that oral magnesium replacement (2.5 g magnesium chloride for 16 weeks) increases insulin sensitivity in diabetic and nondiabetic patients with magnesium deficiency.[148,149]

According to these data, magnesium replacement therapy may be reasonable for individuals who already have or are at risk for the subsequent development of the metabolic syndrome. In the absence of renal insufficiency, dietary intake should exceed the recommended daily allowance to overcome potential renal losses. The absolute dose will likely vary, according to a patient's individual degree of deficiency and renal function. As magnesium is replaced orally, patients should be monitored for the development of diarrhea.

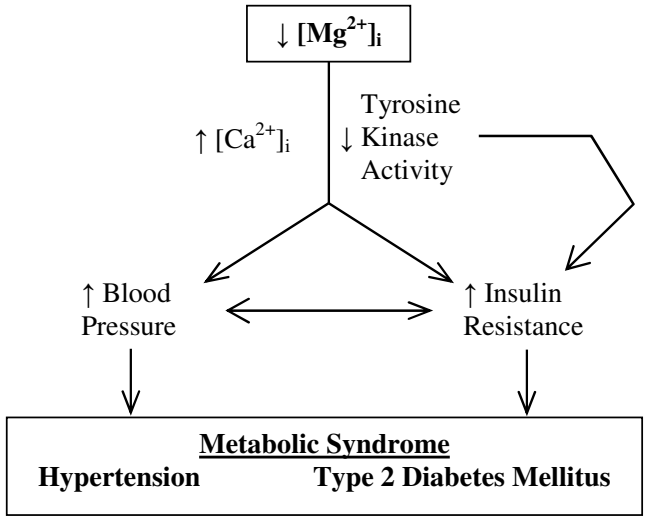

Figure 8. Intracellular magnesium deficiency might mediate the relationship between insulin resistance, hypertension, metabolic syndrome, and type 2 diabetes mellitus. Adapted from *Archives of Biochemistry and Biophysics,* Vol. 458; Barbagallo M, Dominguez LJ; Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance; pp. 40–47; Copyright © 2007, with permission from Elsevier.

Zinc

Zinc is the second most abundant trace metal in the human body.[150] Zinc is a cofactor in over 300 metalloenzymes involved in gene transcription, metabolism, membrane stability, and inflammation.[150] Although tissue zinc status is the critical determinant of zinc deficiency, serum zinc levels may be used clinically as a surrogate marker for overall body stores. Decreased serum zinc concentrations have been observed in obesity, insulin resistance, diabetes, and hypertension.[151–155] Similar to magnesium, zinc deficiency is most commonly precipitated by inadequate dietary intake and increased renal losses.[152] Estimates suggest that only 56% of Americans have adequate zinc intake according to the recommended daily allowance (15 mg for adult men; 12 mg for nonlactating women).[156] The effect of obesity on tissue zinc levels is complicated; therefore, stores may not be reflected accurately by serum levels. In obesity, the expression of multiple zinc transporter proteins in the adipose tissue itself is altered, an effect that may differ appreciably from one region to another (eg, subcutaneous to intra-abdominal adipose tissue).[157]

Zinc status modulates obesity and the metabolic syndrome. In a large clinical study, both low consumption of dietary zinc and low serum zinc levels were associated with an increased prevalence of diabetes, hypertension, hypercholesterolemia, and coronary artery disease.[158] In a recent study of fatty liver disease, patients with steatohepatitis consumed less zinc that those with simple steatosis (a much milder form of the liver disease).[33]

Animal studies have demonstrated potential mechanisms and implied a plausible therapeutic role for zinc in obesity. In mice, a high-fat diet was associated with reduced zinc concentrations in adipose tissue, which, in turn, negatively correlated with serum leptin levels.[159] In rats fed a high-fructose diet, zinc supplementation improved insulin sensitivity and antioxidant status.[160] Zinc is a potent antioxidant and provides a protective effect against ischemia/reperfusion injury, which could be relevant for critically ill obese patients.[161] Acutely, zinc administration stabilizes sulfhydryl groups and antagonizes redox-active transition metals to

decrease free radical formation.[161] Chronically, zinc administration induces metallothioneins, potent free radical scavengers, which have been shown in the past to prevent lipotoxic cardiomyopathy.[161,162] Zinc deficiency causes cell-mediated immune dysfunction, proinflammatory cytokine dysregulation, and increased TNF-α expression.[163] Zinc supplementation, on the other hand, is protective against TNF-α-mediated hepatotoxicity, protects small bowel structural integrity and barrier function, and ameliorates symptoms of diarrhea.[164]

The fact that obesity has been associated with decreased serum zinc levels has important implications in the therapeutic management of inflammation, oxidative stress, and the genesis and progression of the metabolic syndrome. Although data are insufficient to make evidence-based dosing recommendations for zinc supplementation, a 220 mg daily dose of zinc sulfate is commonly used for wound healing applications, and might be a starting point for treatment in obesity. Common side effects are limited to nausea, which may be reduced by prescribing alternate forms of zinc (such as zinc acetate).

α-Lipoic Acid

α-Lipoic acid (ALA), a potent antioxidant synthesized by both plants and animals, is a cofactor for several mitochondrial enzyme complexes.[165] When reduced to α-dihydrolipoic acid (DHLA) by intracellular enzymes, the compound directly interacts with reactive oxygen and nitrogen species.[165] DHLA restores the antioxidant activity of glutathione, vitamin C, and coenzyme Q_{10},[165] and has been shown to increase cellular uptake of cysteine (thereby enhancing glutathione synthesis).[165]

ALA also has numerous immunomodulatory or anti-inflammatory effects. ALA has been shown to attenuate LPS-induced monocyte activation, reduce production and subsequent tissue damage by TNF-α, and protect against endotoxin-induced oxidative stress.[166,167] ALA increases cyclic AMP in human T cells and natural killer cells, which serves to reduce inflammation.[168]

ALA may have an emerging clinically relevant therapeutic role in metabolism. In rats, ALA binds and activates the insulin receptor[169] and has been shown to restore insulin sensitivity during high-fructose feeding.[170] In obese patients with type 2 diabetes, 600 mg of ALA by mouth twice daily nearly doubled peripheral insulin sensitivity over a 4-week period.[171] In an animal model of obesity, ALA reduced body weight and prevented triglyceride accumulation in skeletal muscle and pancreatic islets, an effect that helped prevent diabetes.[172] In the same model, ALA was shown to prevent skeletal muscle lipotoxicity by increasing fatty acid oxidation.[173] Diabetic neuropathy symptoms were improved in 26% to 62% of patients treated with oral ALA at doses ranging from 600 to 1800 mg daily over 5 weeks.[174] Through modulation of AMP-activated protein kinase in the hypothalamus of rodents, ALA has been shown to promote anorexia and reduce food intake, enhance energy expenditure, and promote significant weight loss.[175]

ALA may also help ameliorate long-term complications of cardiovascular disease. In patients with diabetes and end-stage renal disease requiring hemodialysis, ALA supplementation significantly reduced plasma levels of ADMA, mentioned earlier in this manuscript to be a marker of endothelial dysfunction and cardiovascular outcome in these patients.[176] In obese rats, ALA mitigated endothelial dysfunction[177] and diet-induced hypertension.[170] In a genetic, murine model of cardiac lipotoxicity, ALA normalized cardiac triglyceride accumulation to restore myocardial function.[178]

Human studies indicate that an oral dose of 600 mg daily provides the optimal risk:benefit ratio. Side effects of treatment are limited to a dose-dependent increase in nausea, vomiting, and vertigo.

Potential Role of Pharmoconutrition in Obesity

Interesting generalizations can be made from this body of information. It is clear that adipose tissue is not clinically "inert" but represents a true endocrine organ with active secretory capabilities. Obesity serves to up-regulate systemic inflammation, creating a low-grade systemic inflammatory response syndrome (SIRS) response. The location where fat is deposited in a situation of excess caloric provision relates to the subsequent morbidity. Subcutaneous fat (as seen in peripheral adiposity) appears to be tolerated best and may represent the optimal response to excess calories. Fat surrounding the viscera (seen in central adiposity) is more deleterious and more likely to be associated with the metabolic syndrome. But fat deposited within the viscera is the worst-case scenario, leading eventually to organ dysfunction at sites such as the liver, pancreas, heart, and skeletal muscle. In fact, excess lipid accumulation exceeding the innate storage capacity of these ectopic organs is what causes lipotoxicity and leads to cellular and ultimately organ dysfunction.

The SIRS associated with obesity drives the metabolic syndrome. The clinical picture of insulin resistance, central adiposity, and organ dysfunction is similar in cytokine profile, picture of inflammation, and morbidity to that seen with gram-negative sepsis, although less severe than that seen in sepsis. Dietary intake can further modulate the level of inflammation. Exogenous long-chain saturated fat, *via* the TLR-4 receptors, can further increase inflammation (and polyunsaturated or monounsaturated fat may decrease inflammation). In fact, the immune system appears to have difficulty distinguishing between saturated fat in the diet and bacterial endotoxin.

The existence of a preexisting state of inflammation as a result of obesity may fulfill the classic 1-hit–2-hit pattern of immune stimulation. The first hit is the obesity itself, which increases the production of NFκB and TNF, essentially "priming" the immune system. The second hit is the clinical insult itself: the bariatric surgery, trauma, burn, pneumonia, or myocardial infarction. The clinical manifestation of the 1-hit–2-hit phenomenon is that the priming of the immune system from the preexisting disorder (obesity) leads to an exaggerated immune response with the secondary injury (surgery, trauma, sepsis, etc). The significance of this condition at the bedside means that the critically ill, obese patient hospitalized for any reason will have greater risk for organ failure, hyperglycemia, insulin resistance, infectious morbidity, longer ICU LOS, prolonged duration of mechanical ventilation, and greater mortality compared with their lean counterparts.

The challenge, therefore, is developing a nutrition formula for obesity that is capable of altering the metabolic state, removing fat from the liver, improving organ function, down-regulating systemic inflammation, and attenuating the morbidity associated with this disease process. The downside to developing any obesity formula is the realization that providing more calories to a patient who is already in obvious excess energy balance may have a deleterious effect. Weight loss may not be required, however, to convert an obese, unhealthy patient to a similar-weight but healthier patient, as long as there is improvement in insulin sensitivity, organ dysfunction, and level of inflammation.

The design of a regimen for obesity overall should be low-calorie, high-protein, preferably 0.75 kcal/mL or less in caloric density. The macronutrient composition should include a mix of specific proteins, including arginine, leucine, and soy protein. The fat should be predominantly ω-3 PUFAs, preferably in the form of fish oil. To this overall mixture, specific additives (such as SAMe or betaine, carnitine, magnesium, zinc, and ALA) should be added in appropriate doses.

In the outpatient setting, it would be unrealistic to provide such a formula to the general obese population. The cost would be prohibitive and these patients might not be motivated to lose weight. In fact, these are the types of patients that would be less likely to "budget" their intake to accommodate the extra calories in an oral specialty-designed supplement. But a motivated, ambulatory, obese patient in an outpatient weight-loss program might benefit substantially from a specialty formula designed for obesity. The formula could be substituted for 1 or 2 meals a day, and thus would have to be palatable and good-tasting enough for oral consumption. The regimen should promote "safe" weight loss by optimizing fatty acid oxidation and preventing fatty accumulation in the liver as the patient loses weight. In the patient awaiting bariatric surgery, it might even be worth delaying the surgery to decrease the SIRS response, improve insulin sensitivity, and remove fat from the liver preoperatively.

In the critically ill patient, a pharmaconutrition formula for obese patients might provide even greater benefit with respect to patient outcome. Here, a formula would be designed for tube feeding. Current recommendations from the 2001 Summit on Immunonutrition clearly identified "candidates" for immune-modulating formulas according to the strength of the literature that indicated for which patient population use of immunonutrition would clearly change outcome.[179] Certainly, prospective, randomized trials would have to be performed in obese patients before clear conclusions and a specific design could be made. However, the information culled from the current animal studies and limited clinical experience suggests that such a formula is a plausible therapeutic strategy and that "obesity" would be added to this list of indications for a pharmaconutrition formula in the future.

The limitations of this concept are considerable. It would be preferable to evaluate each component separately to determine the specific optimal dose. Findings in animal studies do not always correlate to clinical studies, or at least, the clinical studies may not see as dramatic an effect. Compatibility issues, solubility, and stability may preclude the simple addition of some of these agents. Interaction or synergism between agents with regard to their profile of side effects again may prevent the combination of certain individual nutrients. Any benefit gained from a pharmaconutrition formula could be offset by excess net caloric intake and subsequent weight gain. Finally, the enthusiasm generated from small early studies may be lost if results cannot be replicated when larger clinical studies are eventually conducted.

Conclusions

The current obesity epidemic presents unique problems in healthcare. Obese patients who sustain trauma or develop critical illness seem to be at increased risk and are more likely to experience adverse outcomes than their lean counterparts. Obesity is associated with organ dysfunction and chronic smoldering inflammation. These effects are mediated by lipotoxicity and adipokine dysregulation. New data indicate that obesity-induced inflammation may be mechanistically similar to chronic, low-grade, gram-negative sepsis. This inflammatory response may partially explain worse ICU outcomes in obese patients. The current state of the art for the nutrition therapy of obese patients is limited to high-protein, hypocaloric enteral or parenteral feeding. This approach probably does little to reverse obesity-associated inflammation. Key immunonutrients in categories ranging from specialized fat and protein derivatives to individual minerals and anti-

oxidants can potentially attenuate the inflammatory state and correct the metabolic derangements in obese patients even in the absence of weight loss. We advocate the implementation of this knowledge to develop rationally designed pharmaconutrition regimens that could lessen the morbidity and improve outcome for patients with obesity.

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Invited Review

Neurodegeneration and Inflammation

Mark H. DeLegge, MD, FACG, FASGE, AGAF; and Addy Smoke, RD, CNSD
Section of Nutrition, Digestive Disease Center, Medical University of South Carolina, Charleston, South Carolina

ABSTRACT: Recent studies have demonstrated a strong link between neurodegeneration and chronic inflammation. The central nervous system (CNS) has very limited regenerative capacity. Neural cell death occurs by apoptosis and necrosis. Necrosis in the CNS usually follows ischemic or traumatic brain injury. Apoptosis is known as programmed cell death and often demonstrates histologic features of acute and chronic neurologic diseases. The innate immune response is protective to the CNS to defend against pathogens. Temporary up-regulation of inflammatory events is natural and does not lead to cell death. If this inflammatory process is up-regulated, neurodegenerative changes may occur. There has been a proven link between the inflammatory response, increased cytokine formation, and neurodegeneration. Both pharmaceutic and nutrition interventions for treating chronic neurodegenerative diseases, such as Alzheimer's disease or multiple sclerosis, will be focused on reducing or terminating the chronic inflammatory response.

The adult brain contains 10^{11}–10^{12} neurons supported by at least twice as many neuroglial cells.[1] There are different types of glial cells: oligodendrocytes, microglial, and astrocytes. These cells, especially microglia, are the equivalent monocytes/macrophages of the central nervous system (CNS).

Recent studies have demonstrated a strong link between chronic inflammation and neurodegeneration. Alzheimer's disease (AD) is characterized by the death of cells in the hippocampus and the frontal cortex secondary to chronic inflammation. In Parkinson's disease (PD), chronic inflammation leads to loss of dopaminergic receptors in the substantia nigra. Amyotrophic lateral sclerosis (ALS) is another inflammatory condition in which motor neurons are ultimately destroyed. Multiple sclerosis (MS) is an autoimmune disorder in which inflammatory cells attack the myelin sheath. Although activation of an acute inflammatory event is a necessary self-defense mechanism of the CNS against foreign antigens, prolonged activation of the inflammatory response can lead to chronic inflammation and cell death.[2]

Correspondence: Mark H. DeLegge, MD, FACG, FASGE, AGAF, Medical University of South Carolina, 96 Jonathan Lucas St, Ste 210, Charleston, SC 29425. Electronic mail may be sent to deleggem@musc.edu.

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Cell Death

The CNS has very limited, if any, regenerative capacity; therefore, it is very important to limit cell death in that region.[3] Neural cell death occurs by necrosis or apoptosis.[4] In necrosis, there is often a definitive temporal cause of the death of the cell. In apoptosis, the stimulus for death initiates a cascade of events that ultimately leads to cell destruction.

Necrosis in the CNS generally follows an acute ischemic or traumatic injury to the brain.[5] Abrupt biochemical collapse in an area of the CNS leads to the generation of reactive oxygen species (ROS) and excitotoxins such as glutamate, calcium, and cytokines. The hallmark histologic features of necrotic cell death are mitochondrial and nuclear swelling, and chromatin dissolution. This ultimately leads to nuclear and cytoplasmic membrane degeneration.[6]

Apoptosis is also known as programmed cell death and often demonstrates histologic features of acute and chronic neurologic diseases.[7] After an acute insult in the CNS, apoptosis often occurs in areas that are not as severely affected by the acute injury. Apoptosis is the secondary cause of the neuronal cell death after an acute CNS injury, such as ischemia.[8] In contrast, in chronic neurodegenerative diseases, apoptosis is the predominant form of cell death.[9]

In an apoptotic event, a cascade of biochemical reactions occurs, activating proteases that destroy molecules necessary for cell survival. Histologically, the cytoplasm condenses, mitochondria and ribosomes aggregate, the nucleus condenses, and chromatin aggregates. Within the apoptotic process, intracellular acidification occurs and ROS are generated.

Caspases

The major executioners in apoptosis are proteases known as caspases.[10] Upstream caspases are acti-

vated by cell-death signals (eg, tumor necrosis factor [TNF]). The upstream caspases activate downstream caspases that directly lead to the death of the cell.[11]

In the cascade of apoptosis, cytochrome T (from the mitochondrial electron transport chain) is released. Members of a group of proteins, known as the BCL-2 family, are either apoptotic or antiapoptotic. The balance of these proteins is crucial in stimulating or blocking the release of cytochrome T and initiating or blocking the apoptosis cycle.[12] In chronic neurodegenerative diseases, caspase-mediated apoptotic pathways have the dominant role in causing cell dysfunction and cell death.[13]

Immune Response in the CNS

Early reports defined the brain as immune privileged, being separated from the peripheral blood system by the blood-brain barrier. This separation was theorized to protect the brain from inflammatory disorders present in the peripheral blood system. It is now known that most neurodegenerative diseases are characterized by a local inflammation from resident cells in the brain (microglia) and infiltration of leukocytes from the peripheral blood.[14]

The immune system is a complex network of molecular, chemical, and cellular mediators that function to protect the body against the environment. The front line of defense is the innate (natural) immunity. This component of the inflammatory response is essential to recruit cells of the immune system to a compromised area. Microglial cells, which can be activated to a phagocytic state, are the main cellular components of innate immunity in the brain.[2] Astrocytic cells contribute to the protection offered by the blood-brain barrier by forming processes that surround the endothelial cells of the cerebral microvasculature and protecting it against foreign invasion.

Microglial Cells

It is generally accepted that microglia are derived from myeloid origin.[15] In mature brains, resting microglia are responsible for immune surveillance. The majority of microglial function goes unnoticed as these cells perform general maintenance functions and clean cellular debris. Microglia become activated in response to injury or immunologic stimulus, undergoing significant morphologic alterations[16] (Table 1). This is known as reactive gliosis. Surface molecules, such as complement receptors and major histocompatibility complex molecules, are up-regulated.[17] Activated microglia are capable of releasing a variety of soluble factors that are proinflammatory in nature. These factors include superoxide, nitric oxide, and TNF-α, in addition to others.[18] Microglial cells also have the potential for increasing neuronal survival by the release of trophic and anti-inflammatory factors.[19] This "schizophrenic" personality of the microglia underscores the complexity of its interaction with the CNS.

Table 1 *Triggers of microglial activation and neurodegeneration*

Environmental
Rotenone
Paraquat
Particulate airborne material
Bacterial, fungal, viral invasion
Endogenous
β-Amyloid
α-Synuclein
Matrix metalloproteinase-3
Neuromelanin

Innate Immune Response

In the innate immune response, microglial transmembrane receptors, known as Toll-like receptors (TLRs), have been identified as being very important in the inflammatory response. These TLRs are activated by unique structures present on pathogens known as pathogen-associated molecular patterns (PAMPS).[20] Stimulation of TLRs leads to the production of nuclear factor-κ B (NFκB), a transcription factor involved in the innate immune function response.[21] NFκB promotes expression of genes involved in inflammation such as TNF, interleukin-1 (IL-1), inducible nitric oxide synthase (iNOS), and complement factors.[22]

Microglial cells may also be stimulated by other known environmental factors such as rotenone (a pesticide), diesel exhaust particles (DEP), and paraquat, or by direct neuronal injury.[23] The first event, after activation by this environmental antigen, is production of ROS.[24] Microglial ROS production is followed by the release of cytokines such as TNF-α, IL-1β, nitric oxide, and prostaglandin E-2 (PGE2), all important in promoting inflammation and neuronal injury. ROS all stimulate the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, creating inflammatory cytokines, thromboxane, and prostaglandin. These cytokines generally peak in quantity approximately 6–12 hours after microglial stimulation.

The innate immune response is protective to the CNS to defend against pathogens. Temporary up-regulation of inflammatory events is natural and does not lead to neuronal cell death.[25] Endogenous factors such as glucocorticoids moderate the inflammatory response by negative feedback.[26]

As the brain ages, inflammatory events of the CNS are up-regulated, most likely due to the compromise of the blood-brain barrier (BBB).[27] Cytokines such as IL-1, IL-6, and TNF-α are synthesized at an increasing rate, resulting in the recruitment of microglial cells, astrocytic cells, and macrophages to a site of infection, injury, and inflammation. An

irresolvable stimulus may lead to the chronic production of cytokines, resulting in neuronal toxicity. Activated glial cells produce oxygen-free radicals (OFR), exhausting antioxidant stores and leading to neuronal cell injury and death.[28]

Inflammatory Mediators

Increased activation of COX, LOX, and epoxygenases (EPOX) under pathologic conditions, such as AD, PD, and ALS, produces neuroinflammation that creates either vasodilation or vasoconstriction, platelet aggregation, leukocyte chemotaxis, a release of cytokines, and oxidative stress. These metabolic reactions are closely linked to neural cell injury. Cytokines and chemokines are key regulators of the inflammatory process and the pathogenesis of neurodegenerative diseases.[29] They regulate the activity and survival of inflammatory cells and mediate communication of immune cells with each other and with other cells of the body.

Three forms of COX enzymes, designated COX-1, COX-2, and COX-3, occur in mammalian tissue.[30] COX-1 is involved in a number of homeostatic processes and is know as the housekeeping enzyme. Inflammatory mediators, such as cytokines and bacterial endotoxin, rapidly induce COX-2, which is normally undetectable in human tissue.

Altered expression of specific inflammatory factors triggers or modulates the development of neurodegenerative disease. Overexpression of COX-2, an enzyme involved in the first steps of prostanoid synthesis, has been linked to neuronal apoptosis.[31] Astroglial overexpression of TNF-α2, interferon A, or IL-6 results in neurodegenneration, gliosis, and progressive neurologic disease.[32] It has been shown that cytokine-transforming growth factor-β (TGF-β) and mRNA levels in postmortem AD brains correlate with the degree of neurodegeneration.[33] Much of the cell damage that occurs with neural cell injury is disseminated through OFR. At low levels, ROS function as signaling intermediates in the regulation of fundamental cell activities such as growth and adaptation. At higher levels, OFRs contribute to damage of neural membranes by the process of oxidative stress.[34]

The link between cytokines, the inflammatory response, and neurodegeneration would be best proven by the demonstration that a particular treatment targeted at these cytokines results in an improvement or prevention of a chronic neurologic disease process. There is a profound difference between various nonsteroidal anti-inflammatory drugs (NSAIDs) and their selectivity for either COX-1 or COX-2. There have been reports of COX-2 inhibitor medications (NSAIDs) reducing AD risk by blocking the accumulation of degenerating proteins, blocking proinflammatory cytokines, and reducing microglial activation.[35] The use of NSAIDs has been shown to improve both cognitive and motor function in the animal model.[36] Treatment of rats with indomethacin, a drug inhibiting both COX-1 and COX-2, reduces infarct size after local ischemia with subsequent reperfusion.[37] Ibuprofen, which also inhibits COX-1 and COX-2, also has been shown to reduce neuronal injury and improve cerebral blood flow and neurologic outcome after severe ischemia in an animal model.[38,39] Future research in this area may allow us to have an impact on the outcome of previously chronic, debilitating neurologic diseases.

Recent data suggest that inflammation related to neurodegenerative disease is directly affected by nutrition. Investigators are studying the links between diet and risk of disease, as well as the impact of diet on the progression of disease. The vast majority of research currently focuses on the dietary factors such as calorie intake, ω-3 fatty acid intake, and intake of antioxidants and chemical compounds that may increase or decrease oxidative damage and affect the inflammatory response to damage.

AD and PD

Neurodegeneration in AD occurs as a result of the accumulation of aggregated amyloid plaques in the brain, which steadily increase with age. The inflammatory response resulting from plaque accumulation leads to oxidative damage by generation of ROS and injury to the surrounding cells.[40] Along with the increase in amyloid plaque accumulation, the AD brain exhibits increased levels of proinflammatory cytokines, such as PGE2 and γ-secretase, which may function as stimulators of neuroinflammation.[41] Mitochondrial dysfunction related to plaque accumulation and glial recruitment also occurs as a result of oxidative damage occurring in AD.[1] The brain of a patient with AD is damaged in the regions responsible for learning and memory process, which is exhibited by the cognitive decline resulting from the disease.

In PD, neurodegeneration is a result of the loss of dopaminergic neurons in the substantia nigra, leading to motor dysfunction. Inflammatory processes, including lipid peroxidation and superoxide dismutase (SOD) activity and generation of ROS, are increased in the substantia nigra.[41] Pathogenesis of the disease may also be associated with alterations in LOX, COX, and EPOX pathways, thereby exacerbating the inflammatory response. Mitochondrial dysfunction in untreated PD patients involves decreased function of complexes I and II/III of the electron transport, which may lead to altered oxygen tension and the generation of isofurans *via* nonenzymic oxidation of docosahexaenoic acid (DHA).[1,41]

There have been attempts to either intervene or prevent AD or PD by the use of nutrition-based interventions (Table 2). Numerous studies have evaluated the effects of dietary restriction on neurodegenerative disease. In some animal studies, restricted animals are fed at 30%–40% fewer calories than control animals, which has been shown to increase life span in rats and in mice.[42] In the aging

Table 2 *Intervention and prevention of Alzheimer's and Parkinson's disease*

	Alzheimer's disease		Parkinson's disease	
	Prevention	Treatment	Prevention	Treatment
Dietary restriction	+++		+++	
Docosahexaenoic acid (DHA) supplementation	++			
Curcumin		++		
Coenzyme Q10				+
Folic acid	+		+	
Acetylcarnitine		++		

+, Weak evidence; ++, moderate evidence; +++, strong evidence.

brain, neuronal cell death occurs as a result of oxidative stress, mitochondrial dysfunction, and apoptosis. Restriction of food intake may in fact decrease free radical production by slowing oxidative damage and suppressing oxidative stress. Dietary restriction maintains levels of 18:2 acyl side chains, inhibits cardiolipin composition change, retards aging-associated oxidative damage, decreases mitochondrial oxidant generation, and decreases antioxidant defenses.[43] The protective benefit of dietary restriction also includes an increased production of neurotrophic factors and improved resistance to genetic and environmental causes of inflammatory damage and can actually increase neurogenesis. Consensus data suggest that caloric restriction leads to a decrease in the rate of oxidative damage accrual, decreased cellular injury, and increased heat tolerance. In fact, it has been suggested that dietary restriction may actually slow the aging process by changing the metabolic process of protein turnover and oxidative damage.[44] Epidemiologic evidence strongly correlates the risk of PD and AD to caloric intake, with people in countries consuming 1600–2000 kcal/d having a lower incidence of AD than those that consume 2500–3000 kcal/d.

Depletion of DHA, an ω-3 fatty acid, can lead to increased oxidative damage. Decreased DHA intake is a risk factor for AD, and repletion is correlated with a decrease in amyloid aggregation and accumulation.[44] DHA-supplemented mice have demonstrated and benefited from a decrease in arachidonic acid. The action is similar to that of NSAID activity in reducing proinflammatory intermediates. Epidemiologic data suggest that intake of ω-3 fatty acids and levels of inflammation are inversely related. The typical Western diet is composed of a high ω-6:ω-3 ratio, which may promote inflammatory processes. Low ω-3 fatty acid intake, in the face of increased ω-6 fatty acid intake as is commonly seen in the Western diet where intakes of DHA are $<30\%$ of the recommended intake, may contribute to increased proinflammatory cytokines. However, when high levels of both ω-3 and ω-6 fatty acid intake are present, the combined effect may be that of a stronger reduction in inflammatory marker levels.[45] This evidence disputes the hypothesis that the absolute ω-6:ω-3 ratio is the influential factor. Therefore, DHA/fish oil supplementation, which is safe for long-term use, may be of great benefit in the treatment and possible prevention of AD, and decreasing intake of ω-6 fatty acids is not necessary. At this time, no specific guidelines exist regarding the amount of DHA required in the diet to receive these benefits. However, increasing consumption of fish oils should be encouraged.

Curcumin is a polyphenolic tumeric component that has been the focus of possible treatment of neurodegenerative disease. The compound has multiple functions that may prove to be beneficial. Curcumin has antioxidant functions, including acting as a free radical scavenger, inhibiting lipid peroxidation, limiting oxidative damage induction, and expressing β-secretase (BACE1), in addition to inhibiting expression of proinflammatory markers COX2 and iNOS. Curcumin may have a direct benefit in AD as it appears to actively inhibit the accumulation of aggregated amyloid plaques *via* the binding and labeling of amyloid plaques.[40] In addition to this direct benefit, the AD patient may also receive an immunomodulatory benefit *via* an increase in mRNA and immunostaining of markers that may help to remove amyloid deposits.[44]

Coenzyme Q10 (CoQ10), which is reduced in PD patients, is associated with the mitochondrial oxidative phosphorylation enzymes that serve an antioxidant function. Evidence suggests that PD patients may benefit from CoQ10 supplementation as it may provide a protective resistance of the midbrain dopaminergic neurons to oxidative damage. Several pilot studies have supported that 1200–2400 mg per day of CoQ10 is well tolerated and may be beneficial in slowing disease progression in early PD patients.[46] Supplementation in rats has proven to enhance learning and memory, which may also have some implications for AD patients. Although evidence is inconclusive, supplementation of CoQ10 may prove to be beneficial in slowing the progression of many neurodegenerative diseases.[42]

Levels of homocysteine are increased in patients with AD and PD, and 1 epidemiologic study showed an increased risk of AD with high homocysteine levels. Increased folic acid intake and decreased calorie intake can have a negative effect on homocysteine levels. Studies suggest that decreasing homocysteine levels may actually be neuroprotective. High homocysteine levels may lead to increased vulnerability to oxidative stress, thereby increasing risk of AD and PD. Dietary folic acid supplementation may be beneficial in reducing risk of AD and PD by decreasing homocysteine levels and improving the neuron's ability to defend against oxidative stress. However, there is little evidence that folic

acid supplementation is beneficial in slowing progression of already established disease.[47]

Abnormal mitochondrial metabolism and cell death occur due to free radical generation. Increased mitochondrial DNA mutations are associated with both AD and PD. Acetylcarnitine facilitates the uptake of acetyl-CoA into mitochondria during fatty acid oxidation, enhances production of acetylcholine, and stimulates protein and membrane phospholipids synthesis. Neuroprotective benefits of acetylcarnitine include antioxidant activity, improved mitochondrial energetics, stabilization of membranes, and cholinergic neurotransmission. Studies show that AD patients may benefit from supplementation with acetyl-L-carnitine, improving cognitive function. In animal studies, acetylcarnitine supplementation led to increased cardiolipin levels and restored mitochondrial oxidant/antioxidant function.[43]

Plasma antioxidant levels are significantly lower in AD patients when compared with healthy subjects, which may mean that they have decreased ability to defend against oxidative damage. Specifically, plasma levels of vitamin C, uric acid, vitamin E, vitamin A, lutein, zeaxanthin, β-cryptoxanthin, α-carotene, plasma-SOD, plasma glutathione peroxidase, and erythrocyte SOD have measured significantly lower in the AD patient. This suggests that AD patients may be unable to defend against oxidative stress due to a decrease in antioxidant enzymatic activity. A diet high in antioxidants may prove beneficial in the prevention or delay of cognitive decline. According to epidemiologic data, increased vitamin C and vitamin E intake may decrease AD risk, although experimental studies are not conclusive as to the benefits of supplementation.[48] Vitamin E acts to suppress membrane lipid peroxidation and, in some clinical trials, has shown possible slowed progression of disease in AD patients.[42] Conversely, in a study of vitamin E use in combination with NSAIDs, minimal to no effect on cognitive decline and progression of the disease was found. Therefore, there is no consensus on the use of antioxidants as treatment of AD. Studying the ability of vitamin E to control oxidative damage and inflammation is difficult due to safety issues related to the supplement. Due to the limited evidence related to vitamin C supplementation, it should not be recommended at this time for prevention of AD.[49]

Other Neurodegenerative Disorders

ALS is a disease of damaged motor neurons, primarily affecting the brain and spinal cord. Pathogenesis of the disease may be related to formation of ROS and production of oxidative stress. Both sporadic and familial ALS is related to oxidative stress, although the exact etiology is not known. A mutation in the copper/zinc SOD (Cu/Zn SOD) gene may be the cause of inflammation and oxidative stress in some cases of ALS.[41]

Research has examined the beneficial effects of lipoic acid in cardiovascular disease, cancer, and diabetes. However, it has been suggested that ALS patients may benefit from supplementation. α-Lipoic acid functions as a coenzyme with antioxidant and cytoprotective benefits. One study demonstrated that supplementing α-lipoic acid in Cu/Zn-SOD mutant mice improves length of survival, decreases weight loss, and delays loss of motor function.[50] Theoretically, creatine supplementation may be beneficial in increasing muscle strength in these patients by improving cellular energetics and decreasing oxyradical production.[42] However, in animal and human studies, treatment with creatine supplementation showed no benefit.[51,52]

ALS disease progression is not slowed by dietary restriction, as is seen in AD and PD models. In fact, animals fed at restricted calorie levels experience no change in age of onset and a more rapid disease progression and earlier mortality.[42]

MS results from the interruption of myelinated tracts in the CNS, causing a myriad of symptoms including weakness, sensory disturbance, optic neuritis, diplopia, gait instability, and ataxia. The inflammatory process in MS is well characterized. Lymphocytes are activated in the periphery and mobilize in the CNS, where they attach to receptors and cross the BBB. T cells activate and express surface molecules called integrins. Integrins mediate binding to specialized capillary endothelial cells. The activated T cells then cross the BBB, expressing gelatinases that act as mediators in cell traffic and may increase the inflammatory response. Pathogenic T cells are reactivated and release proinflammatory cytokines, which open the BBB and stimulate chemotaxis. Recruitment of inflammatory cells then occurs.[53]

Patients with MS exhibit lower serum vitamin E and selenium, in addition to increased levels of inflammatory markers in the cerebrospinal fluid. The hypothesis has been stated that supplementation with antioxidants such as vitamin E and C may reduce risk of MS. However, most epidemiologic data do not support this hypothesis.[54] Antioxidant supplementation in diagnosed MS patients may be an important focus of future research.

Prevention of Disease Progression/Occurrence

Although definitive dietary guidelines have not been established relative to prevention and treatment of neurodegenerative disease, emphasis should be placed on restricting dietary calories, increasing DHA intake through fish consumption or through supplementation, and on eating a diet high in fruits and vegetables for increased antioxidant consumption.

Importance should be placed on avoidance of oxidative stress and strengthening of antioxidant defenses. Further research on the uses of vitamins

A, E, and C, selenium, L-carnitine, α-lipoic acid, DHA, and CoQ10 will further elucidate the possibilities of preventing neurodegenerative disease progression and occurrence.[1]

Conclusion

Inflammation, a common denominator among the diverse list of neurodegenerative diseases, has been implicated as a critical mechanism responsible for the progressive nature of neurodegeneration. Alterations in the body's immune response, ultimately leading to cell injury or cell death, encompass a wide variety of metabolic alterations, resulting in a chronic inflammatory state. Therapies for future treatment of neurodegenerative diseases, including nutrition-based therapies, will be targeted against this chronic inflammatory state and its mechanisms of action.

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Invited Review

Inflammatory Mediators and Home Parenteral Nutrition

Mary Hise, PhD, RD, CNSD*; Charlene Compher, RD, CNSD†; and John Brown, PhD‡
**Department of Dietetics and Nutrition, University of Kansas Medical Center, Kansas City, Kansas; †University of Pennsylvania, School of Nursing, Philadelphia, Pennsylvania; and the ‡Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas*

ABSTRACT: Individuals who have sustained intestinal failure due to trauma or disease are able to survive through the use of parenteral nutrition (PN). Although home PN (HPN) is a lifesaving therapy, patients may, over the long term, be at risk for liver, bone, and immune dysfunction. A limited number of human studies and a large number of animal studies suggest that there may be a chronic inflammatory condition and additionally a potentially lower T-lymphocyte immune function associated with PN administration. This article will primarily focus on a review of the limited clinical literature that examines the effect of long-term PN on the occurrence of inflammatory mediators in HPN patients, and will discuss the factors that are currently hypothesized to contribute to the potential inflammatory sequelae.

Individuals who experience intestinal failure due to trauma or disease are able to survive through the use of parenteral nutrition (PN). This lifesaving form of nutrition delivery is transiently used in the hospital setting as a form of nutrition support and is also used long-term by individuals who, because of disease or trauma, have irreversibly lost intestinal function. However, there are indications that this treatment, particularly over long periods, may place the individual at risk for liver or metabolic bone disease or immune dysfunction, perhaps through an increased and chronic, sustained production of proinflammatory mediators. Approximately 15% of individuals receiving home PN (HPN) develop end-stage liver disease, and the incidence of metabolic bone disease among these patients has been reported to occur at a rate between 40% and 100%.[1–3] Catheter-associated bloodstream infections occur at a rate of 0.8/1000 catheter-days[4] and are a major cause of morbidity among HPN patients.[5–7] These infections have been linked to several factors including active inflammation, loss of intestinal function, and the possibility of chronic immune dysfunction. PN has been shown to alter immune and inflammatory mediators in pig, mouse, and rat models; however, the current article will primarily focus on a review of the limited clinical literature that examines the effect of long-term PN on the occurrence of inflammatory mediators in HPN patients.

Correspondence: Mary Hise, PhD, RD, CNSD, University of Kansas Medical Center, Department of Dietetics and Nutrition, 3901 Rainbow Boulevard 4096 DELP, Mail Stop 4013, Kansas City, KS 66160-7250. Electronic mail may be sent to mhise@kumc.edu.

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Is Inflammation Associated With HPN Use?

There are only a limited number of human studies that have examined the extent of inflammation among patients receiving long-term PN. However, data obtained among these investigations suggest that there may be a chronic inflammatory condition and a potentially lower T-lymphocyte immune function among HPN patients relative to individuals with intact intestinal function. In a study of cholestasis in patients receiving HPN, Reimund et al[8] found that persistent inflammation was associated with the disorder. The patient population examined (n = 17) received HPN for a range of 6–132 months. Among this patient population, in comparison with healthy control subjects, 82% had abnormal liver function as determined by significant increases in serum levels of alkaline phosphatases, γ-glutamyltranspeptidase, and aspartate aminotransferase. The level of circulating inflammation markers was also examined among these patients compared with healthy control subjects. The presence of increased circulating concentrations of tumor necrosis factor-α (TNFα) and neopterin is an accepted indicator of inflammation and increased general immune activation, and has been identified among various disease states. A persistent state of inflammation in association with cholestasis was present among the HPN patients examined by Reimund et al[8]; this was indicated by the significant, positive correlations among erythrocyte sedimentation rate, circulating TNFα concentration, and circulating neopterin concentration when compared with the circulating level

of alkaline phosphatases as a measure of liver dysfunction. The positive correlation of TNFα and neopterin concentrations with alkaline phosphatases, in addition to a similar positive correlation with these 2 measurements in relation to the amount of infused calories, suggested a significant inflammatory condition in association with HPN use. In an earlier publication, Reimund et al[9] studied 21 HPN patients and found positive correlations among the circulating concentrations of TNFα, interleukin-6 (IL-6), and neopterin with the circulating manganese concentration of these patients. The level of circulating manganese may be an indicator of liver dysfunction (cholestasis) because the liver is the main organ involved with manganese excretion and maintenance of homeostasis for this trace element in the blood. One important observation made in this particular investigation was that of the 21 patients examined, the circulating manganese concentration within 8 of these patients was able to be determined before or within a few weeks of initiation of PN. Among these patients, the manganese concentration in the blood was within normal range (0.84 ± 0.3 μg/L, $p = .7$ *vs* controls). After a minimum of 3 months of HPN use (range, 3–132 months), this value rose among HPN patients to 1.96 ± 1.1 μg/L *vs* 0.81 ± 0.4 μg/L in controls, $p < .001$. Consequently, underlying disease may not entirely explain the occurrence of hypermanganesemia. Thus, the summary of data of Reimund et al[8,9] support the view that the use of HPN is correlated with liver toxicity to the extent that cholestasis is a significant risk factor for such patients. That an underlying inflammatory condition contributes to this disorder is supported by the positive correlation between HPN calories delivered with the level of cholestasis observed. The discussion issue in the studies by Reimund et al[8,9] is, of course, whether the level of inflammation among HPN patients relative to healthy control subjects as identified by markers such as TNFα, blood neopterin, and IL-6, for example, is independent of the level of inflammation associated with dysfunctional hepatic activity.

Additional investigations have also identified an apparent ongoing occurrence of inflammatory activity among patients who use HPN. A study by Ling et al[10] examined the effect of HPN use on the circulating and urine cytokine levels among 12 clinically stable patients who received HPN for a minimum of 1 year (range, 1.3–19.5 years). No evidence of liver abnormalities or active underlying disease activity was apparent among these patients. In this study, the circulating levels of albumin, C-reactive protein (CRP), temperature, body weight, and white blood cell (WBC) counts were determined. As additional, sensitive measures of inflammation, the blood and urine concentrations of TNFα and the 75-kDa form of the soluble receptor of TNFα (sTNFR-II), as well as IL-6, were determined.

Interestingly, the most commonly used indicators of inflammation (ie, circulating albumin and CRP levels, WBC count, and body temperature) were not significantly different for HPN patients relative to the determinations found among control subjects. In the study by Ling et al,[10] no statistical difference between the circulating CRP concentration among HPN patients compared with healthy control subjects was observed. As a confirmation of CRP determinations among HPN patients, 2 levels of CRP sensitivity assays were used for comparison. The range of CRP concentration among HPN patients in this study determined by conventional assay ranged from <4.0 to 4.0 mg/L (normal level: <8.0 mg/L), whereas the high-sensitivity assay yielded a range of 0.2–2.2 mg/L (normal range: 0.1–1.9 mg/L) among these patients. Similarly, the relative serum concentrations of TNFα among patients and control subjects were not detectable. When 24-h urine samples were tested, TNFα concentrations determined for HPN patients were not statistically different compared with those in control subjects (3.30 ± 0.29 ng *vs* 3.86 ± 0.44 ng, respectively). These data were in agreement with CRP determinations and indicated a stable, noninflammatory condition. In contrast to these data, other indicators of inflammation, IL-6 and sTNFR-II, revealed an inflammatory state among HPN patients.

The general association of the cytokine IL-6 with disease or infection, and the presence of this cytokine as a prognostic indicator of inflammation including the acute phase response, is well established. Indeed, the overproduction of IL-6 may be involved in the onset and maintenance of several disorders, including rheumatoid arthritis, various types of cancer, and other inflammatory diseases.[11] Therefore, the increased production of IL-6 among otherwise stable HPN patients provides evidence for a chronic, proinflammatory response among these patients. In this study, IL-6 levels within the blood and urine were significantly increased among HPN patients relative to control subjects. Serum and 24-h urine IL-6 levels among HPN patients averaged 1.45 ± 0.37 pg/mL and 5.45 ± 1.17 pg/mL, respectively, whereas serum and 24-h urine IL-6 levels averaged 0.29 ± 0.14 pg/mL and 2.48 ± 0.28 pg/mL, respectively, among control subjects. Therefore, compared with control subjects, the IL-6 level in HPN patients was more than 5-fold higher in serum and more than 2-fold higher in 24-h urine ($p < .01$ serum, and, 24-h urine).

There are 2 membrane receptors for TNFα, and these receptors can be released in soluble form as a result of metalloproteinase activity and can enter the circulation. Soluble TNFR-I (55 kDa) and sTNFR-II have been demonstrated to occur at low levels in the serum and urine of healthy humans.[10,12] An increase in circulating sTNFRs, however, has been shown to be an important prognostic indicator of disease activity and is associated with deterioration of nutrition and immune activity.[13] Both soluble TNFα receptor forms have been linked to the acute-phase response, malnutrition, or

death in a variety of clinical states, including human immunodeficiency virus (HIV),[14] cancer,[13] and arthritis.[15] These shed forms of cell-surface TNFα receptors also seem to play a dual role in regulation of the formidable inflammatory activity of this cytokine. Soluble TNFRs in relatively higher concentration can likely function as physiologic inhibitors of TNFα activity by interaction with circulating TNFα and subsequent interference with TNFα binding to membrane TNFRs.[16,17] Alternatively, lower relative levels of TNFα bound to sTNFRs may extend the half-life of TNFα, and such complexes may serve as a reservoir of the cytokine, and therefore could potentially prolong TNFα biologic—and thus inflammatory—activity.[18–20] In the Ling et al[10] study, sTNFR-II determinations yielded the following data. Serum and 24-h urine sTNFR-II levels among HPN patients averaged 7.84 ± 1.68 ng/mL and 17.64 ± 3.97 mg, respectively, whereas serum and 24-h urine sTNFR-II levels among control subjects averaged 1.32 ± 0.28 ng/mL and 0.91 ± 0.18 mg, respectively. Therefore, the sTNFR-II level was 6-fold higher in the serum and 17-fold higher in 24-h urine samples in HPN *vs* control patients ($p < .001$, serum and 24-h urine).

Further studies of inflammation in HPN patients were performed by Hise et al.[21] In this study, 10 stable HPN patients who required PN for at least 2 years and had no evidence of active disease were compared with healthy control subjects with respect to a number of parameters. These parameters assessed inflammatory activity (serum TNFα, sTNFR-I and sTNFR-II, CRP, and IL-6 concentrations), enumeration of peripheral blood T lymphocytes (CD3+) and T-lymphocyte subsets (CD4+ and CD8+), and T-cell function (proliferation activity in the presence of phytohemagglutinin). Similar to the data obtained by Ling et al,[10] when the high-sensitivity CRP assay was used, Hise et al[21] found that circulating CRP levels were not statistically different among HPN patients compared with control subjects, with values in the normal range (0.1–1.9 mg/L) for each population: 0.99 ± 0.14 mg/L *vs* 1.2 ± 0.19 mg/L, respectively. In addition, the circulating TNFα concentration was relatively low and not significantly different in HPN subjects relative to controls (5.4 ± 0.95 *vs* 5.4 ± 0.88 pg/mL, respectively). At first glance, these data and that of Ling et al[10] suggest that there is an absence of ongoing inflammatory condition among HPN patients. However, this conclusion is not supported when one further examines the issue. Like Ling et al,[10] the Hise et al[21] study found that sTNFR-II concentrations in serum were significantly elevated among HPN patients. The Hise study also determined a similarly elevated level of circulating sTNFR-I in these patients compared to control subjects. The values obtained for sTNFR-I in HPN patients and control subjects were 1311.2 ± 93.1 pg/mL *vs* 452.0 ± 40.2 pg/mL, respectively, $p < .001$. The values obtained for sTNFR-II in HPN patients and control subjects were 1592.0 ± 105.0 pg/mL *vs* 666.0 ± 82.0 pg/mL, respectively, $p < .001$. When the values for IL-6 determinations were compared, there was a consistently elevated serum level of this cytokine among HPN patients relative to control subjects, but the difference did not reach statistical significance. The values obtained for IL-6 among HPN patients and control subjects were 3.21 ± 0.28 pg/mL *vs* 2.50 ± 0.24 pg/mL, respectively.

Both Ling et al[10] and Hise et al[21] found that serum TNFα concentrations were not statistically different between HPN patients and healthy control subjects. However, these values of TNFα detected may be deceiving with respect to evidence for inflammation. In light of the statistically significant differences in sTNFR concentrations between the HPN and control subject populations found in both the Ling et al[10] and Hise et al[21] studies, it is possible that although detection of the cytokine in the unbound state by the assay used (monoclonal TNFα specific antibody enzyme-linked immunosorbent assay [ELISA]) was accurate, the assessment of total TNFα (bound + unbound) was not.[16] As discussed above, the activity and perhaps half-life of TNFα may be significantly affected by the relative level of soluble receptors for this cytokine.

In addition to these findings, Hise et al[21] observed that a potential immune dysfunction was associated with HPN use. A lower percentage of $CD4^+$ (38.03 ± 4.70 HPN patients *vs* 51.19 ± 2.77 controls; $p < .05$) and $CD8^+$ (17.35 ± 2.52 HPN patients *vs* 20.87 ± 1.72, but did not reach statistical significance) lymphocytes was present among HPN patients relative to control subjects. Although the $CD4^+/CD8^+$ ratio between the 2 populations did not differ significantly (2.5 ± 0.43 HPN patients *vs* 2.8 ± 0.40 controls), the proliferative response as determined by percent proliferating cells of HPN patient T cells in response to mitogen stimulation was substantially reduced relative to the T cells of control subjects (18.04 ± 4.24 HPN patients *vs* 48.05 ± 4.50 controls). These data suggest that in addition to chronic inflammation among HPN patients, a condition of suppressed immune function may also be present.

An additional result obtained from the Hise et al[21] study was determined from further investigation of the low level of chronic inflammation observed as assessed by soluble TNFα receptor concentrations. When the levels of both sTNFR-I and -II were each independently examined for each patient relative to the length of time of HPN use among the patient population, there was a significantly positive correlation between sTNFR values and duration of HPN use. The regression analyses indicated that when control subjects were excluded from the analyses, for each year of HPN use, one could predict an increase in sTNFR-I of 28 pg/mL and sTNFR-II of 29 pg/mL (sTNFR-I *vs* years requiring HPN, $R^2 = 0.63$, $p = .0001$; and for sTNFR-II *vs* years requiring HPN, $R^2 = 0.72$, $p = .0001$). If control subjects were included, the regression analyses predicted an

increase in sTNFR-I of an additional 55 pg/mL and sTNFR-II of 59 pg/mL per year of HPN use (sTNFR-I *vs* years requiring HPN, $R^2 = 0.55$, $p = .0001$; and for sTNFR-II *vs* years requiring HPN, $R^2 = 0.62$, $p = .0001$). These data support Reimund et al,[8] who detected a positive correlation between HPN calories delivered with the level of cholestasis observed. In addition, it supports the hypothesis that the number of years of HPN use or permanence of intestinal failure increase the risk for liver dysfunction and failure as suggested by Chan et al[3] and Cavicchi et al.[22]

What Factors Are Currently Hypothesized to Contribute to the Inflammation Associated With the Long-term Use of PN?

The lack of significant small intestinal mass (as in short bowel syndrome) or the absence of small intestine stimulation (as in dysmotility syndromes) through normal enteral feeding alone could lead to the increased level of inflammation or immune interference observed among HPN patients. Alternatively, a component or components within the PN solution could lead to these results, and each condition could interact and exacerbate the other. It is also possible that the immune system interacts with the catheter as a foreign body and responds with inflammation. It is impossible in humans to completely remove the influence from the absence of, or lack of use of, small intestine from that of the PN solution's influence on inflammatory activity or immune function because dysfunctional or lack of small intestine simultaneously requires PN to sustain life. However, this difficulty can at least be partly overcome through the use of animal models. Consequently, it is instructive to examine some of the recent data available from several elegant animal studies that assessed the physiologic effects of PN. In most of these studies, animals with intact small intestine received PN in the presence or absence of enteral nutrition (Table 1).

Teitelbaum and co-workers have generated several excellent studies which provide evidence that the absence of intestinal feeding in animals with intact small intestine significantly alters cytokine expression and mucosal immune parameters.[23–25] A recent study by Wildhaber et al[26] that is particularly representative of many of Teitelbaum et al's findings, provided substantive evidence that IV PN given to mice in the absence of enteral feeding significantly increased the following parameters: inflammatory cytokine mitochondrial RNA (mRNA) levels (IL-4, IL-6, interferon γ [IFN-γ], transforming growth factor β_1 [TGF-β_1], and TNFα) expressed by intraepithelial lymphocytes (IEL), bacterial translocation, and epithelial cell apoptosis, when compared with parenterally + enterally fed and to only enterally fed control mice. In addition to these effects of PN when provided in the absence of enteral feeding, alterations in mucosal immunity, reflected in the phenotype and function (mRNA cytokine expression) of IEL, were observed. Both the IEL $CD4^+CD8^-$ and $CD4^+CD8^+$ populations were significantly reduced, as were the CD8$\alpha\beta$ heterodimer$^+$ (thymus-dependent) and $CD8^+CD44^+$ (mature IEL) in the parenterally fed, non–enterally fed group when compared with the control (enterally fed, non–parenterally fed) group and to the combined enterally + parenterally fed group. These data provide strong evidence that the lack of enteral feeding, rather than the PN solution *per se*, induced these changes. These observations are supported by a similar study that examined IEL phenotype and cytokine expression among mice that were subjected to massive small bowel resection (MSBR) in the absence of any PN use postoperatively.[27] In this study, mice were subjected to a 70% mid–small bowel resection. After 1 week of standard mouse chow diet provided in liquid form, IEL were isolated and examined by flow cytometry for phenotypic changes. In addition, cytokine mRNA levels and the proliferative response to T-cell activation were determined for the IEL population. The results of this investigation revealed that MSBR led to significant decreases in specific IEL T-cell subpopulations, including CD8$\alpha\beta^+$ T cells, $CD44^+$ and $CD69^+$ (identifies activated T cells) T lymphocytes. When compared with control mice, IEL TNFα mRNA expression increased 84%, whereas IL-2 and IL-10 mRNA expression decreased by 69% and 72%, respectively. Although the percent proliferation of the IEL in the MSBR group when removed from the milieu of the remaining epithelial layers was significantly higher than controls, when these cells were further stimulated, proliferation failed to increase.

Similar data have been generated in rodents with intact small intestine that show a significant effect of the lack of enteral feeding (that requires the presence of PN) on intestinal immunity and related sequelae. Kudsk and co-workers demonstrated in a series of excellent investigations that the lack of enteral feeding in IV-fed animals significantly alters gut-associated lymphoid tissue (GALT) mass as a result of depletion of Peyer's patches, lamina propria, and the intraepithelial region that contains T and B cells. In addition, this treatment also results in a decrease in the $CD4^+/CD8^+$ ratio among lymphocytes within the lamina propria.[28] In addition to the decrease in IEL, significant lowering of both intestinal and respiratory immunoglobulin A (IgA) levels simultaneously occurs in concert with the loss of IEL.[29] Furthermore, a decrease in the levels of both IL-4 and IL-10 within intestinal tissue was observed.[30,31] When these animals received enteral feeding in the form of chow or a complex enteral diet, normal parameters returned in that both chow and a complex enteral diet maintained normal GALT cell populations, IgA level, and antibacterial immunity. And, although gastrically supplied PN solution led to atrophy of GALT and a reduction in $CD4^+/CD8^+$ ratio that was similar to IV-supplied parenteral

Table 1 *Immune function in animal models of parenteral nutrition*

Author	Design/model	Immune status alterations
Kiristioglu and Teitelbaum, *J Surg Res*, 1998[23]	Mice received IV saline + chow or PN	Increase in bacterial translocation in PN mice and significant decrease in CD4+ and CD8+ IEL populations in PN mice when compared with control.
Yang et al, *Crit Care Med*, 2003[24]	Adult wild-type and IFN-γ knockout mice received PN or enteral diet (control group)	PN significantly increased small bowel permeability in wild-type mice and appeared related to IFN-γ production because small bowel permeability within IFN-γ knockout mice was decreased.
Yang et al, *Am J Physiol Gastrointest Liver Physiol*, 2003[25]	Mice received PN or oral feeding	PN significantly increased intestinal epithelial cell apoptosis.
Wildhaber et al, *J Surg Res*, 2005[26]	Mice received oral feeding, PN alone, or PN plus oral feeding	Relative to control and PN plus oral feeding group, PN alone resulted in significant increases in bacterial translocation, epithelial cell apoptosis, as well as IL-4, IL-6, IFN-γ, transforming growth factor β-1 and TNFα mRNA expression, whereas similar comparison revealed a decrease in CD4+ and CD8+ lymphocytes, IL-2 and IL-10 mRNA.
Yang et al, *J Surg Res*, 2003[27]	Mice received MSBR	MSBR significantly decreased specific IEL subpopulations in addition to IL-2 and IL-10 mRNA expression and increased TNFα mRNA expression.
Li et al, *J Trauma*, 1995[28]	Mice received either oral chow (control), oral PN feeding, or IV PN	Enterally and parenterally fed PN groups showed a decrease in total T cells, CD4+ T cells, and a reduction in the $CD4^+/CD8^+$ ratio in lamina propria populations.
King et al, *Arch Surg*, 1997[29]	Mice received chow for 2 days, followed by PN for 0–5 days	T- and B-cell yields in Peyer's patches and lamina propria were significantly reduced by day 2 and thereafter; lamina propria $CD4^+/CD8^+$ ratio declined significantly by day 4, and small intestinal and respiratory IgA were significantly diminished by day 3.
Wu et al, *Ann Surg*, 1999[31]	Mice received either chow, IV PN, intragastric PN, or complex enteral diet	Severely impaired mucosal immunity with IV PN. Chow and complex enteral diets maintained balance between IgA-regulating cytokines.
King et al, *Ann Surg*, 1999[32]	Mice received either chow, IV PN, intragastric PN, or complex enteral diet	Protection against bacterial pneumonia by immunization was preserved within chow and complex enteral feeding groups and lost with IV PN group.

PN, parenteral nutrition; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; mRNA, mitochondrial RNA; MSBR, massive small bowel resection; IEL, intraepithelial lymphocyte; IgA, immunoglobulin A.

solution, this means of feeding partially corrected respiratory defense against intratracheal *Pseudomonas*.[32]

The possibility also exists that a specific individual component of PN solutions is associated with alterations in immune and inflammatory mediators. Excessive lipid, dextrose, or total calories have all been linked to worsening of liver function. The lipid component of the PN prescriptions has been the most studied and debated in the literature. And recently, an elegant review of the effect of IV fat emulsions on immune and inflammatory mediators has been published.[33] Whether lipid components of HPN prescriptions over a significant period of time could contribute to a low-grade inflammatory process remains unclear. Meta-analysis of a variety of patient populations does not support the premise that long-chain triglyceride (LCT) emulsions significantly affect immunologic status or mortality.[34] However, alterations in fatty acid profiles, including an increase in the proinflammatory fatty acid arachidonic acid, have been shown to be elevated in

patients receiving HPN.[35] Additional studies suggest that lipid emulsions with varying fatty acid compositions may influence metabolic systems by alteration of membrane structure and function, by modulation of immune function through ω-6 and ω-3 metabolites, and by stimulation of inflammatory cytokines. Research describing alterations in immune and inflammatory function with primarily soybean-based emulsions has led to investigations of alternative forms of lipid solutions for HPN patients. Recently, an olive oil lipid emulsion containing 80% olive oil and 20% soybean oil was examined in HPN patients for safety, efficacy, and inflammatory potential.[36] Nutrition status, clinical and biologic tolerance, and systemic inflammatory markers were analyzed for 13 HPN patients over a 3-month period. The olive-based emulsion replaced the lipid component of the PN prescription that was either 100% LCT emulsions or a 50% LCT–50% medium chain triglyceride (MCT) emulsion. Over the course of the study, the olive oil emulsion was well tolerated; there were no significant inflammatory or immune changes noted in a variety of measures, including TNFα, erythrocyte sedimentation rate, soluble receptor for IL-2, erythrocyte glutathione peroxidase, IL-6, IL-8, CRP, malondialdehyde, or blood neopterin levels.

There is considerable doubt whether the effect of lipids alone on inflammatory and immune sequelae can explain the observed responses to HPN that have been demonstrated in this population. For example, essential fatty acid deficiency may be prevented in HPN patients with provision of as low as 0.2 g/kg/d of soy-based emulsion. In addition, a subpopulation of HPN patients may not receive lipids as part of their prescription, and finally, in the animal studies discussed above that were performed by Kudsk and co-workers,[28–32] lipid was purposefully eliminated from the parenteral formulation.

An additional factor that may contribute to inflammation in HPN patients may be the presence of an indwelling catheter. Little is known about real-time catheter conditions and how the catheter itself may affect immune function or inflammation. Microorganisms attach to surfaces, including those of medical devices, such as central venous catheters, to form biofilms. These biofilms may be composed of gram-positive or gram-negative bacteria or yeasts. Biofilm formation has been noted on virtually all indwelling central venous catheters.[37] Excess accumulation of organisms on the catheter tip is related to bloodstream infection. It is not known whether a lower concentration of organisms could elicit a low-grade inflammatory response within the host.

In summary, there is convincing evidence provided from among many animal studies and although limited, consistent evidence among the few human studies performed that HPN leads to a low-level, chronic, inflammatory response. In addition, there is similar evidence that immune function may be impaired. Although the precise, interdependent physiologic mechanisms that lead to these conditions is not yet understood, the loss of small bowel, including the constituent lymphoid mass, appears to be the most significant contributor to these conditions. There of course remains the additional possibility that a component or components within the parenteral formula or bacteria adhering to the catheter may exacerbate or maintain the inflammatory condition. If a loss of immune function is consistently present among HPN patients, the potential sequential episodes of infection may additionally lead to a continuous, self-sustaining loop of inflammation activity.

Together, the currently available data strongly support the view that the inflammatory and immune status of HPN patients should be more closely and thoroughly studied. It is important that the studies include clarification of any impact of specific PN components, catheters, and low-grade bacterial exposure. The long-term health risks of such an inflammatory state should also be described. According to information derived from both animal and human studies, it may be important that additional, perhaps more sensitive, parameters be considered to assess inflammation within this patient population. Such parameters may include monitoring of soluble receptors of TNFα (sTNFR-I and sTNFR-II) or enumeration of circulating $CD4^+$ and $CD8^+$ lymphocytes.

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Invited Review

A Review of Complementary and Alternative Approaches to Immunomodulation

John O. Clarke, MD; and Gerard E. Mullin, MD
Division of Gastroenterology, The Johns Hopkins University School of Medicine, Baltimore, Maryland

ABSTRACT: Current Western therapies for inflammatory diseases are suboptimal; increasingly, patients are turning to complementary and alternative medicine for symptom relief and improved quality of life. There is emerging evidence that many of these therapies have the ability to modulate the immune system and disrupt the proinflammatory cascade through a variety of mechanisms, including antioxidant effects, alterations in cell signaling (in particular the nuclear factor (NF)-κB pathway), cytokines, proinflammatory mediators, and disruption of bacterial flora. Using inflammatory bowel disease (IBD) as a model of inflammation, we explore the principal complementary and alternative medicine treatments that show promise in this regard, namely, resveratrol, green tea, curcumin, boswellia, fish oil, vitamin D, and probiotics. With each agent, we detail the mechanisms that have been described with regard to immune modulation, discuss the medical conditions for which it has been evaluated, and explore the data to date for the prevention or treatment of IBD.

The majority of reimbursed care in the United States today is *via* Western medicine, a tradition that harkens back, in a primitive form, only to the Renaissance. Complementary and alternative medicine (CAM) refers to medical practices that are not currently considered to be part of conventional medicine. However, these "alternative" and "natural" approaches have significant time-proven history, just not in Western literature. Traditional Chinese medicine stretches back 5000 years, and traditional Indian (Ayurvedic) medicine can trace its history for over 2000 years. At the start of the 20th century, in fact, there were already 30,000–40,000 books regarding these practices already in existence.

With all the focus on drug development and marketing, it is easy to forget that nutrition represents the world's earliest medicinal therapy. In the words of Hippocrates (obviously translated) "He who does not know food—how can he cure the disease of man?" Many of the medicinal agents used for therapy today are directly derived from food sources. The role of functional foods in health and disease prevention is a rapidly growing field.[1] Who knows how many other agents are present in everyday foods that have not yet been tapped?

This article will aim to clarify what is known about alternative and nutrition therapies for immunomodulation. Obviously, this is a broad therapy, and so discussion will be restricted to a few key categories: polyphenols (including resveratrol, epigallocatechin, curcumin, and boswellia), ω-3 essential fatty acids (EFA; fish oil), vitamin D, and probiotics. Although many diseases can be examined as a model for inflammation (including inflammatory bowel disease [IBD], rheumatoid arthritis, and multiple sclerosis, to name a few), we have elected to focus on IBD exclusively because: (a) we are gastroenterologists and this is our bias, and (b) to dwell on every inflammatory condition would make this paper too unwieldy to be readable without coercion.

In the words of Hippocrates: "Let food be thy medicine."

Polyphenols

Polyphenols are phytochemicals that are found in food substances produced from plants. Polyphenols are separated from essential micronutrients in that a deficiency state has not been identified; nevertheless, these chemicals are believed to play a biologically active role and have been shown to be potentially immunomodulating.[2] Although numerous polyphenols have been identified, 4 in particular have a preponderance of evidence in the role of immune modulation and will be addressed in this review: resveratrol, epigallocatechin, curcumin, and boswellia. The findings of polyphenols to prevent and treat animal models of IBD are summarized in Table 1.[3–22]

Correspondence: Gerard E. Mullin, MD, The Johns Hopkins Hospital, Division of Gastroenterology, 600 North Wolfe Street, Carnegie Building, Room 464, Baltimore, MD 21287. Electronic mail may be sent to gmullin1@jhmi.edu.

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Table 1 *Prophylactic and therapeutic effects of polyphenols in animal models of IBD*

Polyphenol	No. of studies	Dose; route	Results
Resveratrol	2	5–10 mg/kg; 2/2 IG	2/2 Improvement: clinical, path, mediators, cytokines
EGCG	3	5 g/L, 50 mg/kg/d; 1 IP, 2 PO	3/3 Improvement: clinical, path, mediators, cytokines
Curcumin	6	2%, 30–300 mg/kg/d; 6 PO, 1 IP	6/6 Improvement: clinical, path, mediators, cytokines, markers; 4/7 ↑ survival
Boswellia	3	5.0–34.2 mg/kg/d; 2 PO, 1 IP	2/3 Improvement: clinical, macroscopic, microscopic, mediators; 1/3 no improvement

Resveratrol, ECGC, curcumin, and boswellia have been evaluated in mice for their ability to treat and prevent chemical-induced colitis. Quercetin has been evaluated in 6 trials, with mixed results, and was not included in the table for this reason.[3–22]
↑, increased; EGCG, epigallocatechin gallate; IBD, inflammatory bowel disease; IG, intragastric; IP, intraperitoneal; PO, by mouth.

Resveratrol

Resveratrol, trans-3,5,4′-trihydroxy-trans-stilbene, is a phytochemical produced by plants. It has been identified in >70 plant species, including grapes, peanuts, berries, and pines; however, it is believed to be most abundant in the skin of red grapes, contributing to a high concentration in red wine and grape juice.[23] Since the initial report linking resveratrol to the possible cardioprotective benefits seen with red wine, hundreds of papers have been published showing purported health benefits.[24] These have encompassed a wide array of illnesses, including cardiovascular disease,[25–29] cancer,[23,30–33] immunomodulation,[34–38] and longevity.[39,40]

Numerous mechanisms for resveratrol have been proposed, including inhibition of cyclooxygenase (COX), hydroperoxidase, protein kinase C, Bcl-2 phosphorylation, Akt (an anti-apoptic kinase), focal adhesion kinase, nuclear factor (NF)-κB, matrix metalloprotease-9, and cell cycle regulators.[23] With regard to anti-inflammatory and immunomodulatory effects, the exact mechanism by which resveratrol works has not been clearly established; nevertheless, significant interest has been paid to this potential role, given that COX inhibitors are commonly used as anti-inflammatory drugs and resveratrol is a potent inhibitor of COX activity *in vivo*.[41,42] However, the effect of resveratrol on the immune system does not seem to be mechanistically as simple as nonspecific inhibition of inflammation; resveratrol seems to enhance the immune response of mice treated with the arylating substance dinitrofluorobenzene and prevents immunosuppression by ethanol.[36] Resveratrol also appears to protect mice from infection with herpes simplex viruses.[43,44] The exact mechanisms by which resveratrol differentially inhibits and enhances the immune system have not been clearly elucidated.

In rodent models of inflammatory colitis, intragastric resveratrol given acutely before and after colonic injury has been shown to reverse weight loss, increase stool consistency, improve mucosal appearance, improve histopathology, decrease inflammatory infiltrate, and decrease mucosal levels of interleukin (IL)-1β, COX-2, and prostaglandin (PG) D_2.[45] In another study by the same group, intragastric resveratrol was given for a 14-day period after colonic injury and was shown to increase stool consistency; improve colonic appearance and histopathology; decrease tumor necrosis factor-α (TNFα), NFκB, and colonic myeloperoxidase (MPO) activity; and normalize prostaglandin E2 (PGE2) levels.[4] To date, resveratrol has not yet been studied in human subjects with IBD; however, given its impressive results in the rodent model, it seems like a reasonable next step, if issues of cost, bioavailability, and toxicity can be ironed out.[24,46]

Catechins

Catechins refer to monomers of flavonols with similar composition such as catechin, epicatechin, epigallocatechin, epicatechin gallate (EGC) and epigallocatechin gallate (EGCG). These compounds are particularly abundant in green (nonfermented) tea, whereas black tea contains theaflavins and thearubigins.[47,48] Given that tea is the most consumed beverage in the world other than water,[49,50] the health benefits present in these chemicals may translate to significant public health benefits on a global scale. Reports have linked green tea to beneficial effects in the prevention or treatment of cancer (breast,[51,52] ovarian,[53] prostate,[54] stomach,[55–57] and lung[58]), hypertension,[59–61] cardiovascular disease,[56,62–66] oral health (dental caries, periodontal disease, and tooth loss),[67] skin disease,[68,69] weight management,[70,71] osteoporosis,[48] and glucose tolerance.[72,73] There is also significant data evaluating the role of catechins in immune modulation, which will be detailed below.

The mechanisms by which catechins achieve their beneficial effects is still not entirely clear; however, there is mounting evidence that they likely work through a combination of both antioxidant effect and alteration of intracellular signaling (primarily through inhibition of the NFκB pathway). Catechins, particularly EGCG, are effective free radical scavengers *in vitro*[74]; however, it has been suggested by some researchers that these compounds may play a relatively minor role as antioxidants *in*

vivo due to low circulating levels and rapid metabolism.[75] This has led to investigation into the role of catechins in cell signaling, and it has now been demonstrated that EGCG can modulate and inhibit NFκB activity.[76] Given that expression of IL-8, a major human inflammatory mediator, is dependent on IL-1β activation of NFκB, it stands to reason that inhibition of the NFκB cascade may result in a profound effect on inflammation. To support this, studies have shown that administration of EGCG can affect and inhibit the infiltration of CD8+ T cells into sites of inflammation.[77]

Using IBD as a marker of a chronic inflammatory disease, the current data on catechin administration are promising. Using a murine colitis model with IL-2 deficiency, investigators were able to show that oral administration of green tea extract for 6 weeks after disease presentation resulted in weight gain, improved colonic histopathology, decreased colonic weight, and increased hematocrit (Table 1).[6] In another study involving a murine IBD model, it was shown that green tea polyphenol extract given for 3 days before and 7 days after a caustic trigger resulted in decreased weight loss, improved diarrhea, improved histopathology, decreased serum inflammatory cytokines, and improved hematocrit.[5] Similar findings were reported in a rat model of colitis given green tea polyphenols extract for a period of 5 days.[3] An *in vitro* study involving human colonic tissue showed that administration of EGCG resulted in decreased proinflammatory cytokine production and down-regulation of genes involved in inflammation.[78] To date, there are no *in vivo* human studies evaluating the role of green tea extract in IBD; however, given the encouraging results above and the excellent safety profile of these agents, it is hard to imagine that these studies are far away.

Curcumin

Turmeric, the major spice in curry, is a natural spice made from the herb *Curcuma longa*, a member of the ginger family. Besides being a culinary staple, it has been used in Ayurvedic medicine since ancient times. The major chemical constituents of turmeric are curcuminoids, the most prominent of which is curcumin. In traditional medicine, it has been used as an oral and topical agent to treat a wide variety of ailments, including—but not limited to—pain, rheumatism, amenorrhea, liver disease, common colds, and pulmonary diseases.[79–81] Given the longstanding history of this medication, patient preference for a "natural" remedy and the excellent safety profile in studies to date,[82,83] research has exploded in the use of curcumin for medicinal treatment, and there is emerging literature for gastrointestinal disease. To date, over 1900 papers have been published on curcumin (and most of these have been published in the last 4 years). Studies to date have suggested possible benefits in the prevention or treatment of numerous diseases, including atherosclerosis,[84,85] cancer,[86,87] neurodegenerative diseases including Alzheimer's dementia,[88,89] pancreatitis,[90,91] and rheumatoid arthritis.[92,93]

The number of mechanisms by which curcumin acts seems to be rivaled only by the number of disease processes in which it has been shown to be of benefit. Its antioxidant activity was initially demonstrated in 1976,[94] and it has been shown to be a potent free radical scavenger both *in vitro* and *in vivo*.[95] Recently, investigational focus has shifted toward the role of curcumin as an intracellular signaling agent, and studies have demonstrated that curcumin, much like green tea polyphenols, is an inhibitor of NFκB[96,97] and leads to downstream regulation and inhibition of proinflammatory genes and cytokines (Figure 1).[47] Interestingly, the cell signaling effects of curcumin seem to be pleiotropic as administration of curcumin has also been reported to modulate a host of other cytokines and signaling pathways, including inducible nitric oxide synthase (iNOS), matrix metalloproteinase-9 (MMP-9), TNFα, c-Jun N-terminal kinase (JNK), p38, Akt, Janus kinase (JAK), extracellular signal-regulated protein kinase (ERK), and protein kinase C (PKC).[47,98,99] Given the wide array of pathways affected by curcumin, it is difficult to distinguish whether the anti-inflammatory effects of this agent are due primarily to inhibition of one specific pathway or due to the combination of multiple interlocked systems. Hopefully, this will be clarified with ongoing and future research.

Given that curcumin may act through NFκB inhibition and would be expected to down-regulate proinflammatory genes and decrease cytokines involved in inflammation, it would stand to reason that IBD would be a natural avenue to explore for possible therapeutic efficacy. Not surprisingly, the studies to date examining this have been encouraging. Studies involving curcumin to date in the field of IBD have been consistently positive. Four studies involving curcumin administration to murine colitis models showed clinical and histopathological improvement and, where measured, decreased inflammatory cytokine production.[8,9,12,100] These findings were echoed in 3 studies involving rodent models of colitis.[7,10,11] The natural next step would be a pilot study in human subjects with IBD. Holt and colleagues[101] reported in 2005 the preliminary results of a pilot study involving open-label administration of curcumin preparation to 5 patients with ulcerative colitis and 5 patients with Crohn's disease. Of the 10 patients, 9 reported improvement at the conclusion of the 1-month study. Four of the 5 patients with ulcerative colitis were able to decrease or eliminate their medications. In a larger, randomized, double-blind, multicenter trial involving 89 patients with quiescent ulcerative colitis, administration of 1 g of curcumin twice daily resulted in both clinical improvement and a statistically significant decrease in the rate of relapse.[102] Given its excellent safety profile, plausible mechanism for

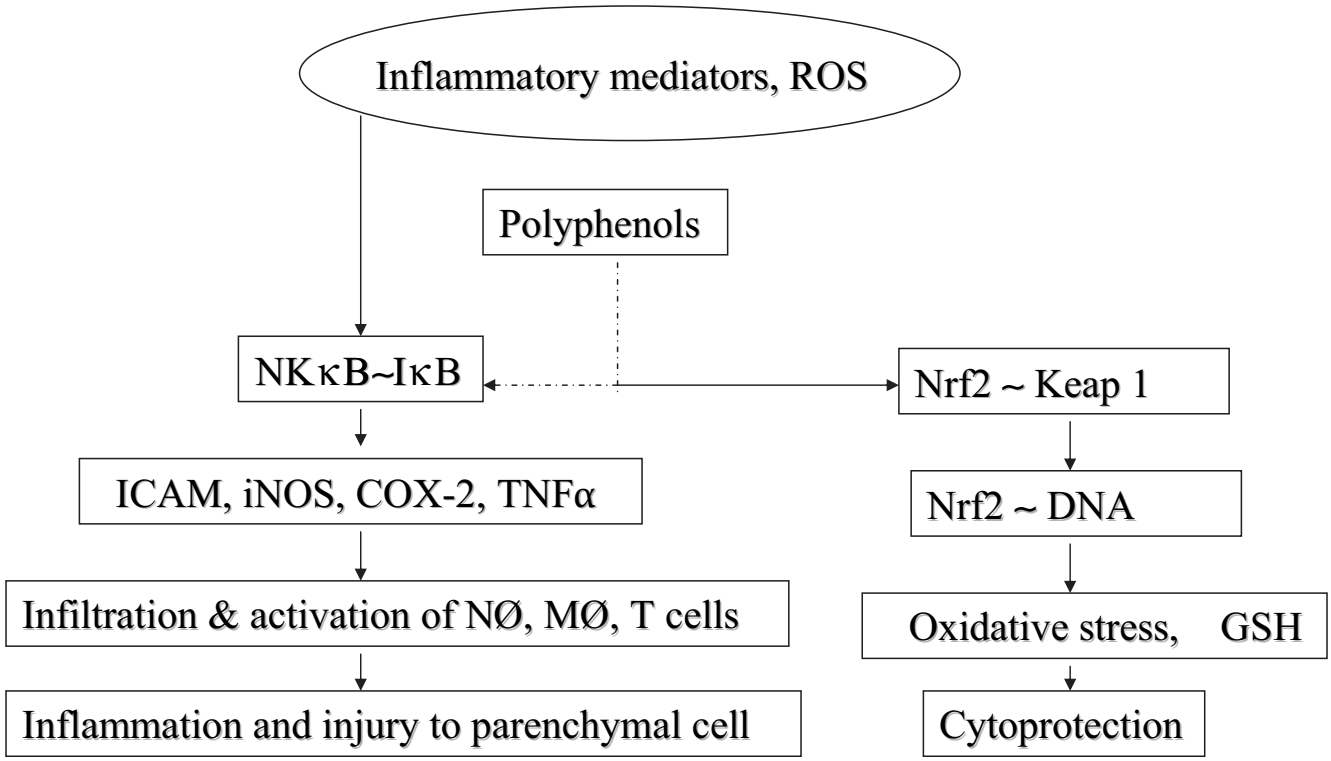

Figure 1. Polyphenols attenuate inflammation and injury. Polyphenols attenuate injury to parenchymal cells by down-regulating inflammatory genes and up-regulating cytoprotective ones. COX-2, cyclooxygenase-2; DNA, deoxyribonucleic acid; GSH, reduced glutathione; HO-1, haem-oxygenase-1; IκB, inhibitor of κB; ICAM, intercellular adhesion molecule; iNOS, inducible nitric oxide synthase; NFκB, nuclear factor κB; Nrf2, nuclear factor E2–related factor; ROS, reactive oxygen species; TNFα, tumor necrosis factor-α.

affecting inflammation, and the results above, curcumin is poised to have a prominent role in the future management of IBD.

Boswellia

The lipophilic fraction of the gum from the tree *Boswellia serrata*, termed "frankincense," is a traditional Ayurvedic remedy. It has been used in Asia and Africa as a medical therapy for at least 3500 years and has been used to treat a wide variety of ailments, including respiratory problems, diarrhea, constipation, flatulence, central nervous system disorders, rheumatism, liver disease, wound healing, fat reduction, and fevers. It has also been used as a mental tonic, taste enhancer, and even as an aphrodisiac.[103,104]

When the resin of different *Boswellia* species is analyzed, over 200 different compounds can be identified. However, the main biologic effects of the *Boswellia* species are thought to be derived from a group of chemicals referred to as tetracyclic triterpenes and pentacyclic triterpenes. These substances are referred to commonly as boswellic acids (BA).

How these agents work is not completely understood. It has been shown that BA interfere with the 5-lipoxygenase pathway, with a resultant decrease in leukotriene formation (Figure 2). This has been demonstrated in a number of *in vitro* experiments[103,105]; however, there is considerable debate as to whether suppression of 5-lipoxygenase and leukotriene production is of pharmacologic relevance *in vivo*.[103] Other postulated molecular targets for BA include human leukocyte elastase, CYP 2C8/2C9/3A4, topoisomerase I, topoisomerase IIa, and IKK α/β. In addition, recent reports have also suggested that BA may also exert some effect through calcium mobilization and mitogen-activated protein kinase phosphorylation.[103] A recent paper has also shown that BA, similar to the previously discussed polyphenols, may have a role in inhibition of NFκB and down-regulation of the proinflammatory cascade.[106] The relative contribution of each of the above mechanisms to the *in vivo* anti-inflammatory activity of *Boswellia* has not been clearly established at this time.

There are now emerging data to suggest that *Boswellia* may have a role to play in the management of IBD. In a study involving a rat model of colitis, investigators showed that oral administration of *Boswellia* extract or acetyl-11-keto-β-BA (AKBA) over a 2-day period resulted in a dose-dependent decrease in rolling (up to 90%) and adherent (up to 98%) leukocytes. In addition,

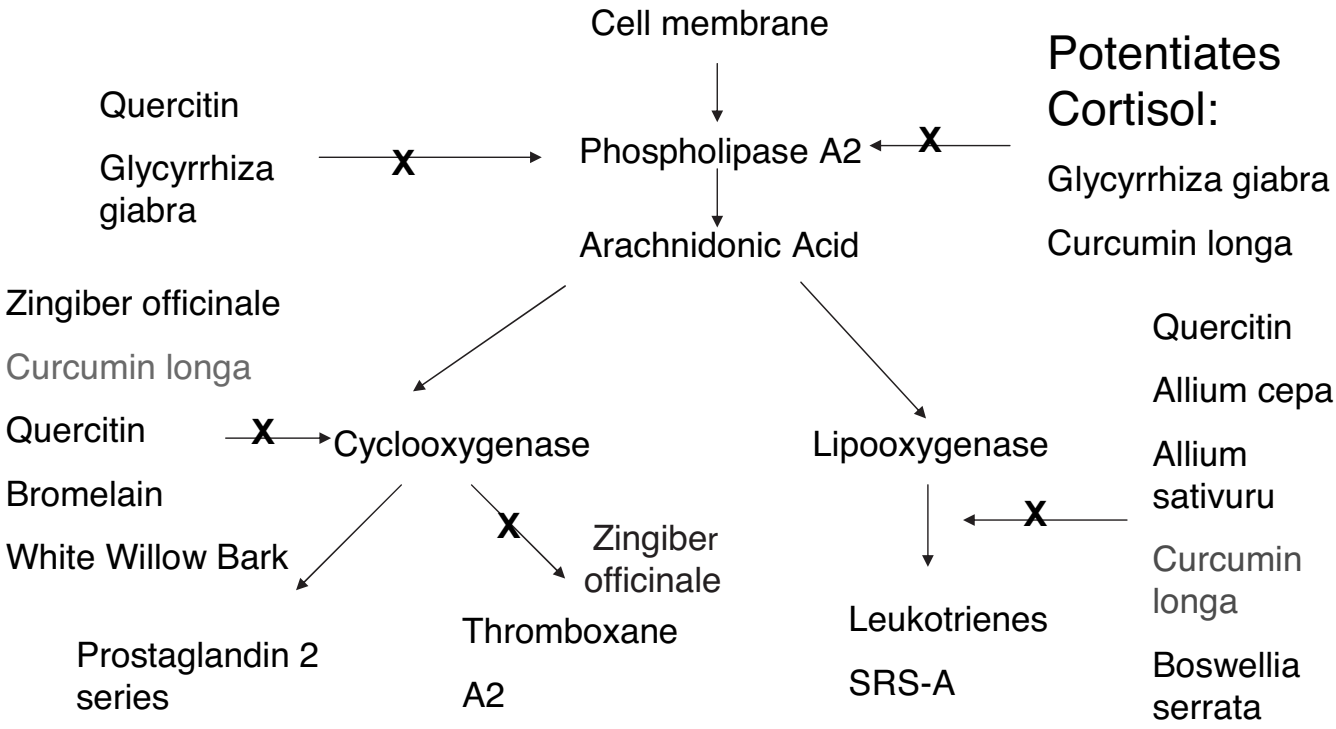

Figure 2. Botanical modulation of arachidonic acid cascade. Polyphenols have a number of different mechanisms for down-regulating inflammation and modulating immunity. A number of botanicals, including polyphenols (curcumin, boswellia, quercetin, and ginger) interfere with the production of noxious proinflammatory eicosanoids such as prostaglandin-2 series (PGE2), leukotrienes (LTB) such as slow reactive releasing substance (SRS-A) and thromboxane A2 (TXA2) *via* inhibition of the enzymes cyclooxygenase-2 (COX-2) and 5′-lipooxygenase (5′LPO).

necropsy showed improvement of inflammatory changes on both a macroscopic and microscopic level.[20] In a murine model of colitis, a semisynthetic form of AKBA was shown to blunt disease activity both grossly and histologically, reduce recruitment of adherent leukocytes and platelets, and prevent up-regulation of P-selectin (normally observed with this particular model of colitis). These anti-inflammatory effects were comparable to effects seen in the same murine model treated with corticosteroids.[22] However, a benefit was not observed in a study by a different investigational group evaluating the effects of BA on a murine model of chemically-induced colitis, although the formulation by which BA was derived varied and this may have partially explained the negative results of this study.[21]

Few studies have been performed evaluating the role of *Boswellia* in human subjects with IBD. Researchers from India compared administration of *Boswellia* (350 mg 3 times daily for 6 weeks) to sulfasalazine (1 g 3 times daily for 6 weeks) and found similar improvement in clinical, laboratory, and histopathological parameters.[107] The same investigators reported a subsequent study comparing 30 patients treated with *Boswellia* or sulfasalazine (at the same doses as above). Of the 20 patients treated with *Boswellia*, 18 (90%) had improvement in at least 1 secondary endpoint, and 14 (70%) went into remission. In contrast, in the 10 patients treated with sulfasalazine, only 6 (60%) had improvement in at least 1 secondary endpoint and only 4 (40%) went into remission.[108] Investigators from Germany compared *B serrata* extract H15 with mesalazine for the treatment of Crohn's disease in a randomized, double-blind controlled trial involving 102 patients. The primary outcome was the change in the Crohn's Disease Activity Index, which decreased by 90 in the H15-treated group and decreased by 53 in the mesalazine-treated group. The authors concluded that *B serrata* extract H15 "appears to be superior over mesalazine in terms of a benefit-risk-evaluation"[109]; however, as the study was powered only to be a noninferiority study, these conclusions must be interpreted with caution, and further research, hopefully in the form of a multicenter, randomized, controlled trial, is necessary to definitively evaluate the role of *Boswellia* in the therapeutic armamentarium of IBD.

Essential Fatty Acids

Essential fatty acids (EFA) refer to dietary constituents that cannot be synthesized endogenously and must be obtained *via* the diet for optimal health. By definition, these EFA can be subdivided into ω-3 and ω-6 polyunsaturated fatty acids according to the position of the initial double bond from the methyl

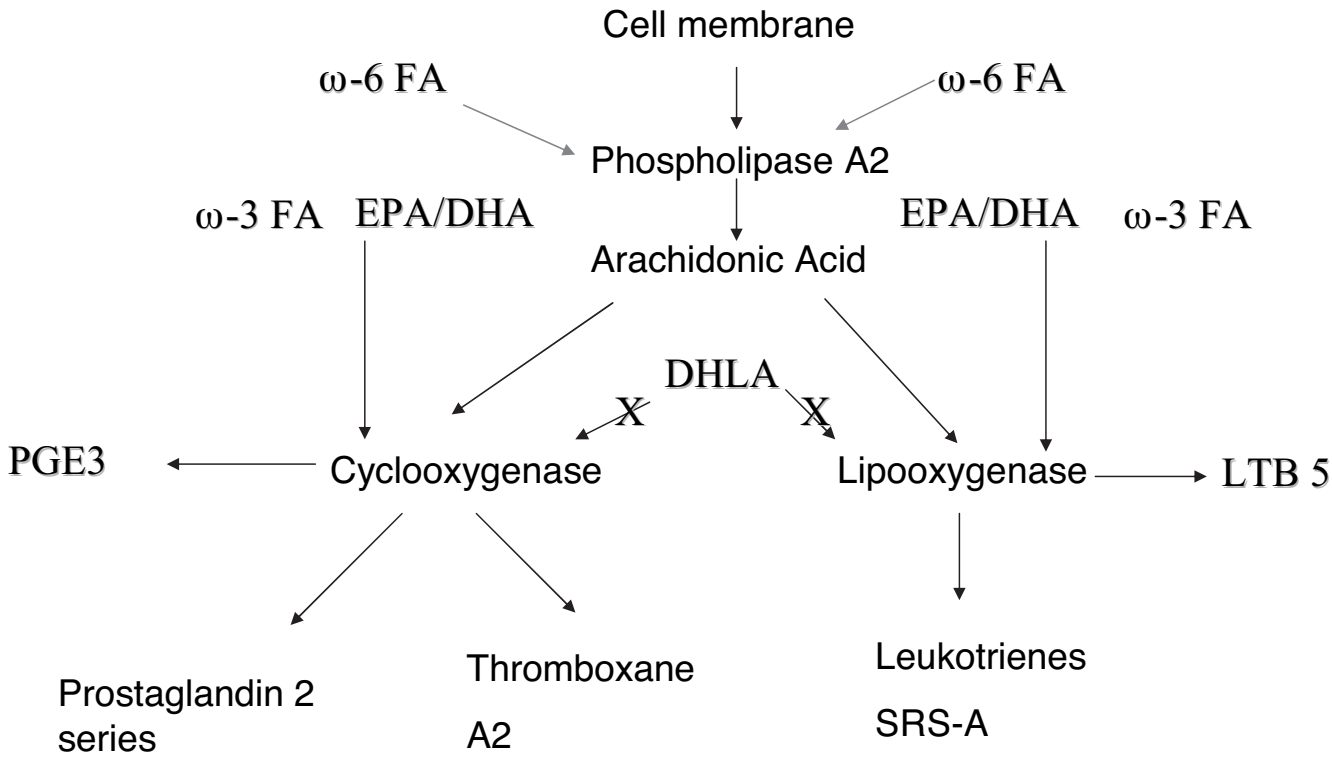

Figure 3. ω-3 Modulation of the arachidonic acid cascade. ω-6 Fatty acids appear to promote the production of phospholipase A2 (PLP A2), arachidonic acid (AA) and production of noxious proinflammatory eicosanoids such as prostaglandin-2 series (PGE2), leukotrienes (LTB) such as slow reactive releasing substance (SRS-A), and thromboxane A2 (TXA2). ω-3 Fatty acids, in contrast, downregulate production of proinflammatory eicosanoids by competitive inhibition of the enzymes cyclooxygenase-2 (COX-2) and 5′-Lipoxygenase (5′LPO) for AA, thus leading to preferential production of the prostaglandin-3 series (PGE3) and leukotriene-5 series (LTB5).

end of the fatty acid. ω-6 EFA are markedly more common in the current Western diet and may have a proinflammatory effect. Although ω-3 EFA are found in a wide variety of foods, including wild plants, eggs, nuts, and berries, they are particularly abundant in fish, and, not surprisingly, ω-3 EFA supplementation has become synonymous with fish oil.[110,111] Typical fish oil is extracted from fish bodies and is composed of a variety of long-chain ω-3 EFA. The 2 most common EFA are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which comprise 18% and 12% of fish oil, respectively, in typical marine fish.[112,113] Fish oil can also be obtained from cod liver; however, cod liver oil has slightly less EPA (10%) and DHA (10%) than other marine oils and can be associated with vitamin A toxicity at high doses. Administration of fish oil has not been associated with any serious acute treatment-related syndromes; however, long-term use raises theoretical concerns for possible increased bleeding, lipid peroxidation, and toxicity of mercury and halogenated biphenyls.[112,114] Fish oil can be administered as either raw fish oil or as an enteric-coated capsule. A dose of up to 3 g per day of EPA plus DHA has been determined to be safe for general consumption.[112]

The health benefits of fish oil in a broad array of disease processes are widely heralded. Hu and colleagues have reported that in a cohort of 84,688 women enrolled in the Nurses' Health Study and followed for a period of 16 years, deaths related to cardiovascular disease were 50% lower in women who consumed fish 5 times per week, and a significant reduction in cardiovascular disease was noted even with fish consumption as infrequently as 1–3 times per month.[115] In fact, a PubMed search of "fish oil" and "cardiovascular disease" results in more than 2000 entries. Other disease processes in which fish oil has been postulated to be of benefit include hyperlipidemia,[116,117] asthma,[118,119] cystic fibrosis,[120,121] rheumatoid arthritis,[113] depression,[122,123] and dementia.[124,125] This is by no means an exhaustive list, as more than 11,000 papers have been published to date on the benefits of fish oil.

ω-3 EFA seem to work through a plethora of mechanisms. To begin with, eicosanoids seem to affect both the COX pathway (primarily COX-2) and the 5-lipoxygenase pathway (Figure 3). Prostaglandin E_2 is a proinflammatory, nociceptive factor that is produced through the COX-2 pathway. Arachidonic acid (AA) is the usual substrate for this pathway. EPA is a chemical homolog that differs from AA by only the presence of the ω-3 double bond. Therefore, EPA represents both an inhibitor of AA and an alternate substrate for COX. In addition,

through similar means, EPA also results in inhibition of the 5-lipoxygenase pathway and decreased production of leukotriene B_4.[112,125] In addition to decreasing production of proinflammatory mediators, it has been recently shown that EPA and DHA can act themselves as substrates for the formation of novel protective mediators, termed E- and D-series resolvins, that may have direct anti-inflammatory effects.[127–129] ω-3 EFA are also thought to play a role in the control of transcription factors such as peroxisome proliferator-activated receptors (PPARs), with resultant down-regulation of inflammatory processes. Through these, and possibly other mechanisms, ω-3 EFA inhibit NFκB and decrease the release of the proinflammatory cytokines IL-1β and TNFα.[113,130]

Fish oil and inflammation are closely intertwined. A PubMed search for the 2 terms results in more than 600 publications and, given the mechanisms detailed above, this is hardly surprising. Although there are abundant data evaluating multiple disease models of inflammation, including rheumatoid arthritis, asthma, and multiple sclerosis, the discussion in this paper will be restricted to IBD. Interestingly, the rate of IBD has traditionally been very low in the Japanese population; however, this appears to be changing, and one theory as to why this change is occurring is the dietary shift from an ω-3 EFA–based diet to an ω-6 EFA–based diet.[131]

Numerous studies have evaluated the effects of fish oil on ulcerative colitis. Several early studies supported the notion that enteral fish oil supplements led to improvement in IBD in animal models,[132,133] and these findings were corroborated in small clinical trials.[134–136] Although a variety of studies have been performed exploring the roles of ω-3 EFA in the treatment of ulcerative colitis, the methodology and endpoints have been varied, and it is difficult to directly compare the results obtained. When clinical scores were used as an outcome (Disease Activity Index, Ulcerative Colitis Activity Index, or undefined "clinical score"),[137] 3 of 5 studies showed significant clinical improvement in the fish oil arm of the study at some point during the course of therapy[138–140] (although only 2[138,140] of these 3 studies showed significant benefit at the predetermined endpoint of the study). Two studies showed no significant change between the 2 groups.[134,141] When endoscopic endpoints were used to evaluate the role of fish oil in the treatment of ulcerative colitis, 3 of 3 studies showed statistically significant improvement in the study group that received fish oil supplementation[134,140,141] (although it should be noted that one of the studies[134] included patients with both ulcerative colitis and Crohn's disease and statistical significance was not met when the 2 subgroups were analyzed individually). When examining the endpoint of histologic improvement, only 1[141] of 3[138,141,142] studies reported significant improvement in the fish oil–treated arm of the study.[137] However, the data that pertain to the effects of ω-3 fatty acids on steroid requirements suggest that ω-3 fatty acids may reduce the need or dose for corticosteroids among patients with IBD. Future studies should assess the effects of pharmaceutical grade enteric-coated ω-3 fatty acids on clinical outcomes in IBD, including requirements for corticosteroids.[142]

Recently, a randomized, controlled trial evaluated a "nutritionally balanced oral supplement enriched with fish oil, fructooligosaccharides, gum arabic, vitamin E, vitamin C, and selenium" on disease activity and medication use in patients with mild to moderate ulcerative colitis. A total of 121 patients were randomized to this dietary supplement or placebo. The subjects were instructed to consume 18 oz of the oral supplement daily for a 6-month period, with a resultant planned fish oil intake of 3.27 g of EPA and 1.38 g of DHA daily. Clinical and histologic parameters, as well as medication usage, were assessed at 3 and 6 months. Eighty-six patients completed the study. Both treatment groups (oral supplement and placebo) showed similar improvement in clinical and histologic indices. However, the group treated with the supplement containing fish oil showed a significantly greater rate of decrease in the dose of prednisone required to control clinical symptoms when compared with the group that received placebo.[144] This type of integrated approach with synergistic nutraceuticals may achieve superior outcomes in future IBD studies.

The relationship of fish oil and Crohn's disease has also been extensively evaluated. The Cochrane Collaboration recently published a systematic review evaluating this topic.[145] A total of 214 publications were evaluated and 15 randomized, controlled trials were identified. After exclusionary criteria (including the use of non–enteric-coated fish oil supplementation), the researchers felt that 4 studies were of sufficient quality to be included in the analysis.[146–149] When all 4 studies were reviewed, the Cochrane Collaboration found that enteric-coated ω-3 EFA supplementation reduced the 1-year relapse rate by half with an absolute risk reduction of 31% and a number needed to treat (NNT) of only 3. The conclusion of the review was that the limited available data suggests that daily oral therapy with enteric-coated ω-3 EFA supplementation is safe and may be effective for maintenance of remission in Crohn's disease. However, emphasis was made that the data are limited and a larger multicenter, randomized, controlled trial is needed to definitively evaluate the issue.[145]

Vitamin D

Vitamin D is recognized as essential for optimal bone mineralization and the maintenance of a healthy skeleton. Normal acquisition is *via* direct exposure to sunlight, which induces the production of cholecalciferol (vitamin D_3). Vitamin D can also be

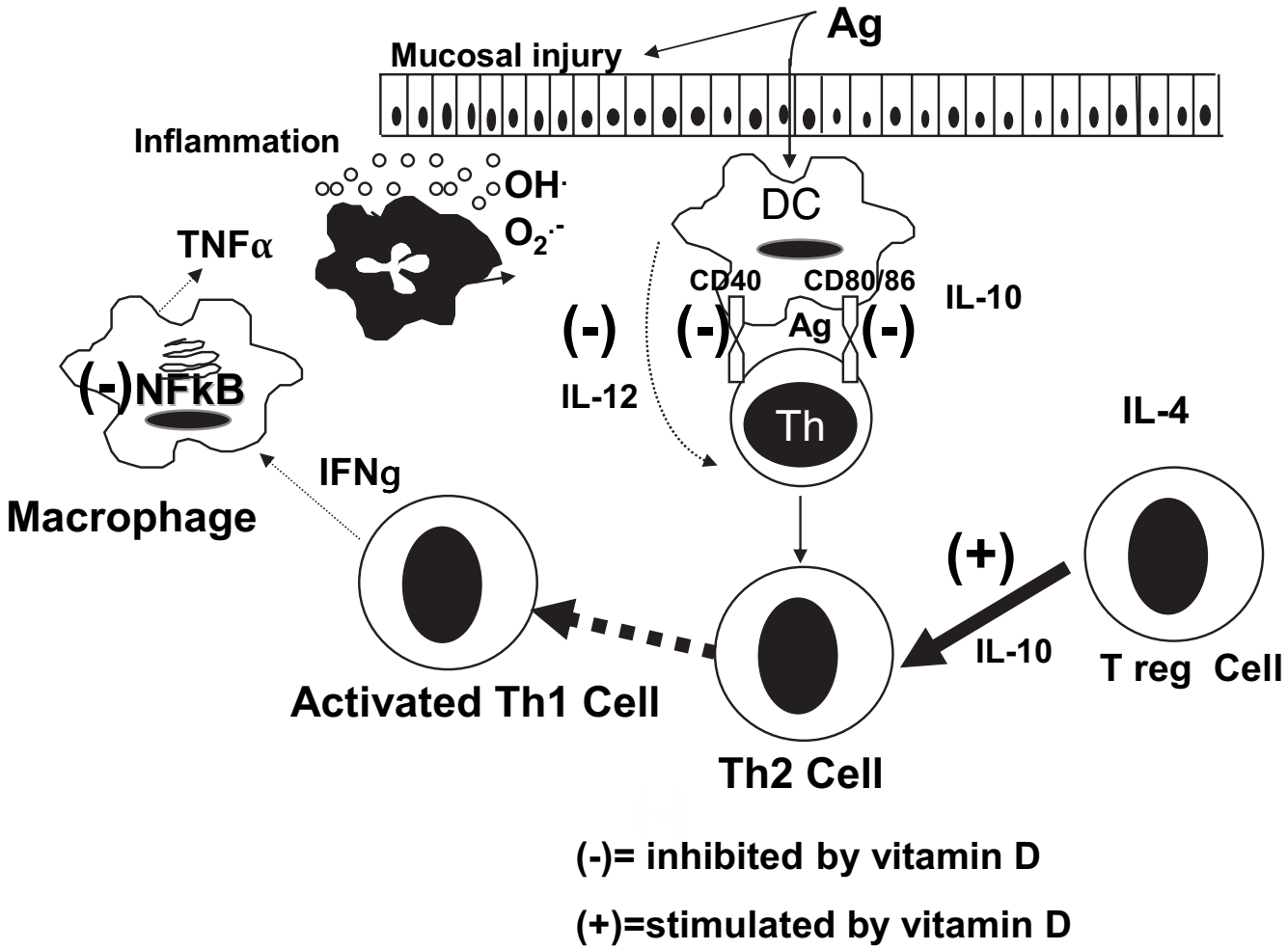

Figure 4. Immunomodulatory role of vitamin D in inflammatory bowel disease. In Crohn's disease, bacterial antigens drive antigen-presenting cells (DCs) to produce cytokines such as interleukin-12 (IL-12) to drive a T-helper 1 (Th1) proinflammatory response to induce macrophages, which produce TNFα and neutrophil chemoattractive agents, which ultimately result in the production of noxious agents and tissue injury. The damaged intestinal tissue is more permeable to antigens that drive the vicious cycle of antigen-presentation, local immune activation, and tissue injury. Anti-inflammatory cytokines such as interleukin-10 (IL-10), made by regulatory T cells (T regs) antagonize Th1 proinflammatory processes by stimulating T-helper 2 function. Vitamin D antagonizes Th1 proinflammatory responses by interfering with antigen-presentation and Th1 activation, up-regulating Th2 cytokines, and down-regulating NFκB in macrophages.

ingested orally as either ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3). Recently, there has been recognition that vitamin D receptors are present in tissues not believed to be involved with calcium and phosphate metabolism. This has led to renewed investigation into the role of vitamin D, and there is now increasing evidence that vitamin D is involved in regulation of the immune system and cancer prevention.[150]

The mechanisms by which vitamin D modulates the immune system are being studied extensively. Vitamin D receptors have been identified on almost all cell types involved in immune modulation. Currently, it is believed that the main mechanism by which vitamin D affects inflammation is through T-cell regulation, specifically through modulation of the Th1 and Th2 pathways (Figure 4). Vitamin D deficiency favors a Th1, or proinflammatory, response, whereas supplementation of vitamin D (at least *in vitro*) appears to shift T-cell activity toward a Th2 response. Numerous studies have been done to elucidate these mechanisms, and these studies have recently been reviewed by one of the authors, along with the role of Th1/Th2 responses in IBD (G.E.M.).[150,151] In addition, like most of the substances reviewed in this paper, vitamin D has also recently been shown to be an inhibitor of the NFκB pathway (with resultant decrease in proinflammatory cytokines).[152]

One interesting observation that may suggest a role for vitamin D in the pathogenesis of IBD is the fact that the prevalence of IBD appears to be highest in North America and Northern Europe, where

direct sunlight exposure is lower.[153] Further, vitamin D deficiency is common in patients with IBD, even when their disease is well controlled.[154,155] To date, there have been at least 3 animal experiments that have evaluated the role of either vitamin D elimination (*via* knockout of vitamin D receptors) or vitamin D supplementation on the development and severity of IBD. All 3 studies have shown a consistent link between the presence of vitamin D and either improved parameters or delayed development of colitis.[156–158]

Probiotics

The gastrointestinal tract is a sterile environment at the time of birth; thereafter, the situation rapidly changes and the human gastrointestinal system is colonized by at least 300–500 different bacterial species, with concentrations of bacteria in the large intestine that can reach 10^{12} cells/g of luminal contents.[159] This dynamic system of intestinal microflora plays a vital role in the maintenance of intestinal health, and, increasingly, data are emerging to show that this community plays an important role in the regulation of the mucosal inflammatory cascade.

Probiotics are defined as "living microorganisms that, upon ingestion in certain numbers, exert health benefits beyond those of basic nutrition."[160] Although the concept of oral consumption of bacteria was initially described over a century ago by the Russian Nobel Prize winner Metchnikoff (who espoused the benefits of yogurt consumption on longevity), it is only in recent years that this theory has gained credence and received serious scientific attention.[161] In the past 5 years, over 2000 papers have been published on probiotics and a wide variety of medical conditions, including, but not limited to, IBD, pouchitis, traveler's diarrhea, hepatic encephalopathy, nosocomial infections, prevention of infection after pancreatitis, allergic diseases, irritable bowel syndrome, prevention of preterm labor, asthma, and other etiologies.

The mechanisms by which probiotics exert their effect are not entirely clear and several theories abound. In patients with IBD, the bacterial microflora become aberrant, and this may contribute to some extent to the underlying pathogenesis of the disease.[162] One route by which probiotics may exert benefit is through competition with the native microbial pathogens for limited epithelial receptors, resulting in inhibited epithelial attachment and, thus, decreased intracellular invasion by a large variety of toxic bacteria.[163] Probiotics can also stabilize the intestinal barrier and epithelial tight junctions.[164] However, it seems that probiotics act in a systemic manner and do not simply perform a barrier function. There is now evidence to suggest that probiotics modulate the mucosal immune response to IBD through a number of different pathways, including inhibition of NFκB, modulation of PepT1 activity, reduction of the number of CD4 intraepithelial lymphocytes, regulation of the anti-inflammatory effect *via* the Toll-like receptor-9 (TLR9) signaling pathway, modulation of immune cell apoptosis and proliferation *via* TLR2 signaling, and modulation of the PPAR-γ pathway (Table 2). In addition, certain probiotics may be active secretors of antimicrobial agents and may serve a role in decomposition of luminal pathogenic antigens.[162]

Table 2 *Immunomodulator effects of probiotics in IBD*

Up-regulate Th2 cytokines (IL-10)
Down-regulate Th1 cytokines (IL-12)
Inhibit IFNγ by T cells
Inhibit NFκB
Stabilize IκB levels
Induce T-regs

Probiotics have been shown to modulate the ability of T-regs to rebalance cytokines in the mucosal immune system. T-regs induce anti-inflammatory cytokines such as IL-10 to antagonize Th1 proinflammatory processes mediated by IFN-γ and IL-12 by stimulating T-helper 2 function. Probiotics also down-regulate the expression of NKκB and stabilizing IκB, which results in dampening proinflammatory responses.
IBD, inflammatory bowel disease; IL, interleukin; INF, interferon; IκB, inhibitors of κB; NFκB, nuclear factor κB; T-regs, T-regulatory cells.

Over 20 trials have been published in the last few years evaluating the role of probiotics in the prevention, treatment, and maintenance of IBD, and several excellent reviews have been recently published on the matter.[161,165–167] The results to date have been mixed, with probiotics thus far benefiting ulcerative colitis more so than Crohn's disease.[168–176] The reader is referred to these excellent reviews for a full analysis of this topic.

Conclusion

Over 30% of the Western population are now using some form of CAM.[177] In the field of IBD, these numbers are estimated to be even higher (50%), given the data present in this discipline and the questionable efficacy of existing medical therapies.[178] Although many of the treatments in this paper are not included in the texts of most medical training institutions, it is important to recognize that there is a mountain of scientific data behind these supplements. A PubMed search for resveratrol, green tea, curcumin, boswellia, fish oil, vitamin D, or probiotics results in over 50,000 publications, and the quality of the data, in many cases, is excellent.

The immune system is a complicated process, and there are many ways to disrupt and gently modulate this equilibrium. Polyphenols, fish oil, probiotics, and vitamin D all promise a novel approach to attenuation and possible reversal of the inflammation cascade and do so from the safety of (in some cases) centuries of usage. Rigorous randomized, controlled, multicenter studies are needed to clarify the

role of these agents in the armamentarium of Western therapy; however, the data are promising, and these therapies should be considered as adjunct therapies for select patients with IBD and other inflammatory conditions.

With increasing data regarding these agents, it is tempting to speculate on a world in which patients would supplement their diet with natural agents designed to treat their individual ailments and modify their diets early in life according to familial and environmental risk factors. Inflammation would be a carefully regulated phenomenon acting at the discretion of the patient, rather than being an unwieldy albatross. A new term would have to be devised for CAM as it would be neither complementary nor alternative any longer. The term "integrative medicine," devised by Dr Andrew Weil for incorporating aspects of sound diet, lifestyle factors, and nutraceuticals, together with conventional care for superior outcomes, may be the future direction for clinical trials and patient care. Given the exciting research detailed above, perhaps this day is coming sooner than we think.

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Invited Review

Polycystic Ovary Syndrome (PCOS) and Other Androgen Excess–Related Conditions: Can Changes in Dietary Intake Make a Difference?

George U. Liepa, PhD, FACN; Aditi Sengupta, MBBS, MS; and Danielle Karsies, BS, RD
School of Health Sciences and Department of Chemistry, Eastern Michigan University, Ypsilanti, Michigan

ABSTRACT: Polycystic ovary syndrome (PCOS) is a condition that involves the excess production of androgens. It affects up to 10% of all American women and can lead to the development of acne, hirsutism, and infertility. It has also been associated with coronary heart disease, diabetes, and metabolic syndrome. Over half of the women who are diagnosed with PCOS are overweight or obese. Recommendations are made for overweight/obese women to lose weight *via* diet and exercise. Women with PCOS should also consider maintaining a diet that is patterned after the type 2 diabetes diet. This diet includes an increase in fiber and a decrease in refined carbohydrates, as well as a decrease in trans and saturated fats and an increase in ω-3 and ω-9 fatty acids. Foods that contain anti-inflammatory compounds (fiber, ω-3 fatty acids, vitamin E, and red wine) should also be emphasized. Evidence is provided for the impact of these dietary changes on improvements in the androgen profile of PCOS patients.

Polycystic ovary syndrome (PCOS), also know as Stein-Leventhal syndrome, is the most common endocrine disorder found in women of reproductive age.[1] Excessive body weight is a major risk factor for PCOS, with 40%–50% of patients with PCOS being obese [body mass index (BMI) >30 kg/m^2].[2] PCOS is characterized by the presence of hirsutism, acne, irregular or absent menses, ovarian cysts, and a concomitant increase in infertility. If left unchecked, PCOS can lead to a variety of inflammatory metabolic disorders, including coronary heart disease (CHD) and metabolic syndrome (MBS). Its cause is not known; however, it has often been shown to be associated with excessive release of luteinizing hormone (LH) by the pituitary gland or hyperinsulinemia and insulin resistance.[3] It has been suggested that hyperinsulinemia (a) interrupts normal signals that tell eggs to grow, (b) induces increased androgen production, and (c) creates a hormonal milieu that can lead to hirsutism. Diagnosis of PCOS can be difficult because it is a heterogeneous disorder that typically manifests with androgen excess and ovulatory dysfunction. Although it is treatable, there is currently no cure for this syndrome. The present review focuses on the prevalence, diagnosis, pathogenesis, and current dietary treatment modalities of PCOS, as well as other conditions that are related to an excessive production of androgens.

Correspondence: George U. Liepa, PhD, FACN, School of Health Sciences and Department of Chemistry, Eastern Michigan University, Ypsilanti, MI. Electronic mail may be sent to george.liepa@emich.edu.

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Prevalence

The overall prevalence of PCOS in premenopausal American, Australian, and European women is estimated to be 5%–12%, with variability due to reported differences in diagnostic criteria and in various ethnicities and races.[4] There does not seem to be a statistically significant difference between white and black women with regard to incidence of PCOS when controlling for BMI and age.[4] Numerous European studies have shown similar PCOS incidence patterns in Europe, with 22% of the women having polycystic ovaries (PCO) and, within this population, 30%–76% having PCOS.[5] Although research has not shown a definitive PCOS-related genetic predisposition,[6] it has shown a familial pattern of occurrence, with 22%–40% of premenopausal, first-degree, women relatives of PCOS individuals also exhibiting PCOS.[4]

Diagnosis

Although a precise and uniform diagnosis for PCOS is not yet established secondary to the heterogeneity of presenting signs and symptoms, androgen excess seems to be a relatively uniform characteristic of this syndrome. There are 2 accepted diagnostic criteria for PCOS, one being the Rotterdam 2003 criteria that give a positive diagnosis when 2 of the following criteria are met: oligo- or

Table 1 *Diagnostic hormonal changes noted in the serum of patients with polycystic ovarian syndrome (PCOS)*[15,16]

Hormone	Normal laboratory value	Status in PCOS
Luteinizing hormone (LH)	2–14 IU/L	Increased
Androstenedione	4.9–9.0 nmol/L	Increased
Testosterone	6–86 ng/dL	Increased
Dehydroepiandrosterone sulfate (DHEAS)	12–535 μg/dL	Increased
Follicle-stimulating hormone (FSH)	3–26 mIU/mL	Decreased
Sex hormone-binding globulin (SHBG)	27–109 nmol/L	Decreased

anovulation, elevated levels of circulating androgens, or clinical manifestations of hyperandrogenism such as hirsutism, male pattern hair loss, acne, and polycystic ovary (PCO) as defined by ultrasonography.[7,8] The other set of diagnostic criteria is the National Institutes of Health 1990 criteria, which defines PCOS as having evidence of biochemical or clinical hyperandrogenism and ovulatory dysfunction.[9] This process also involves ruling out other related disorders such as hyperprolactinemia, nonclassic congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting neoplasm, and acromegaly. Primary diagnostic hormonal changes are shown in Table 1.

Additional laboratory and anthropometric measurements are also commonly used to assist in the diagnosis of PCO/androgen excess (Table 2). The mean serum concentrations of LH and various androgens [androstenedione (A), testosterone (T) and dehydroepiandrosterone sulfate (DHEAS)] are elevated in PCOS.[10–14] In addition, mean serum concentrations of follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG) are typically low in individuals with PCOS.[15,16]

BMI and waist:hip ratio (WHR) are commonly obtained because of the high prevalence of obesity in PCOS and the predisposition for central adiposity. The latter has been shown to be related to elevated androgen levels, hyperinsulinemia, or leptin.[10,16,17] Normal laboratory values commonly used as PCOS indicators are shown in Table 2.

Comorbidities of PCOS/Androgen Excess

Untreated PCOS has been associated with a number of health disorders, including hypertension, coronary heart disease (CHD), and diabetes. All of these conditions are also associated with the obesity and with an increase in inflammation, followed by a concomitant rise in the primary marker that is used to diagnose inflammation, C-reactive protein (CRP).[18] In a recent study by Kelly et al,[19] serum CRP concentrations of PCOS patients were significantly higher than levels in controls.

Table 2 *Normal values of commonly used polycystic ovarian syndrome indicators*

Indicator	Normal values
Body mass index	18.5–24.9
Waist:hip ratio	>0.80
Free androgen index	1.0%–4.5%
Fasting serum insulin	60 pmol/L
Normal leptin levels	18–250 ng/mL

Hypertension and vascular dysfunction appear readily in PCOS patients and cannot be explained by obesity alone. This phenomenon is also seen with obstructive sleep apnea (OSA). Despite its association with obesity and the predisposition for PCOS patients to be obese, OSA rates are higher in the PCOS population when compared with age- and weight-matched normal controls.[20] This pattern has also been shown to be present in PCOS patients with a BMI $\leq$32.3 kg/m^2. The higher propensity for OSA in women with PCOS is likely due to the elevated androgen levels in this patient population.

Women with PCOS have been shown to have a number of negative changes in their cardiovascular biomarkers. Typically, they have lower serum high-density lipoprotein cholesterol (HDL-C) or HDL2-C concentrations, as well as higher triglyceride (TG) and low-density lipoprotein (LDL) concentrations than age- and weight-matched control women.[21,22] This characteristic lipid profile in women with PCOS is largely related to the degree of insulin resistance or hyperinsulinemia, independent of androgen levels and BMI.[23] Plasminogen activator inhibitor (PAI) levels are also elevated[24,25] and, together with alterations of serum lipids, may be partly responsible for the increased incidence of hypertension, CHD, and thrombosis in women with PCOS.[21,26]

The PCOS population also has an increased incidence of impaired glucose tolerance and early-onset type 2 diabetes (40% and 10%, respectively). In addition, studies have shown that these subjects also have an 8-fold greater risk of developing type 2 diabetes, which increases to a 10-fold risk when using the 2003 diagnosis criteria.[1] In a recent study, it was shown that premenopausal women with type 2 diabetes have an increased incidence of PCO but not of PCOS when compared with age- and BMI-matched healthy premenopausal women.[27] These conflicting findings may be related to the fact that the latter study only included type 2 diabetic patients and had a smaller sample size. Diamanti-Kandarakis et al[28] studied the relationship between

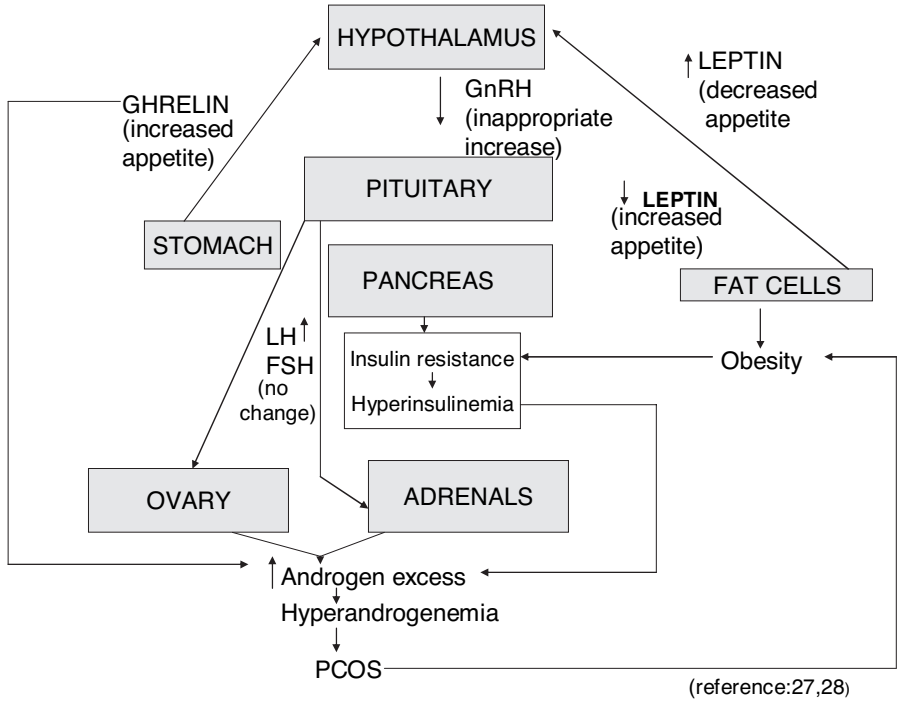

Figure 1. A schematic diagram demonstrating the relationship between PCOS and obesity. The role of organ specific hormone effects on the development of PCOS.
FSH, follicle-stimulating hormone; GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome.

insulin resistance and PCOS in Greek women and discovered that as severity of fasting hyperinsulinemia increased, the severity of the clinical phenotype of hyperandrogenism increased; this finding was independent of obesity. PCOS was found to occur in approximately 7% of the women studied.

Subjects with PCOS have been shown to have an increased incidence of MBS. MBS is a clinical diagnosis in which the following 4 risk factors exist in a patient: insulin resistance, hypertension, abnormal serum cholesterol levels, and obesity. Apridonidze et al[1] reported that women with PCOS who were between 20 and 39 years old displayed a 4-fold increase in MBS; a subset of the younger women showed an 8-fold increase when compared with matched controls. The most prevalent MBS factors noted were high BMI and low serum HDL-C. In PCOS patients with MBS, serum androgen and free testosterone concentrations have been shown to be higher and serum SHBG concentrations significantly lower than those found in non-MBS PCOS patients, even when correcting for BMI and age.[1]

Some studies suggest that an association exists between PCOS and certain types of cancer.[2] Present data do not support an increased risk of breast cancer with this condition.[29] In an elegant review on PCOS and cancer, Balen[30] concluded that a relationship exists between endometrial cancer incidence and PCOS. He suggests that women with PCOS who have amenorrhea and oligomenorrhea should have artificial withdrawal bleeds induced, if necessary, to prevent endometrial hyperplasia and decrease the risk of endometrial cancer. Balen also suggests that the possibility of an association between ovarian cancer and PCOS is unlikely because research in this area is conflicting at best.

Interrelationships Between Serum Androgen Concentrations and Obesity

The primary underlying factor that has been associated with PCOS is hyperandrogenemia. The cause for this well-documented hormonal shift is still poorly understood. Although a significant number of women with excessively high serum androgen concentrations have increased adrenal gland androgen production, numerous studies have shown that insulin resistance and the concomitant production of excessive amounts of insulin are also tied directly to increased ovarian production of androgens.[31] These abnormally high serum insulin concentrations seem to work synergistically with LH to enhance androgen production.[32] To exacerbate this problem, hyperinsulinemia also has been shown to inhibit hepatic synthesis of SHBG, the protein that binds with T. This change causes an increase in free T concentrations which, in turn, leads to the development of secondary characteristics associated with PCOS.[31]

More than 60% of women who have PCOS are also classified as being either overweight or obese, 2 conditions that lead to an increased incidence of

hyperinsulinemia. In a study of premenopausal Spanish women, investigators found that 28.3% of overweight and obese women had PCOS, compared with only 5.5% of their lean counterparts.[33] Similar observations have led to studies which focused on the interrelationship between hormones such as ghrelin and leptin, compounds that have been shown to be related to weight gain, increased appetite, and androgen excess. Ghrelin is manufactured in stomach cells and released in increased quantities when a person is fasting. It signals the hypothalamus to stimulate appetite. Leptin is produced by adipocytes in greater quantities after a person eats and is associated with a decrease in appetite.

Various studies have found that women with PCOS have lower ghrelin concentrations when compared with normal controls. This does not seem to be due solely to increased serum androgen concentrations, because women with hyperandrogenemia have been shown to have insignificantly lower concentrations of ghrelin when compared with controls.[34,35] In a study by Glintborg et al,[36] a significant decrease was shown to occur in PCOS patients, and this was indirectly related to increases in serum testosterone, insulin, and leptin levels.

In addition to ghrelin, leptin may also play a role in the decreased satiety and the androidal fat composition found in women with PCOS. Although obesity is a common characteristic of PCOS and high leptin levels are associated with obesity, serum leptin concentrations in PCOS individuals are higher than expected for their BMI.[37] Leptin plays an important role in the regulation of body-fat mass in animals and humans,[38] and visceral fat has been shown to secrete less leptin than subcutaneous fat, potentially leading to decreased satiety.[39] In addition, high serum leptin concentrations have been associated with insulin resistance[40,41] and could contribute to hypersecretion of insulin by the pancreatic β cells in obese, insulin-resistant subjects.[42] The interrelationships between obesity and various hormonal changes in PCOS patients are shown in Figure 1.

Because centrally located (waist) obesity is associated with both hyperinsulinemia and high DHEAS concentrations, the current effort related to PCOS treatment has focused on decreasing both body weight and, concomitantly, insulin resistance. Although estrogens are known to enhance insulin sensitivity, elevated androgens, specifically dihydrotestosterone (DHT) and T, exacerbate insulin resistance,[43–47] possibly accounting for some of the insulin resistance seen in obese people and people with PCOS. In a study by Mather et al[48] in which women with PCOS were compared with healthy, age- and BMI-matched controls, the PCOS group had greater insulin resistance then controls, suggesting that both the PCOS and BMI contribute to the observed insulin resistance. Studies have shown that a very modest reduction in calorie intake seems to be effective at reducing insulin levels and increasing SHBG.[49] In a study by Kumar et al,[50] black women and white women who were either eumenorrheic and nonhirsute or were diagnosed with PCOS were studied to establish if a relationship existed between BMI values and androgens. DHEAS concentrations were shown to be elevated in 20% of all white women with PCOS and in 30% of all black women with PCOS. The white women with PCOS in the study also had DHEAS concentrations that were negatively associated with BMI and fasting insulin concentrations, suggesting that weight control could play a role in decreasing serum DHEAS concentrations.

Treatment of PCOS

The optimal treatment for PCOS is yet to be determined, but literature supports a multifactorial approach, including any combination of one or more of the following interventions: diet and lifestyle management, use of pharmaceuticals (oral contraceptives and cyclic progestins, antiestrogens, gonadotropins, and insulin sensitizers), and surgery. In a recent survey of 138 endocrinologists and 172 gynecologists that was conducted by Cussons et al,[51] the majority of respondents stated that the first line of treatment for all presentations of PCOS should be diet and exercise.

Role of Diet and Weight Loss

Central visceral obesity is present in more than 40% of patients with PCOS.[2,52] In general, both obese and nonobese PCOS patients tend to be insulin resistant.[53] Thus, the recommended diet composition for PCOS patients is drawn from current recommendations for dietary management of type 2 diabetes. A high-fiber, low-glycemic-index diet with adequate protein and an emphasis on fatty acids that are monounsaturated instead of saturated is recommended.[54] In addition, a hypocaloric diet is also warranted for obese PCOS patients in order to induce weight loss. A hypocaloric diet is not indicated for all PCOS patients[55]; a eucaloric diet that is focused on healthy food choices is recommended for lean PCOS patients. Recent success with this type of diet was shown in a study by Berrino et al,[56] in which 104 postmenopausal women were selected from 312 healthy volunteers according to high serum T concentrations, then randomly assigned to either an intervention group or control group. The intervention group began receiving an *ad libitum* diet that was low in animal fats and refined carbohydrates and high in low-glycemic-index foods, monounsaturated and ω-3 fatty acids, and phytoestrogens. Over a 4-month period, the intervention group showed a significant increase in SHBG, whereas T decreased. The intervention group also showed a significant decrease in WHRs, as well as serum total cholesterol and fasting glucose concen-

Table 3 *Role of major minerals and trace elements in PCOS*

Element	Function based on literature review	Further research	Recommended supplementation
Magnesium and high calcium:magnesium ratio	Literature shows that PCOS patients have lower serum magnesium levels than normal controls.[68] Low levels are associated with insulin resistance, cardiovascular problems, diabetes mellitus, hypertension.		Magnesium, 300 mg twice daily for insulin resistance in PCOS
Calcium	Calcium is required for the interrelationship of calcium metabolism, egg maturation, and normal follicular development.[83,84]		1000-1300 mg/d (RDA for age)
Boron	Low levels induce hyperinsulinemia in vitamin D–deprived rats.[69] Adequate boron enhances efficacy of vitamin D.[70] Boron may enhance vitamin D effect on PCOS patients.		Not available
Chromium	Daily supplementation of chromium has been shown to improve glucose tolerance in PCOS patients.[71]	A small study (5 women) found a 38% improvement in glucose disposal rates with 1000 μg/d trivalent chromium picolinate.[72]	200 μg/d
Zinc	High levels of zinc have been shown to increase testosterone levels in zinc deficient individuals.[73,74]		Zinc supplementation should be discouraged in PCOS patients.

PCOS, polycystic ovarian syndrome.

trations. This study showed that a radical modification in diet that was designed to reduce insulin resistance also decreased serum sex hormones in postmenopausal hyperandrogenic women.

Role of Anti-inflammatory Diet

PCOS has been shown to be an inflammatory condition that involves the development of increased serum CRP concentrations. The proven relationship between inflammation, increased serum CRP concentrations, and many of the chronic diseases that PCOS patients often develop makes it critical that this population consume a diet that emphasizes anti-inflammatory food products. In a review by Liepa and Basu,[18] key dietary ingredients are mentioned as compounds that lower serum CRP concentrations. These items include fiber, ω-3 fatty acids, vitamin E, and moderate levels of alcohol. Red wine is suggested as a preferable alcohol source because it is high in antioxidants, raises HDL-C levels, and decreases platelet aggregation.[18,57]

Proteins

High-protein diets have been used for many years in successful weight loss programs and have, therefore, been of interest in the treatment of PCOS patients. In a study by Stamets et al,[58] a high-protein, low-carbohydrate diet was compared with a normal control diet and no difference was noted in serum androgen concentrations. These results were attributed to significant positive changes noted in weight loss with both diet modalities. In a separate study that investigated the impact of individual dietary components on weight loss, it was shown that protein ingestion increased serum insulin concentrations less than glucose, and that the dietary protein suppressed ghrelin concentrations significantly longer than glucose.[59] These findings indicate that, whereas a high-protein, ketogenic diet is not necessary, protein does play an important role in the control of hyperinsulinemia and satiety, and should be included in a balanced diet that includes complex carbohydrates, proteins, and essential fatty acids.

It has also been suggested that processed dietary proteins in convenience foods may impact on the increased incidence of PCOS. A study investigating the effects of food processing on protein structures in grains found that modern food processing methods altered the protein structure of these grains, resulting in faster digestion, followed by excess release of insulin.[60]

Table 4 *Role of various vitamins in the treatment of polycystic ovarian syndrome (PCOS)*

Vitamin or mineral	Function based on literature review	Recommended supplementation	Precautions
Vitamin A	Vitamin A may play a role in the treatment of PCOS-related acne. Literature supports that individuals with severe acne have low serum vitamin A levels.[75]	Supplementation up to the daily recommended intake for each of these vitamins and minerals is recommended to prevent deficiency related effects and to promote adequate intake for optimal function of each nutrient.	
Vitamin D	Vitamin D may play a role in calcium and insulin metabolism.[76] There is an inverse relationship between glucose tolerance and serum vitamin D levels in elderly men.[77] Low vitamin D levels are seen in both hyperinsulinemic patients and subjects with type 2 diabetes.[78,79] Insulin resistance, as well as increased BMI and serum leptin levels, were seen in women with hypovitaminosis D.[80] Vitamin D deficiency impairs biosynthesis and release of insulin, and supplementation improved insulin response.[81] Addition of vitamin D to theca cells removed from the ovaries resulted in decreased androstenedione production basally and in the presence of luteinizing hormone.[82]		Increased consumption of milk products (milk, sherbet, cottage cheese, cream cheese) to increase vitamin D and calcium intake is not recommended in the PCOS population because increased milk consumption causes increased blood levels of ILGF-1 and may play a role in promotion of acne by hyperkeratinization.[85]
Vitamin E	Vitamin E lowered C-reactive protein levels and therefore improved the inflammation status of PCOS patients. In combination with fish oils, vitamin E has been shown to decrease insulin concentrations.[66]		

BMI, body mass index; ILGF-1, insulin-like growth factor.

Carbohydrates

Carbohydrates have been shown to have an impact on PCOS primarily *via* their effect on insulin concentrations in the blood. Studies indicate that a high-fiber, low-glycemic-index diet will lead to an overall weight loss and a decrease in insulin resistance.[61] A 6-month, low-carbohydrate, ketogenic diet was used by Mavropoulos et al[62] to study the impact of a decreased intake of carbohydrates on obese and overweight PCOS patients. Improvement was noted in weight (12% decrease), free T (22% decrease), LH:FSH ratio (36% decrease), and fasting insulin (54% decrease). The impact of low-glycemic-index foods on androgens in men was recently shown in a study by Smith et al.[63] It was noted that diets emphasizing high-fiber, complex carbohydrates improved insulin sensitivity and thereby altered androgen levels and decreased the incidence of acne in young men. Serum androgens (T, DHT, A, and DHEAS) were also shown to be lowered in white men who had been consuming a high-fat diet, then began consuming an isocaloric, high-fiber, low-fat diet for 8 weeks.[64]

Table 5 *Role of supplements and nutraceuticals in the treatment of polycystic ovarian syndrome (PCOS)*

Supplement/nutraceutical	Function based on literature review	Recommendations
Garlic	For its hypoglycemic, anticoagulative, antihypertensive, antihyperlipidemic effects[86–91]	Increased consumption of fresh garlic and cinnamon in cooking is recommended.
Cinnamon	For its effect on insulin resistance and plasma glucose levels[92,93]	

Fatty Acids

Dietary fat has also been investigated in relation to PCOS. Fish oil, when used as a supplement, has been shown to have insulin-sensitizing effects.[65] Fish oils in conjunction with vitamin E have been shown to decrease levels of insulin, as well as growth hormone and DHEAS in healthy men.[66] In a study by Kiddy et al,[49] it was shown that when women lost 5% or more of their body weight *via* a low-fat, low-calorie diet, they had a reduction in hirsutism.

Minerals and Vitamins

Various trace elements have been shown to have an impact on PCOS primarily *via* 2 mechanisms: insulin resistance and T binding (Table 3). Most research relating vitamins to PCOS treatment has focused on the fat-soluble vitamins. Although 3 of the 4 fat-soluble vitamins have been shown to have an impact on PCOS, vitamin D clearly seems to play a major role (Table 4). Specifically, a provocative recent study investigated the role of calcium and vitamin D dysregulation on follicular arrest seen in women with PCOS. In this study, it was shown that vitamin D (ergocalciferol 50,000 units weekly or biweekly to attain a targeted serum 25-vitamin D concentration of 30–40 ng/mL) and calcium supplements (calcium carbonate, 1500 mg of elemental calcium per day) led to normalized menstrual cycles in 7/13 subjects within 2 months and pregnancies in 2/13 subjects.[67] These data suggest that calcium and vitamin D supplementation, up to the daily recommended intake (DRI), should be considered in the dietary management of PCOS. Table 4 provides additional information focusing on vitamin D and the 3 other fat-soluble vitamins in the treatment of PCOS.

Supplements/Nutraceuticals

This food category is an area of growing interest for its role in the management of various diseases. There is strong evidence to support the addition of fresh garlic and cinnamon to the diet of PCOS patients (Table 5).

Concluding Comments

Whether in conjunction with pharmacotherapy or as a standalone treatment, diet and lifestyle modification should be included in the treatment of women with PCOS. Women with PCOS who are overweight should lose weight *via* diet and exercise. Their diet should contain high-fiber complex carbohydrates, moderate levels of protein, and adequate fat to meet essential fatty acid needs. It should also focus on ω-3 fatty acids and monounsaturated fatty acids (MUFA) and contain limited amounts of trans and saturated fats, as recommended by current literature. In addition, a multivitamin and mineral supplement providing no more than 100% of the DRI for all nutrients, with additional calcium, vitamin D, and magnesium, may also be beneficial. Dietary food items that contain compounds with anti-inflammatory properties (fiber, ω-3 fatty acids, vitamin E, red wine, etc) should also be emphasized in order to maintain normal serum CRP concentrations. These dietary adjustments should significantly improve many of the symptoms that accompany PCOS. Exercise has also been shown to have a positive effect on PCOS by reducing serum testosterone concentration and by promoting weight loss.[94,95] Because symptoms of PCOS usually occur with menarche, it is also important that adolescents who are at risk be screened, and dietary changes for the prevention of PCOS and associated comorbidities be implemented. This is especially important with rising childhood obesity rates because research has shown a strong correlation between obesity and PCOS, although PCOS is found in nonobese adolescents and adults alike.

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Clinical Observations

Screening for Celiac Disease in Short Bowel Syndrome

Rajesh N. Keswani, MD; Kimberly Neven, MS, RN; and Carol E. Semrad, MD
Section of Gastroenterology, Department of Medicine, University of Chicago, Chicago, Illinois

ABSTRACT: *Background:* Malabsorptive diarrhea due to short bowel syndrome (SBS) results in nutrition compromise, often requiring parenteral nutrition (PN). Activation of latent celiac disease can occur after gastrointestinal surgery. Our objective was to determine whether undiagnosed celiac disease contributes to malabsorption in patients with SBS. *Methods:* Adult subjects with SBS were tested for celiac disease using immunoglobulin A (IgA) tissue transglutaminase (TTG) antibody and total IgA level. Subjects with an elevated IgA tissue transglutaminase were offered upper endoscopy with biopsies of the duodenum. *Results:* Eighteen subjects were enrolled. The subjects were predominantly white, and the most common cause of SBS was Crohn's disease. The mean length of remaining small bowel was 93.1 ± 54.6 cm. All subjects had undergone surgeries, resulting in loss of the ileocecal valve. Five subjects were found to have an elevated total IgA. A single patient was found to have an elevated IgA tissue transglutaminase antibody, and subsequent endoscopy demonstrated active gastroduodenal Crohn's disease, without features of celiac disease. *Conclusions:* No subjects were IgA deficient, but 5 subjects were found to have elevated IgA levels. Undiagnosed celiac disease did not contribute to malabsorption in our small cohort of predominantly white SBS patients. Larger studies are warranted.

Short bowel syndrome (SBS) in adults is due to surgical resection or bypass of small intestine, resulting in the inability to maintain adequate nutrition.[1] Patients with this syndrome usually have <200 cm of remaining small intestine. In clinical practice, patients are differentiated between those with and those without a colon in continuity.[2] In general, patients with an intact colon are better able to tolerate a foreshortened small intestine.[3]

Correspondence: Rajesh N. Keswani, MD, 600 S. Euclid Ave., Campus Box 8124, St. Louis, MO 63110. Electronic mail may be sent to rkeswani@wustl.edu.

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Malabsorptive diarrhea due to SBS results in nutrition compromise. When this occurs, patients may require parenteral nutrition (PN), a therapy associated with complications including sepsis, deep venous thrombosis, and liver failure.[4] Due to its associated morbidity and decreased quality of life, other causes of diarrhea must be investigated that may allow patients with SBS to discontinue or reduce PN requirements.

Celiac disease is a genetically determined inflammatory disease of the small bowel that is triggered by the ingestion of wheat, rye, oats, or barley.[5] Initially thought to be an uncommon disease, the prevalence now has been variably estimated between 0.4% and 1% in white populations.[5,6] The advent of sensitive and specific serologic testing has allowed for noninvasive screening for celiac disease. The ELISA antibody test for tissue transglutaminase (TTG), the autoantigen eliciting endomysial antibody, has been shown to be a sensitive and specific screening test for celiac disease.[7–9] The classic symptoms associated with celiac disease include malabsorptive diarrhea and abdominal gas with bloating.[5] However, because these symptoms overlap with those of SBS and other gastrointestinal disorders, a diagnosis of celiac disease may be missed in clinical practice.[6,10]

Some individuals may have silent or asymptomatic celiac disease. Triggers such as pregnancy or gastrointestinal surgery may activate silent celiac disease.[11–13] In particular, activation of silent celiac disease with failure to thrive after pancreaticoduodenectomy has been reported.[14–16] It is unknown whether small bowel resection activates silent celiac disease. In patients with severe SBS, underlying celiac disease might contribute to malabsorption and the need for PN. To test this hypothesis, we screened a population of patients at a single institution with clinically significant SBS for celiac disease by serologic testing.

Materials and Methods

The electronic medical records of all adult patients followed in the gastroenterology nutrition clinic for the SBS at the University of Chicago were reviewed by 2 investigators (R.N.K., K.N.). Patients with clinically significant SBS defined as previous resection of the small intestine and the need for PN

Table 1
Patient characteristics

Total number of subjects	17
Mean age ± SD, y (range)	58.3 ± 13.46 (28–79)
Sex	
Female	11 (64.7%)
Male	6 (35.3%)
Ethnicity	
White	13 (76.5%)
Black	2 (11.8%)
Other	2 (11.8%)
Etiology of short bowel syndrome	
Crohn's disease	9 (52.9%)
Ischemic bowel	6 (35.3%)
Small bowel obstruction	2 (11.8%)
Malignancy	1 (5.9%)
Parenteral nutrition requirement	14 (82.4%)
Oral diet	17 (100%)
Mean small intestine length ± SD, y (range)*	93.1 ± 54.6 (30–203)
Gastrointestinal anatomy	
End jejunostomy with ileal resections	3 (17.6%)
End ileostomy with jejunal resections	6 (35.3%)
Small bowel resection(s) with partial colectomy	7 (47.1%)
Small bowel resection with intact colon	1 (5.9%)
Intact ileocecal valve	0 (0%)
Mean number of abdominal surgeries (range)	4.1 (1–14)
Mean time elapsed since last surgery (range)	81 (1–300)

*n = 11 patients.

or oral/parenteral vitamin supplementation were included in the study. Potential subjects were enrolled at the time of routine clinic visit. Patients with a previous diagnosis of celiac disease were excluded from this study.

Enrolled subjects completed a short questionnaire regarding their medical history that focused on previous surgeries and ongoing treatment of SBS. The electronic medical record was also reviewed for additional medical history details. Small intestine length was determined *via* operative report and, when this was not reported, by small bowel imaging. Remaining small bowel length was not determinable in all patients. Phlebotomy was performed at the time of clinic visit. A venous blood sample was submitted to the laboratory for analysis of serum total immunoglobulin A (IgA) level (normal, 100–490 mg/dL) and IgA TTG antibody (normal, <20 U/mL). All testing was performed at the University of Chicago Medical Center laboratory.

Patients with an elevated IgA TTG antibody or IgA deficiency were offered upper endoscopy with biopsies of the duodenum. The biopsies were reviewed by a single experienced gastrointestinal pathologist. This study was approved by the University of Chicago Medical Center Institutional Review Board.

Patient characteristics were described by mean and range for continuous variables and proportions for categorical variables. Differences in patient characteristics and outcomes between groups were compared by using the *t*-test. p Values $< .05$ were considered statistically significant.

Results

Patient Demographics and Baseline Characteristics

In total, 18 subjects were identified that met inclusion criteria for the study. Of these patients, 17 subjects (94.5%) were subsequently enrolled (Table 1). Nearly two-thirds of enrolled patients were female and the majority of patients were white. The etiology of SBS was Crohn's disease in 9 subjects (52.9%), ischemia in 6 subjects (35.3%), small bowel obstruction in 2 subjects (11.8%), and malignancy in 1 subject (5.9%).

Thirteen subjects (76.5%) had undergone >1 previous abdominal surgery. No subject had an ileocecal valve. Nine subjects (52.9%) had undergone previous surgeries, resulting in an end ileostomy or jejunostomy. Small intestine length was available in 11 subjects. Mean length of remaining small bowel was 93.1 cm, and only a single subject had a small intestine length >200 cm. Fourteen patients (82.4%) required home PN, and the remaining 3 patients required only oral and parenteral vitamin supplementation. All subjects were taking an oral diet that included gluten.

Serology

Serology testing was performed in all subjects. In 15 subjects (88.2%), at least 12 months had elapsed

Table 2
Serology results

Total IgA antibody	
Mean ± SD, mg/dL (range)	449.3 ± 211.8 (212–1147)
Subjects with IgA deficiency	0/17 (0%)
Subjects with elevated IgA level	5/17 (29.4%)
IgA transglutaminase antibody	
Negative	16 (94.1%)
Positive	1 (5.9%)

IgA, Immunoglobulin A; SD, standard deviation.

since their most recent small bowel resection (mean, 81 months; range, 1–300 months). Results of serology testing are summarized in Table 2. There were no subjects with IgA deficiency. Five total subjects were found to have elevated serum IgA levels. Four subjects receiving home PN were determined to have elevated serum IgA level. There was no significant difference in mean total IgA level between those subjects requiring home PN *vs* those requiring only oral and parenteral vitamin supplementation (456.4 ± 239.6 *vs* 416.33 ± 77.7; $p = .78$). A single subject was found to have a positive serum IgA TTG antibody level (61 U/mL). This subject was receiving home PN and had undergone previous ileal resections and a subsequent proctocolectomy for ileocolonic Crohn's disease.

Endoscopy

Upper endoscopy was performed on the single subject with positive IgA TTG antibody testing. Endoscopic findings included atrophy of the gastric mucosa and fissuring and scalloping of the duodenal mucosa without ulceration. Gastric biopsies showed focal active gastritis with solitary loosely formed granulomas; duodenal biopsies showed patchy enteritis with mild activity, features most consistent with gastroduodenal Crohn's disease. There were no biopsy features suggestive of celiac disease.

Discussion

Adults with SBS have an increased morbidity and mortality when compared with the general population. The 5-year survival in adults with SBS is 75%, with an average rate of 2.6 complications per year.[17,18] Associated morbidity is, in part, due to dependence on home PN. Complications from PN may account for half of all admissions within the first year of initiation of therapy.[19] Recurrent hospitalizations for catheter-related sepsis, venous thrombosis, and metabolic derangements also result in a significant financial burden.[20] Thus, other causes for diarrhea need to be considered, particularly in those dependent on receiving PN.

Activation of latent celiac disease may be triggered by gastrointestinal surgery in susceptible patients.[12] After all intestinal surgery, a transient increase in intestinal permeability occurs.[21] This increase in permeability might allow increased amounts of gluten peptides to enter the intestinal mucosa.[14] In the susceptible patient, this can initiate an inflammatory cascade, resulting in mucosal damage and subsequent malabsorption. Thus, the stimulation of the innate and adaptive immune response due to increased intestinal permeability to gluten after surgery may be the underlying mechanism of activation of latent celiac disease.[14]

In this study, we examined whether undiagnosed celiac disease contributed to the symptoms of diarrhea and malabsorption in a cohort of patients with SBS. The patients were predominantly white and female. A majority of patients had a history of inflammatory bowel disease. Although this may reflect an institutional bias, as we are a tertiary-care referral center for patients with complicated inflammatory bowel disease, Crohn's disease is the main cause of SBS in the United States. All of the patients identified in this study had undergone previous surgical procedures, resulting in the loss of the ileocecal valve. This finding underscores the importance of the ileocecal valve in maintaining sufficient intestinal transit time for absorption.[22]

Screening for celiac disease was performed using serum IgA TTG level. Total IgA was also examined to evaluate for IgA deficiency. A single patient was found to have an elevated IgA TTG antibody level; however, gastric and duodenal biopsies were suggestive of active Crohn's disease rather than celiac disease. False-positive TTG antibody tests can be seen in patients with inflammatory bowel disease, and this is a known limitation of this screening test.[23] In our cohort of patients, no cases of undiagnosed celiac disease were found.

IgA is critically important in protecting humans from intestinal infections, and in some cases, its deficiency is associated with recurrent infections.[24] IgA is mainly secreted across mucous membranes and constitutes only a small portion of serum immunoglobulin. In this report, 5 patients had an elevated total serum IgA level, 4 of whom were receiving PN. In a previous study, PN initiation in healthy subjects was also associated with a minimal elevation in serum total IgA.[25] It is unclear whether the elevation in total IgA is related to administration of PN or due to short bowel. In animal studies, total PN causes gut-associated lymphoid tissue atrophy and a resultant decrease in mucosal secretory IgA levels.[26,27] Serum IgA serves as a second-line defense system, clearing bacteria that escape intestinal mucosal barriers in the presence of low secretory IgA levels.[28] Thus, an increase in serum IgA level may be secondary to decreased mucosal IgA. These findings may warrant further investigation.

Our institution has experience with SBS and serves as a regional referral center for patients. Thus, although our sample size is small, it is one of the largest in the area, underscoring the decreasing frequency of SBS as medical and surgical care

progresses. This small sample size is a potential limitation of this study because the prevalence of celiac disease in the general population is <1%. Antibody to TTG and total IgA was used to screen for celiac disease. Although the sensitivity of antibody testing is excellent, it is possible that celiac disease was underdiagnosed as endoscopic biopsies were not obtained in all patients. Furthermore, our institution also serves as a referral center for patients with inflammatory bowel disease. Thus, the predominance of patients with Crohn's disease in this study may not be representative of other patient populations.

In summary, we did not find an increased prevalence of celiac disease in our cohort of predominantly white patients with SBS. Our small sample size and the predominance of subjects with inflammatory bowel disease are potential limitations of this study. Given the high prevalence of celiac disease and the morbidity of SBS, larger studies are warranted in patients with SBS and those with bypass surgery for obesity who fail to thrive.

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Biostatistics Primer: Part 2

Brian R. Overholser, PharmD; and Kevin M. Sowinski, PharmD, BCPS, FCCP
Department of Pharmacy Practice, Purdue University, School of Pharmacy and Pharmaceutical Sciences, West Lafayette and Indianapolis, Indiana; and the Department of Medicine, Indiana University, School of Medicine, Indianapolis, Indiana

ABSTRACT: Biostatistics is the application of statistics to biologic data. This article is the second part of a 2-part series on the application of statistics in nutrition science. The first article, published in the December 2007 issue, reviewed descriptive statistics. Inferential statistics, to be discussed in this article, can be used to make predictions based on a sample obtained from a population or some large body of information. It is these inferences that are used to test specific research hypotheses. This article focuses on inferential statistics and their application in the nutrition and biomedical literature. Additionally, this review will outline some of the most commonly used statistical tests found in the biomedical literature.

In the December 2007 issue of *NCP*, the first part of a 2-part series, "Biostatistics Primer," we reviewed basic concepts of statistics and expounded on descriptive statistics. The concluding part of this series will focus on inferential statistics and some of the most commonly used statistical tests in biomedical literature.

Commonly Used Statistical Tests

The appropriate statistical test for any given dataset should be chosen according to certain characteristics of the collected data. Most of the statistical tests that are described in this review are termed *parametric tests*. Although these tests are the most powerful (ie, more likely to detect a difference if one exists), there are certain assumptions that must be met to appropriately use a parametric test. First of all, the collected data should be on an interval or ratio scale and be describable using the mean and standard deviation (SD). If the correct variable is chosen, the 2 primary assumptions of parametric tests are that samples are obtained from a population that is normally distributed and that the sample variances are essentially equal. In many cases, studies with small samples sizes may not meet these criteria. The Kolmogorov-Smirnov and goodness-of-fit tests are 2 statistical tests that can be used to test the normality of data (ie, the underlying assumption of normally distributed data). If this assumption is not met, the data should be transformed to a normal distribution (eg, log transformation for positively skewed data) or a different statistical test should be used, such as a nonparametric test. Statistical tests can also be used to test the homogeneity of variance to ensure that the second assumption of parametric tests is met.

For each parametric statistical test described in this section, a nonparametric equivalent will be briefly described for data that do not meet the assumptions for a parametric test. Nonparametric tests are more robust and do not make any assumptions about the underlying distribution of the data. However, if the assumptions for a parametric test are met, these should be used because they are more powerful. Finally, data that cannot be placed on an interval or ratio scale are generally described as categorical data. Categorical data (nominal or ordinal) can have different underlying distributions than that of continuous data and therefore a different statistical analysis is required. The most common categorical test in the medical literature is the χ^2 test.

t-Tests

One of the most common questions raised in biostatistics is whether 2 groups differ from one another, making *t*-tests very common applications in biostatistics. These tests are used to compare the average response to a drug between 2 distinct groups, to compare the average value in a group to a known standard, or to compare the baseline drug response with the response after receiving a drug in the same individual. The purpose of this section will be to describe 3 statistical tests that generally are referred to as *t*-tests. In essence, these tests use similar calculations for making the comparisons but

Correspondence: Kevin M. Sowinski, PharmD, BCPS, FCCP, Purdue University, Department of Pharmacy Practice, W7555 Myers Building, WHS, 1001 West Tenth Street, Indianapolis, IN 46202. Electronic mail may be sent to ksowinsk@purdue.edu.

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are, in fact, quite different in their underlying assumptions and requirements for study design. Each *t*-test uses the ratio of the difference between the group means in the numerator divided by the variability within the groups to calculate the statistical ratio.[1] In the case of a *t*-test, the statistical ratio is the *t*-statistic. This ratio is shown in the following equation:

$$\text{Statistical Ratio} = \frac{\text{difference between group means}}{\text{variability within groups}}$$

The larger the difference between the group means compared with the variability within groups, the greater the absolute value of the ratio. The larger the ratio, the more likely it is to demonstrate a statistical difference between the 2 groups. This ratio is the general form of each of the *t*-test equations, in addition to equations used in the analysis of variance (ANOVA) F-test. *t*-Tests are among the most commonly used,[2] and sometimes misused, statistical tests in the biomedical literature

All *t*-tests are parametric tests, and as such, the user assumes an underlying distribution or structure when using the tests. The derivation of the *t*-test calculation assumes that the outcome response being measured is normally distributed, although it has been suggested[1] that the *t*-test performs reasonably well if the underlying distribution deviates "moderately" from normality. The 3 types of *t*-tests most commonly used are the 1-sample *t*-test, 2-sample *t*-test, and paired *t*-test. The following discussion will address each one.

The 1-sample *t*-test is used to answer the question: is the average of a set of observations equal to the standard value in the population? The study design for this type of *t*-test includes only 1 sample and has a goal of determining if the observed outcome has the same mean of some standard or reference population. The structure of the *t*-statistic is as follows:

$$t = \frac{\bar{x} - \mu}{s/\sqrt{n}}$$

where $\bar{x}$ is the sample or observed mean, s is the sample or observed SD, n is the sample size, and μ is the population mean. Remember that the statistical ratio described earlier, in this case the numerator, is the difference between the observed mean and the reference mean and the denominator is the variability within the observed group. An example of the use of this type of *t*-test may be used to determine if the mean of 100 blood glucose samples (mean ± SD; 125 ± 15 mg/dL) obtained in the emergency department is different from the established hospital control (115 mg/dL).

A second type of *t*-test, a 2-sample *t*-test, is very commonly used in the biomedical literature. This test is also known as an independent-samples, independent-groups, or unpaired *t*-test. This type of *t*-test is used to compare the responses or outcome measures in 2 independent groups, that is, 2 groups of different subjects. Examples of this in the literature include responses to active drug compared with control, placebo, or standard therapy, or the response to 2 treatments given to different persons. Other examples include comparison of pharmacokinetic parameters between 2 different groups (men *vs* women, young *vs* old, heart failure *vs* control, etc) or comparison of demographics (age, weight, etc) between 2 groups. The question we ask for a 2-sample *t*-test would be: is the mean of one group equal to the mean of the other group (a 2-tailed *t*-test)? Alternatively we may ask, is the mean of one group less than (or greater than) the mean of the other group (a 1-tailed test)? There are 2 types of 2-sample *t*-tests used, one that assumes the variances of the 2 groups are equal (variance homoscedasticity) and one in which the variances are not equal (variance heteroscedasticity). Most statistical software packages perform both of the tests and the analyzer chooses the most appropriate test. The assumption of equal variances can be tested formally or informally. In the latter case, if the ratio of the samples variances (ratio of larger variance to smaller variance) is >2, it is generally assumed that the variances are unequal. This can be formally tested with the F-test for variances, which will not be discussed in this review. If the variances are not equal, the unequal variance *t*-test should be used; otherwise, the equal variance *t*-test is appropriate. The equations below describe the case when the variances are assumed to be equal; interested readers are directed to other reviews[3] for discussion and calculations involved for the unequal variance test and the associated F-test. The structure for the equal variance *t*-test is:

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the means of the first and second groups, respectively, s_p is the pooled SD, and n_1 and n_2 are the number of observations in the first and second groups, respectively. The pooled SD is the weighted average of the SD in the 2 groups as described in the following equation:

$$s_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2) - 2}}$$

Remember that the numerator of the statistical ratio is the difference between the 2 means and the denominator is the measure of variability within these 2 groups. Using the example from Table 1, we wish to know if the gestational age is statistically different between the glutamine group and the control group. In this example, because these groups are separate individuals, we use the unpaired or independent samples *t*-test rather than the paired *t*-test. The assumptions for this test are that the

Table 1 *Baseline and nutrition characteristics (modified with permission from van den Berg et al[4])*

	Glutamine (n = 52)	Control (n = 50)	p*
Antenatal corticosteroids	39/52 (75%)	39/50 (78%)	.72
Vaginal delivery	23/52 (44%)	24/50 (48%)	.70
Gestational age (wk)	29.3 ± 1.7	28.7 ± 1.8	.07
Birth weight (kg)	1.18 ± 0.4	1.16 ± 0.3	.79
Birth weight <10th percentile	17/52 (33%)	12/50 (24%)	.33
Sex (% male)	28/52 (54%)	27/50 (54%)	.99
Clinical risk index for babies (CRIB)	2.5 (0–12)	3 (0–13)	.45
Age at start of study supplementation (d)	2.6 (1.4–4.6)	2.5 (1.8–3.8)	.53
Time to full supplementation dose (d)	10 (4–17)	9 (4–23)	.94
Age at increasing enteral nutrition (d)	3.6 (0.2–11.8)	3.4 (0.7–10.1)	.92

Values are mean ± SD, median (range), or number (%).
*Student's *t*-test, Mann-Whitney *U* test, χ^2 test, and log rank test for continuous normally distributed data, nonparametric continuous data, dichotomous data, and time-dependent data, respectively.

variances are equal and that the data are normally distributed. The first assumption appears to be met because the SDs (and variances) are nearly equal. The second assumption is more difficult to verify without access to the actual data, a common problem when evaluating statistical procedures used in published studies. What we do know about the data are that they do not seem to be highly variable (according to the SD) and thus not likely to be skewed or affected by extreme outliers. Thus the use of a *t*-test seems to be appropriate in this case. If you were the researcher with access to the actual data, with the observations from the study, you would be able to evaluate the normality of the data. As an initial step, many investigators would compare the mean and median value and, if similar, assume that the data are normally distributed. Finally, because the number of subjects studied in this case is relatively large, approximately 50 in each group, most would agree that the use of a parametric test is reasonable in this situation.[5]

The third type of *t*-test, a paired *t*-test, also known as a matched-pairs *t*-test, is used to compare 2 responses or outcome measures in the same person. The alternative to this design is the test described previously, the unpaired *t*-test. Instead of 1 treatment administered to 1 group of subjects and the other treatment to another group of subjects (as in a parallel-groups design), in a paired design, 1 subject receives 2 treatments. A commonly used type of study design using a paired *t*-test is a crossover study where 1 subject has 2 measurements obtained after 2 treatments. The benefit of this type of design is the between-subject variability is reduced as each subject serves as his or her own control. The use of a paired study design and paired *t*-test increases the power of this test as compared with a 2-sample *t*-test or a parallel-group study design. The determination of the test statistic for the paired *t*-test is illustrated in the following equation:

$$t = \frac{\bar{x}_d - \mu_d}{s_d / \sqrt{n}}$$

where $\bar{x}_{\mathrm{d}}$ is the mean of the differences in the 2 treatments, s_d is the SD of the difference between the 2, μ_d is the population mean of the difference in the 2 treatments, and n is the number of pairs observations in the first and second group, respectively.

The previous several paragraphs discussed using *t*-test and hypothesis testing to compare means or to determine if a statistical difference exists. A related alternative approach is the use of confidence intervals. Recall from the previous discussions that the confidence interval represents the interval in which the true population mean or difference between means may exist. In most cases we use the 95% confidence interval, which corresponds to the $p < .05$ level of significance used in hypothesis testing.[6]

ANOVA

ANOVA is a commonly used statistical analysis in the biomedical literature. In this paper, we are discussing introductory statistical concepts, so our discussion will focus mainly on 1-way ANOVA, a brief discussion of repeated measures ANOVA, and 2-way ANOVA. ANOVA is one of several multifactorial analyses; factors are groups or treatments. ANOVA can be applied to independent (1-way ANOVA) and related (repeated-measures ANOVA) groups design. The simplest type of ANOVA is a 1-way ANOVA, in which 3 or more groups are being compared. Therefore, it is an extension of an independent-samples *t*-test, in which only 2 groups are compared. There is 1 factor, and the analysis is performed to test whether any of the means differ. ANOVA is a parametric analysis and thus subject to the same assumptions we discussed for *t*-tests and other parametric tests. The assumptions underlying ANOVA are that the dependent variable is continuous or at least interval scaled, the response data are drawn from a normally distributed population, there is a random unbiased selection of cases and assignment to groups, the intersubject variance (or SD) in all groups or factors is equal, and the factors (grouping or independent variables) are categorical or transformed to be categorical.

One-way ANOVA, sometimes referred to as a single-factor experiment, as described above, is applied to an experiment in which 3 or more independent group means are compared. One-way ANOVA implies 1 factor with 3 or more levels. Table 2 illustrates this concept for a hypothetical data set. In this example, 60 subjects were randomly assigned to treatments 1 through 4, which is a parallel-groups design study. The table depicts the individual data and mean and SD in each group. The 1-way or factor, in this example, is the independent variable treatment and the levels are 1–4. The null and alternative hypotheses for this statistical analysis are:

$$H_0 : \mu_{\text{Treatment 1}} = \mu_{\text{Treatment 2}} = \mu_{\text{Treatment 3}} = \mu_{\text{Treatment 4}}$$

H_A: At least 2 means differ significantly

At first look, one may come to the conclusion that multiple independent *t*-tests could be used to perform this analysis. This is a commonly used and incorrect statistical approach for this type of data. If we were to compare each possible permutation in this example, we would be conducting 6 *t*-tests. Assuming an *a priori* type I error rate of 5%, multiple tests would increase that risk of a type I error to >5% and increase the error rate. Using ANOVA keeps the type I error rate at an acceptable level. The statistical test for ANOVA uses the F-test to perform the analysis. The F-test uses the ratio of the sum of squares between groups to the sum of squares within groups to determine the critical value of the F-test statistic. The analysis is similar to the earlier description of an independent *t*-test, except that we must account for the variation between the groups and within the groups for all of the groups rather than just the 2 groups. Just like a *t*-test, the concept of the statistical ratio can be used to understand the components of the statistical analysis used to perform the analysis. Recall that the statistical ratio relates the difference between the means (intergroup variation) and the variability within groups (intragroup variation). The key concept is that ANOVA separates the error into components. In 1-way ANOVA, the calculations for the analysis, which are usually made using statistical software packages, determine the sum of squares total, sum of squares between groups, and the sum of squares within groups. According to how the hypothesis test is written for ANOVA, it should be apparent that the conclusion from the ANOVA will be that either there is no difference between the means or at least 2 of the means differ. In the example in Table 2, the overall ANOVA was statistically significant, as evidenced by the *p* value of < .001, which is below the $\alpha = .05$ set before the study. The overall ANOVA, using the F-test, will not determine which of the 2 means are different. Instead, we would conclude, according to the alternative hypothesis, that at least 2 of the means differ significantly.

Table 2 *Individual and mean and SD data from a hypothetical study in which 4 treatments were administered to 4 different groups*

	Treatment			
	1 (n = 15)	2 (n = 15)	3 (n = 15)	4 (n = 15)
	277	272	255	277
	270	275	253	267
	279	259	268	273
	272	278	264	275
	274	275	279	273
	289	276	253	279
	282	276	278	278
	278	285	275	264
	285	274	265	259
	275	275	251	275
	277	259	258	273
	269	283	255	274
	276	281	264	282
	285	275	268	272
	255	276	265	292
Mean*	276.2	274.6	263.4†	274.2
SD	8.1	7.2	9.2	7.7

*Overall ANOVA, $p < .001$.
†Treatment C different from treatments 1, 2 and 4; $p < .01$, by Tukey HSD.
SD, standard deviation.

Intuitively when one looks at Table 2, it seems as if treatment 3 differs from the others. The use of post hoc testing is the most common approach to statistically evaluate this situation. This approach controls the type I error rate.[6] Although the specific discussion of the calculation of the various post hoc tests will not be done in this article, the various post hoc tests that may be encountered are Tukey's HSD, Scheffe, Bonferroni, Dunnett, Dunn, Newman-Keuls, and least significant difference tests. Each of these procedures has advantages and disadvantages discussed elsewhere.[5]

A more complex ANOVA technique using an independent groups design is 2-way or 2-factor ANOVA. Two-way ANOVA allows the study of 2 independent variables at the same time. An example would be the extension of our previous 1-way ANOVA shown in Table 3. Instead of 1 independent variable (treat-

Table 3 *Mean and SD data from a hypothetical study in which 4 treatments were administered to 4 different groups in 2 different age groups*

		Treatment			
		1	2	3	4
Age	Mean-young (n = 15)	276.2	274.6	263.4	274.2
	SD-young	8.1	7.2	9.2	7.7
	Mean-elderly (n = 15)	262.4	258.5	235.1	225.3
	SD-elderly	4.6	5.2	4.7	5.9

SD, standard deviation.

ment) now we have 2 independent variables, (treatment 1–4) and age (young and elderly). It should be noted that this is not a repeated-measures design, which is the young and elderly receiving 4 treatments, but instead, 4 independent groups receiving the treatments. Using ANOVA we now ask 3 questions: (1) What is the impact of age independent of treatment? (2) What is the impact of treatment independent of age? and (3) What is the joint effect of age and treatment? Instead of 3 sums of squares terms, now there are 4: sum of squares total, sum of squares due to treatment, sum of squares due to age, and sum of squares due to the interaction between treatment and age.

Repeated-measures ANOVA is an extension of a paired *t*-test, which is a related-samples design rather than an independent-groups design. Instead of the example that we have used for 1-way ANOVA (Table 2), in which 4 different groups (n = 15) received different treatments, now consider the example in which 1 group (n = 15) receives 1 treatment, with 4 samples over time. An example of this approach would be the effect of the impact of a natural herbal remedy on total cholesterol, illustrated in Table 4, over the course of a 6-month time period. The study design is an example of a repeated-measures design, or in other words, a paired-study design. We now ask the questions, (1) what is the impact of treatment (ie, time)? (2) what is the impact of subject? and (3) what is the joint effect of subject and treatment? The 4 sums of squares terms are sum of squares total, sum of squares due to treatment, sum of squares due to subjects, and sum of squares error. This could be expanded to another level by adding another group of subjects (like elderly subjects) and become a 2-way repeated-measures ANOVA. There are numerous other types of ANOVA that are more complex and beyond the intent of this article.[1,5,6]

Correlation

In many applications in the biomedical literature, clinicians and researchers wish to examine the relationship between 2 variables. Instead of asking the question that we previously asked from Table 1—are the gestational ages of subjects in the glutamine group different from the gestational ages of the subjects in the control group?—now we ask the question: what is the relationship between birth weight and gestational age? In correlation analysis, we examine whether pairs of data are associated. In this example, intuitively we may think that as gestational age increases, so does birth weight. This is likely to be true up to a certain gestational age. In correlation analysis, this is not to say that increased gestational age causes increased body weight; this would be evaluated by regression analysis. Correlation analysis is often referred to as the degree of association between the 2 variables. In other words, we are investigating the relationship between 2 variables, not suggesting or studying whether or not one causes the other. The 2 variables investigated are both treated equally, and neither is assumed to be the predictor or the outcome.[7] The null hypothesis for a correlations analysis is that the correlation coefficient is equal to zero (no relationship) or that variable 1 and variable 2 are not related.

Table 4 *Mean and SD cholesterol data from a study in which 15 subjects received an herbal therapy thought to reduce cholesterol*

	Time			
	Baseline	6 wk	3 mo	6 mo
Mean (n = 15)*	275.8	265.5†	273.7	274.4
SD	3.20	3.12	2.79	3.69

Cholesterol was measured at the intervals indicated over the 6-month period.
*Overall repeated-measures ANOVA, $p < .001$.
†Six weeks different from baseline, 3 months, and 6 months, $p < .001$, by Tukey HSD.
ANOVA, analysis of variance; SD, standard deviation.

The statistical parameter quantifying the degree of association between the 2 variables is referred to as the correlation coefficient (r). The correlation coefficient is a unitless measure of the association between the 2 variables. The size and sign of the correlation coefficient communicate important information about the relationship. The correlation coefficient ranges from −1 to +1; a value of −1 indicates a perfect negative relationship (that is, as variable 1 increases, variable 2 decreases). A perfect positive relationship has a correlation coefficient of +1, whereas an r of zero indicates no linear relationship. There is no agreed-upon or consistent interpretation of the value of the correlation coefficient. Table 5 depicts several arbitrary published criteria for defining the strength of the relationship.

The value of r is strongly affected by sample size, measurement error, and the variables being explored. Equally important, the interpretation of the value of the correlation coefficient depends on the type of research being conducted (ie, clinical research, basic research, social science research). The value of r alone also does not communicate any information about whether the relationship between the 2 variables is statistically significant. The formal statistical test for correlation can be performed and is highly dependent on sample size. The larger the sample size, the more likely a statistically significant association will be found. Recall that a statistically significant finding does not necessarily mean that there is a strong relationship, just one that is unlikely to have occurred by chance. Consider Table 6, which illustrates data from 2 correlation analyses. The importance of sample size should be apparent in this example. In study 1, the r value can be described as poor, yet the relationship is statistically significant due to the large sample size. Contrast that with study 2, in which the r value would most

Table 5 *Correlation coefficient descriptions*

r Value	Guilford[8]	Rowntree[9]	DeMuth[6]	Portney[1]
<0.10	Slight correlation, negligible relationship	Very weak, negligible	"Weak"	Little or none
0.10–0.20				
0.20–0.30	Low correlation, definite but small relationship	Weak, low	"Fair"	
0.30–0.40				Fair
0.40–0.50	Moderate correlation, substantial relationship	Moderate	"Good"	
0.50–0.60				Moderate to good
0.60–0.70				
0.70–0.80	High correlation, marked relationship	Strong, high, marked		
0.80–0.90				Good to excellent
0.90–1.00	Very high correlation, very dependable relationship	Very strong, Very high		

likely be described as good, yet there is no statistically significant relationship, due to a small sample size. When evaluating correlation coefficients and associated statistical tests, the reader should pay close attention to these issues and appreciate the factors involved in these analyses.

There are several different types of correlations utilized for statistical analysis. The 2 most commonly employed are the Pearson product-moment correlation and the Spearman rank correlation. The Pearson product-moment correlation is the most commonly used correlation analysis, the statistic generated by this analysis is r, or the correlation coefficient, for the sample and for the population, ρ. This is a parametric statistic, and its use assumes that the 2 variables have underlying normal distributions, are continuous or ratio scaled data, and the variables are linearly related. The second type of correlation, the Spearman rank correlation coefficient, is the nonparametric analog of the Pearson product-moment correlation. This correlation is not subject to the same parametric assumptions as Pearson's correlation, and can be used with ordinal data. Instead of using the observed data, the calculation uses the difference in the ranks of the observations to determine r_s (Spearman's ρ).

Table 6 *Correlation coefficients from 2 different study designs and associated* p *values*

	Study 1	Study 2
r	0.20	0.70
n	102	8
p Value (2-tailed)	<.05	>.05

Crucial to the proper use of correlation analysis is the interpretation of the graphical representation of the 2 variables. Before using correlation analysis, it is essential to generate a scatter plot of the 2 variables to visually examine the relationship. Because standard correlation analyses are only useful for linear relationships, graphical displays are necessary to determine if the relationship is something other than linear, such as curvilinear, or to determine if 1 data point is driving the relationship.[1,10]

Regression

A statistical technique related to correlation is regression analysis. Like correlation, there are many different types of regression analysis, including linear, multiple, weighted, and logistic. These techniques are widely used in all disciplines of the biomedical literature, from clinical to basic research.[5,7] It is beyond the scope of this paper to discuss each type of regression analysis; instead, the

focus will be on simple linear regression, the most commonly used technique in basic biostatistics applications. In correlation analysis, we discussed that the assumption was not for one variable to predict another. Instead, we used correlation analysis to determine if an association or relationship existed between 2 variables. In regression analysis, the intent of the analysis is to make predictions based on the observed relationship. That is, given a value of X, what will the observed regression analysis predict to be the most likely value of Y? Also, regression analysis provides us with some degree of certainty relative to the observed relationship. In regression analysis, we refer to studied variables as either the dependent (outcome) and the independent (causative) variable. In addition to making predictions of the dependent variable from the independent variable, regression analysis also quantifies how well the independent variable predicts the dependent variable. An example of regression analysis is shown in Figure 1. In this figure, the outcome variable is resting energy expenditure (Y-axis) and is dependent on the causative variable, weight (X-axis). These data were extracted from a published study.[11] Naturally, body weight does not totally explain resting energy expenditure but only some percentage of the variability. In this example, we are only considering 2 variables and refer to the example as simple linear regression.

The linear regression model, written in a way that is on one hand familiar and easy to understand and then, also, using statistical nomenclature is:

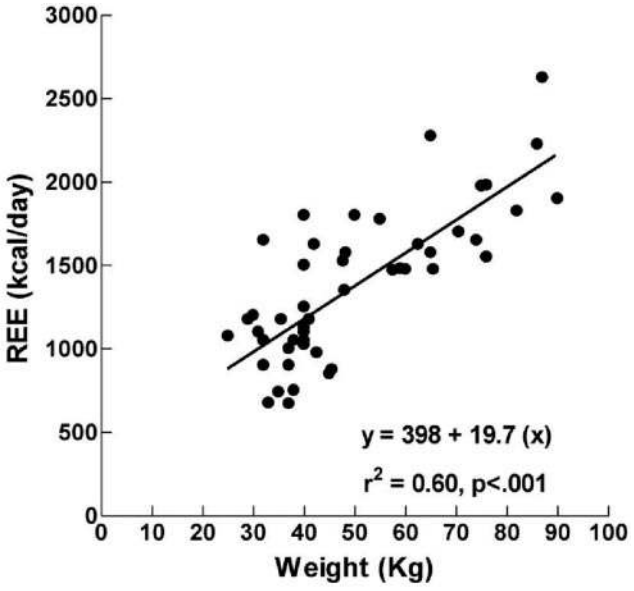

Figure 1. Relationship between resting energy expenditure and body weight. Data were extracted from a previously published study.[11] The coefficient of determination (r^2), *p* value, and regression equation are shown in the figure.

$$Y = mX + b$$

where Y is the dependent variable, X is the independent variable, m is the slope of the relationship, and b is the y-intercept, the location where the regression line crosses the Y-axis. In the sample, in statistical terms:

$$Y = b_0 + b_1X$$

where b_0 is the Y-intercept, the location where the regression line crosses the y-axis, and b_1 is the slope of the relationship. There are numerous ways in which to estimate the linear regression parameters, although the most commonly used in basic statistical analysis and by basic software applications is the method of least squares (ie, least squares linear regression).

The method of least squares regression seeks to minimize the sum of squared differences between the actual values of the dependent variable Y and the predicted values of Y (ie, minimize the sum of squares differences) and provides an estimate of the best-fit regression line, slope, and intercept and regression coefficients. Crucial to the proper use of regression analysis is the interpretation of the graphical representation of the 2 variables. As with use of correlation analysis, it is critical to generate a scatter plot of the 2 variables to visually examine the relationship. Visualization of the data allows us to draw some initial conclusions about the data and helps us to determine what the underlying model for the regression might be. As seen in Figure 1, the relationship between weight and resting energy expenditure appears to be linearly related. The relationship does not seem to be curvilinear or some other relationship.

The assumptions for linear regression are that the dependent variable (Y) is adequately modeled as being linearly related to a single independent variable X. Values of X are "known" or at least observed with negligible error. For each value of X, there is a population of Y values that are normally distributed. The observed values of Y are independent (ie, the value of an observation is not affected by the values of another observation).

The second intent of regression analysis is to determine the extent of variability in the dependent variable that can be explained by the independent variable. The coefficient of determination (r^2) is the ratio of the sum of squares explained by the regression and the total sum of squares. Values of r^2 can range between 0 and 1. The higher the r^2, the stronger the relationship. Like the correlation coefficient used in correlation analysis, the coefficient of determination is a means, albeit with some problems, to quantify the strength of the relationship between the dependent and independent variable. Mathematically, the coefficient of determination is the square of the correlation coefficient. In the example shown in Figure 1, the r^2 is 0.61, suggesting that the body weight explains 61% of the variability in resting energy expenditure.

Like interpretation of r, the interpretation of r^2 is dependent on the scientific arena (ie, clinical, research, and social science research) to which it is applied. In other words, a value of r^2 may have a very different meaning depending on the situation being studied. An r^2 of 0.80 obtained from an analytical standard curve would likely be considered a very poor relationship, whereas the same r^2 in a clinical pharmacokinetic study relating creatinine clearance and drug clearance would indicate a good relationship between the 2 variables.

The biomedical literature is replete with examples of regression analysis being used to make predictions. For example, aminoglycoside elimination rate constants are often estimated from estimated creatinine clearance by the equation:

$$\textit{gentamicin elimination rate} = (0.0024 \times \textit{CrCl}) + 0.01$$

to make initial dosing predictions. The development of this equation utilized linear regression techniques from a study relating the 2 variables.[12] Usually, equations derived from these studies are only used to make future predictions if several "criteria" are met. In general, we would not use the regression results unless that statistical analysis indicated a statistically significant relationship between the 2 variables. Unfortunately, an error that is frequently made in the interpretation of linear regression analysis is using the regression parameters to predict outcomes without examining all of the pieces of the regression statistics. In evaluating regression statistics, one should evaluate the value of r^2, as well as whether or not a statistically significant relationship between 2 variables was observed. There may be some situations in which a significant relationship between the 2 variables is observed but the value of r^2 is too low to meaningfully predict Y from X. Ideally, we would also investigate the standard error of the regression parameters, the distribution of the residuals, although these parameters are seldom presented in published biomedical manuscripts. The most frequent error that is made in regression analysis is to predict values that were not included in the studied relationship. Extrapolation is discouraged because the nature of the relationship outside of the observed range is unknown. An example of this practice would be to use an assay to determine the concentration of drug below the limit of quantification for a standard curve. Extrapolation outside of the observed values in most cases is discouraged.

Regression analyses are important techniques in the biomedical literature. In this paper, linear regression was discussed. Numerous other types of regression analyses are available, including nonlinear regression, multiple regression, and logistic regression.[1,5,6]

Nonparametric Statistics

Thus far, we have discussed the use of parametric statistics, that is, statistical tests that assume the data have an underlying normal distribution and some degree of variance homogeneity. Nonparametric statistics are not as restricted by underlying distributional requirements required for parametric statistics. Due to this fact, nonparametric statistics are often referred to as distribution-free statistics. In general, nonparametric statistics are less powerful than their parametric counterparts. They are generally useful in several situations when the assumptions of a parametric test are violated: when the outcome variable is an ordered (ordinal, nominal) and not continuous variable, or the outcome variable is continuous but not normally distributed, highly variable, or subject to outliers. The goal of this section is to present 4 commonly used nonparametric tests, to present the situations in which these tests may be used and their parametric counterparts. The nonparametric analog of the paired *t*-test is the Wilcoxon signed-rank test. It is useful in situations described above for the paired *t*-test. In this situation, the null hypothesis is that the median difference between the pair of observations is zero. Consider a situation in which we wish to determine if a patient's Acute Physiologic and Chronic Health Evaluation (APACHE) II score[13] improves from admission to day 3 of a hospitalization. The pair of observations would be the APACHE II score at admission and at 3 days. Because APACHE II scores can only take integer values (that is 1, 2, 3, 4, etc) the use of mean and SDs to describe the central tendency of this type of data does not make sense. Therefore, it should then be intuitive that a parametric test should not be used to perform the statistical analysis. The Wilcoxon signed-rank test is a nonparametric test that uses the ranks of the difference between the paired data, rather than the actual data in the analysis. Another nonparametric test that can be used with this type of data is the sign test.

The Wilcoxon rank sum test is the nonparametric analog of the 2-sample or unpaired *t*-test. The test is also often referred to as the Mann-Whitney *U* test. It is applied in situations described above for the unpaired *t*-test, in which 2 independent groups are being compared, and in which the outcome data do not meet the assumptions of the parametric test. The null hypothesis in this case is that the median is not different between the 2 groups. There are 2 examples of the use of this type of statistic in Table 1. First, the test is used to compare the Clinical Risk Index for Babies (CRIB) between the glutamine and control groups. Recall from earlier in this paper that CRIB is an index that can only have values that are whole numbers. Thus, the CRIB index is not a continuous variable, and comparisons between the 2 groups with an independent groups *t*-test would not be appropriate; instead, the Wilcoxon rank sum test should be used. Another example of use of this test is in the comparison of age at study start, time to full dose, and age at increasing dose. One could certainly make a case that days are continuous data, although we seldom record days as 1.1 days or 1.6 days, etc.

On inspection of the data, which are presented as the median and range in the table, it is evident that these data are skewed to the right and likely not normally distributed. In this case, a *t*-test is also not appropriate, and the Wilcoxon rank sum test would be appropriate. The extension of the Wilcoxon rank sum test for application to >2 groups is the Kruskal-Wallis 1-way ANOVA by ranks. This test is the nonparametric analog of 1-way ANOVA.

Finally, as discussed briefly, the Spearman's rank correlation coefficient is the nonparametric analog of Pearson's correlation. Unlike Pearson's correlation, Spearman's correlation does not require continuous data. Instead, it can be applied to continuous (regardless of the underlying distribution) or ordered data to test the null hypothesis that there is no association between the 2 variables.

χ^2 Test

The χ^2 test is one of the most frequently reported statistical tests in the medical literature. This test can be used on nominal or ordinal (categorical) variables that can take on >2 possible outcomes. Ordinal data can be transformed to discrete data by assigning numeric values and are often assessed using nonparametric tests as opposed to the χ^2 test.

Data that can be described by a contingency table are oftentimes nominal and by definition categorical data. Table 7 provides an example of a 2×2 contingency table (pronounced 2 by 2) that has been created using the reported sexes of the very-low-birth-weight (VLBW) infants in Table 1. As previously stated, sex is a nominal variable and can be placed in a contingency table such as the one in Table 7. The inner boxes in this table represent the observed number (percentage) of men and women of the participants in each study group, as reported in Table 1. The outermost boxes are the totals for the rows and columns, and the lower right box is the total of the rows, which is equal to the total of the columns. This is an important attribute of a contingency table.

The χ^2 test is commonly used to test baseline characteristics of treatment groups to ensure that there are no underlying compounding factors between the experimental groups. This is accomplished by testing observed *vs* expected values. The goal of the χ^2 test in the example provided in Table 7 is to test the number of men and women in each study group to ensure there were similar genders in each study group. The null hypothesis for the χ^2 test in this example could state that the sex of the infants in this study is independent of the assigned therapy. Therefore, this is commonly referred to as the χ^2 test for independence. The data presented in Table 7 are the observed values collected from a clinical study. Therefore, expected values are still needed to perform this statistical test. In this example, the expected values can be calculated directly from the observed values in the contingency table.

Table 7 *2 × 2 Contingency table of the sex of research subjects in van den Berg et al*[4]

	Glutamine	Control	Totals
Males	28 (54%)	27 (54%)	55
Females	24 (46%)	23 (46%)	47
Totals	52	50	102

The calculation of the test statistic for the χ^2 test is beyond the scope of this review but it is based solely on observed and expected values. For tests of independence, such as the previous example, the expected values can be calculated from the observed values to test for underlying diseases, sex, or other nominal variables in clinical studies. For studies that have small expected values (<5), the Fisher exact test should be used in place of the χ^2 test.

Summary

Biostatistics are essential tools for all individuals involved in clinical, basic, and translational research. A basic understanding of the underpinnings of biostatistics is necessary for the proper conduct and evaluation of biomedical research papers. This outlines important features of descriptive and inferential statistics as it applies to commonly conducted research studies in the biomedical literature. Additionally, commonly used statistical tests found in the biomedical literature were reviewed.

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Clinical Research

Microbial Contamination of Enteral Feeding Sets Used in the Home of Pediatric Patients

Cristine A. Roberts, RN, PhD(c)*†; and Elizabeth Lyman, RN, MSN†
*University of Missouri-Kansas City, Kansas City, Missouri; and †Children's Mercy Hospitals and Clinics, Kansas City, Missouri

ABSTRACT: Bacterial contamination of enteral feeding sets has been well documented in studies of patients. Much of the literature on this subject validates problems with manipulations of the feeding sets such as mixing formula and handwashing by the caregiver. Rinsing and storing of the set could also have serious implications in the amount of contamination. There is currently no standard recommending the length of time for use of enteral feeding sets for home care patients, particularly in children. Nine homecare patients with ages ranging between 1 and 15 years participated in this study. Cultures of the formula in the feeding set were obtained at zero hours with a new set, and after 24 and 48 hours. The caregivers prepared and administered the formula in their usual manner. Clinical data were collected for 10–14 days before the samplings and for 7 days afterward. Data included weights before, during, and after the culture collection period. Medications, stools, and emesis were recorded during this timeframe. It is difficult to draw statistically significant conclusions based on the small sample size of this study. There was an undesirable level of contamination at 48 hours of enteral feeding set use that was not present at 24 hours in 2 of the patients (22.2%). Neither of these children had diarrhea, vomiting, or other clinical changes, but both showed a small weight loss. The majority of the patients (77.8%) demonstrated that using sets for 48 hours did not increase the amount of contamination.

Background

Enteral feeding therapy is a common method for delivering additional calories to patients who are nutritionally at risk. Supplemental feedings can be given in intermittent bolus form or *via* a pump over longer periods, up to 24 hours per day. This therapy is usually initiated in the hospital or clinic setting, and then is continued in the patient's home. For pediatric patients, parents are taught the proper method of formula preparation, enteral set cleaning, and pump operation by the nutrition support and home health nurses. Poor sanitation in the home, improper feeding set changes or cleaning, and improper length of formula hang time can all contribute to gastroenteritis or similar symptoms.[1] Gastroenteritis, manifested as diarrhea and vomiting, may significantly affect a child's weight gain secondary to intolerance of the feeding as nutrients are lost in stool output. No studies have been reported that correlate gastroenteritis with enteral feeding set contamination in a pediatric homecare population.

A study in Japan linked contaminated formula with diarrhea in adult patients receiving enteral feedings.[1] This prospective study documented clinical diarrhea (a minimum of 3 liquid stools in a 24-hour timeframe) in 2 patients who also had significant microbial contamination. There was a statistically significant difference in contamination levels between these 2 patients and the other 23 patients in the study. The standard safe limit for pasteurized milk from the United States Dairy Association (USDA) and the standard for food-borne disease from the Centers for Disease Control and Prevention (CDC) is bacterial counts not more than 10^4 colony forming units (cfu) per mL.

When the impetus for this research started, the authors could find no consensus among pediatric home health agencies for a standard enteral set hang time. Some patients received a new enteral set daily and some changed the set every other day due to third-party payer guidelines or policies. The concerns that generated this study were the cost of daily set changes to the patients' families and to the healthcare system, the impact on the environment that a higher frequency of set changes would entail, and the risk of contamination to the patient.

Study Aims

The study aims for this project were (1) to compare the level of bacterial contamination in enteral

Correspondence: Cristine A. Roberts, University of Missouri Kansas City, Health Science Building, 2464 Charlotte, Room 3406, Kansas City, MO 64108. Electronic mail may be sent to robertscris@umkc.edu.

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feeding sets used for children in their homes with their diarrhea score, incidences of vomiting, and weight change; (2) to describe the timeframe that enteral feeding sets can be used safely for children in their home without exceeding the USDA standard of a microbial count $<10^4$ cfu/mL, as measured by samples of formula from the distal end of their set tubing at zero hours, 24 hours, and 48 hours.

Methods

This prospective, descriptive study was primarily observational and consisted of pediatric home health patients. The protocol did not require any changes in the current practice of caregivers in the home unless they were changing enteral sets more often than every 48 hours. The only other alteration in the family's routine was a visit from study personnel for specimen collections that occurred on 3 consecutive days.

Permission to proceed with the study was obtained from the institutional review board of the children's hospital of which the home health agency was a department. The participants of this study were selected as a convenience sample from 1 pediatric hospital's home health agency in a large Midwestern metropolitan area. The selection criteria for participants were that the child must be clinically stable, <18 years old, receiving enteral feedings for at least 3 weeks before the start of the study, and could not be receiving an elemental formula or antibiotic treatment. The rationale for exclusion of patients receiving elemental formulas was to minimize any variability that highly osmolar feedings could introduce into the sample. All of the patients who were served by this home health agency and that met these criteria were sent recruitment letters. Three attempts to contact the child's caregiver were also made by telephone. The principal investigator (PI) or trained study personnel went to the homes of those who volunteered to participate. The purpose of the study was explained and informed consent was obtained from the parents or legal guardians.

Data collection consisted of obtaining initial demographics, including age, gender, ethnicity, and diagnosis. Enteral feeding data, including type of formula administered, type of gastrostomy device, and delivery method were also documented. Clinical data, including weights, vomiting, stool pattern, and medication history, were collected at the start and throughout the 3-week study period.

The timeframe for the data set was 21 days. On day 1, demographics were collected and the caregivers began their diary of stool, vomiting, and medication history for the child. The researchers were able to determine if the patient was clinically stable before data collection commenced. None of the children showed symptoms of gastroenteritis (specifically, vomiting, diarrhea, or significant weight loss) during this period. At approximately 10–14 days, the specimen collection began. Specimens were collected with the start of a new enteral set (called time zero), then at 24 hours, and again at 48 hours. Parents continued to collect written data for 7 more days after the third specimen was collected.

The families' current practices for enteral set changes and bag cleaning were assessed by the nurse. Families were instructed to administer the feeding as usual. All of the families used the same type of enteral feeding set and none of them used closed systems. Caregivers were asked to change enteral feeding sets once every 48 hours during the study period. If the patient was receiving intermittent feedings, once the feeding was complete, the parent was instructed to rinse out the bag and tubing with hot water and hang it to dry. If the patient was receiving continuous feedings, the enteral set was rinsed with hot water every 24 hours. The patient's stooling history, including frequency, volume, and consistency as evaluated by the Guenter tool,[2] was recorded for 10–14 days before the specimen collection began, during the data collection period, and then for 7 days afterward by the caregivers. Vomiting was observed and recorded during the same period of time. These data were documented on a standardized flow sheet by the child's primary caregiver. The home health nurse recorded weekly weights on the same scale.

On days 10–14 of the data collection period, when a new enteral set was started, the study nurse arrived at the patient's home to collect the first sample. The patient's parent or primary caregiver prepared formula according to directions established by the physician or nutritionist. A baseline sample of formula was obtained at this time using the following standardized protocol. With 2 alcohol wipes, the nurse cleansed the tip of the tubing used to connect the feeding system to the patient. Then, 5 mL of formula was dispelled through the feeding set and wasted. An additional 5 mL of formula was dispelled into a sterile tube for collection and placed immediately on ice. This formula was transported by the study personnel to the hospital laboratory for culture. The transport time did not exceed 60 minutes. The same method was used at 24 and 48 hours after time zero. The laboratory reported qualitative data (the specific pathogen) and quantitative data to the PI through a patient identifier code.

Results

Patients

Nine children participated in this research study (Table 1). There were 4 boys and 5 girls, with an average age of 5.6 years (range, 15 months to 14 years and 8 months). All 9 children had gastrostomies, 6 of which were low-profile devices (buttons). All children were given tube feedings each day.

Table 1 *Patient demographic and clinical data*

Patient	Gender	Age	Diagnosis	Formula*	Bolus/drip	Tube
1	Boy	2 y 3 mo	Cerebral palsy, failure to thrive, seizures	Peptamen	Bolus	PEG
2	Girl	2 y 8 mo	Gastroesophageal reflux, seizures	PediaSure	Bolus	G button
3	Girl	1 y 3 mo	Bronchitis	PediaSure	Bolus and drip	G button
4	Boy	14 y 8 mo	Spina bifida	Sustacal	Bolus and drip	PEG
5	Girl	3 y 11 mo	Cerebral palsy	PediaSure	Bolus and drip	G button
6	Girl	14 y 7 mo	Cerebral palsy	Ensure	Bolus	G button
7	Boy	6 y	Congenital anomalies	PediaSure	Bolus and drip	G button
8	Boy	4 y 1 mo	Prune belly	PediaSure	Bolus and drip	PEG
9	Girl	1 y 6 mo	Motor/mental retardation	Propeptide	Bolus and drip	G button

PEG, percutaneous endoscopic gastrostomy; G button, gastrostomy button.
Peptamen: Nestle Nutrition, Glendale, CA; PediaSure, Ensure: Abbott Nutrition, Columbus, OH. Sustacal no longer available. Propeptide: CNP Professional, Los Angeles, CA.

Culture Results

Twenty-seven cultures were obtained from the 9 patients (Table 2). Almost 25% of the cultures showed $>10^4$ cfu/mL. Two were contaminated at time zero when a new set and sterile formula were used. These same children with bacterial counts at the threshold at time zero had cultures below the limit at 48 hours. Two were contaminated at 24 hours, but only 1 continued to be contaminated at 48 hours. Three children had cultures above the safe threshold at 48 hours. Two of the children with bacterial counts above the threshold in the 48-hour culture showed no significant contamination on the earlier cultures (22.2%). Only 1 child showed $>10^4$ cfu/mL at 24 and 48 hours.

Four patients had no bacteria isolated on any culture (44.4%). Cultures performed at 24 hours and 48 hours showed 2 patients and 3 patients, respectively, with bacterial counts above the acceptable level of 10^4 cfu/mL. Most of the samples (78%) demonstrated that 48 hours did not increase the amount of contamination from 24 hours.

Weight

No child had more than a 5% weight loss from the baseline during the study (Table 3). Given the population of participants in this study, normal-for-age weight gain is not a reasonable nutrition goal. Therefore, weight stability or minimal loss (defined as $<5\%$) was deemed a reasonable parameter to validate stability of nutrition status. Four patients had a minor ($<3.2\%$) weight loss, 1 remained at the same weight, and 4 gained weight during the study. Two of the children with weight loss did and 2 of the children did not have cultures that were unacceptably contaminated at 48 hours. Two children vomited once during the study, but neither child had microbial growth in their cultures and neither had diarrhea during the course of the study. Medications remained stable during the data collection period, with the exception that 1 child received inhaled antibiotics after the specimen collection was completed.

Diarrhea Scores

Diarrhea scores were calculated using the Guenter scale (Table 3). The Guenter scale uses

Table 2 *Enteral set pathogen count at 3 data collection times*

Patient	Pathogens and cfu/mL at 0 h	Pathogens and cfu/mL at 24 h	Pathogens and cfu/mL at 48 h
1	*Enterobacter cloacoe* $>10^5$ *Pseudomonas fluorescens* $>10^5$	None	*E cloacoe* 6×10^3 *P fluorescens* 2×10^3
2	Diphtheroids 2.7×10^4 *P fluorescens* 7×10^3 Staph, coagulase negative 2×10^3	Staph, coagulase negative 2.1×10^4	Staph, coagulase negative 5×10^3 γ Streptococcus 4×10^3
3	γ Streptococcus 2×10^3	None	*Klebsiella pneumoniae* 2×10^4
4	None	None	None
5	None	None	None
6	None	*E cloacoe-Flavobacterium oryzihabitans* 5×10^3	*F oryzihabitans* $>10 \times 10^5$
7	None	None	None
8	None	None	None
9	None	*E cloacoe* $>10^5$ *Serratia marcenscens* $>10^5$	*E cloacoe* $>10 \times 10^5$ *S marcenscens* $>10 \times 10^5$

cfu, colony forming units.

Table 3 *Patient's clinical data*

Patient	Weight change over 3 wk	Vomiting	Guenter diarrhea score	Culture data
1	2.79% Loss		4.55	$>10^4$ at 0 h
2	1.65% Gain		1.20	$>10^4$ at 0 and 24 h
3	2.43% Loss		2.00	$>10^4$ at 48 h
4	1.25% Gain		2.10	No contamination
5	2.45% Loss	×1	0.38	No contamination
6	3.16% Loss		0.71	$>10^4$ at 48 h
7	No Change	×1	6.24	No contamination
8	1.23% Gain		3.67	No contamination
9	0.08% Gain		2.45	$>10^4$ at 24 and 48 h

quantitative and qualitative measures to assess stool output.[2] Stool output is assigned points according to volume and consistency. A score of 30 is the mean that is associated with clinical diarrhea in adults. In this study, the mean stool output score was 2.59 ± 1.91 (standard deviation [SD]). High scores did not correlate with contaminated cultures, and none of the caregivers classified even the higher scores as diarrhea. None of the Guenter diarrhea scores of these children were within the 95% confidence interval of the mean diarrhea score. Specifically, on the dates that microbial contamination was documented by cultures, the diarrhea scores were 6, 0, 6, 2, 1, 2, and 2, all well below the adult mean.

Discussion

Research with adults in hospital settings demonstrates that high levels of bacteria growth do occur in enteral feeding sets, particularly as the duration of set use is increased.[3] Research has indicated that the incidence of adult patients with microbial contamination of enteral tubes was higher in their homes than in the hospital.[4] Few studies have been conducted with children receiving enteral nutrition. Matlow and colleagues[5] found bacterial growth in 97.3% of feeding hubs of pediatric patients that they cultured (n = 37). In 78% of these patients, the same organisms were cultured in the enteral set. These researchers' concern was cross-contamination in a hospital setting. Roy et al[6] also studied contamination in the pediatric hospital setting. They conducted 2 studies and found significant contamination in 19% and 29% of the enteral sets at the end of the administration cycle (no specific times were given). There were no reports of diarrhea in any of the children studied. No increase in contamination of enteral feeding sets was found by researchers who increased set hang time from 4 hours to 8 hours in a pediatric burn hospital.[7] These children were considered immunocompromised as a result of their injuries. One study was conducted with children receiving enteral nutrition in their homes.[8] Again, the length of hang time of the enteral set was never >12 hours. The actual formula container was contaminated in 15%, the distal set tubing in 45%, and the actual gastric aspirate in 90% of the children. However, there is no convincing data that bacterial growth in enteral feeding sets causes diarrhea, vomiting, or poor weight gain in children in their homes.

In the current study, there was no relationship between the patients' demographic data (age, gender, primary diagnosis, type of formula, administration timeframes) and the results. Two of the contaminated specimens were independent of the enteral set hang time and were more likely due to poor hygiene during handwashing or the water supply to the home. Patient 1 had contaminated samples at 0 and 48 hours, but not at 24 hours. It is possible that pathogens were introduced into the system during handling. Interestingly, 1 child had contamination greater than the safe threshold at 0 and 24 hours but not at 48 hours, which could be accounted for by the natural lifespan of the microbe.

It was speculated during the study that the patients who had bolus-only feedings may have a higher incidence of contamination due to increased manipulation of the enteral feeding sets, but the results were inconclusive. Two of these 3 children did not exceed the safe standard of microbial contamination at 48 hours even though their cultures were above the standard at time zero or at 24 hours. Additionally, only 1 patient received a formula that needed reconstitution. Again, it was speculated that contamination may be increased due to this extra manipulation. This child was 1 of the 3 that had a pathogen count above the limit at 48 hours.

A limitation of the study was that while it was being initiated, the Medicaid Health Maintenance Organization of this hospital changed compensation to allow feeding bag changes from once every 48 hours to every 24 hours. All the patients of this agency were given this new allowance. It became more difficult to convince parents to let their child participate in the study because it meant more labor for the families to clean and store bags for a longer period of time. Most of the eligible subjects were chronically ill and required much care. Therefore, the sample size was smaller than expected. Another concern was that the Guenter diarrhea scale is based on adult studies. However, these pediatric data showed that even with the highest diarrhea scores, the caregivers did not report the child as

having clinical diarrhea, but instead a greater number of normal stools per given day.

Conclusion

These outcomes do not contraindicate 48-hour use of enteral feeding sets with children in their home. This study should be repeated with a larger sample size from different geographic areas. Additionally, a crossover design could be used to compare contamination after 24- and 48-hour set changes. In future studies, cultures should be collected from the distal end of the feeding set, as in this current study, and also from the actual formula container and a gastrostomy aspirate. This would give the researcher more information about the source of the contamination.

Research has been conducted indicating that closed feeding systems demonstrate a lower level of contaminates.[9] But many children require complicated nutrition that necessitates the mixing of formula and precludes the option of a closed system.

The purpose of this study was to identify the therapy that offers the best clinical and cost-effective outcome for the patient. Consideration of the environment is an important variable because the amount of waste produced with 30 set changes per month compared with 15 per month has an impact. The lessons learned from this study are that close attention must be paid to the preparation and handling of formula and the proper rinsing of enteral sets. The results of this study do not support changing practice from a 48-hour enteral set change to a 24-hour change.

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Advances and Controversies in Clinical Nutrition: The Education Outcome of a Live Continuing Medical Education Course

James S. Scolapio, MD*; John K. DiBaise, MD‡; W. Frederick Schwenk II, MD§; Mary E. Macke†; and Rosann Burdette†

**Division of Gastroenterology and Hepatology, Mayo Clinic, Jacksonville, Florida; †Mayo School of Continuing Medical Education, Jacksonville, Florida; ‡Division of Gastroenterology and Hepatology, Mayo Clinic, Scottsdale, Arizona; §Department of Pediatrics, Mayo Clinic, Rochester, Minnesota*

ABSTRACT: *Background:* The aim of this study was to assess participants' nutrition knowledge and practice behavior before and after completing a live continuing medical education (CME) nutrition course designed for practicing nutrition clinicians. *Methods:* Electronic surveys were sent to the first 100 registered participants before and after attending the course. The curriculum consisted of 16.75 hours of live education. The curriculum was revised when the precourse surveys identified a gap in medical knowledge or practice behavior. Knowledge change was assessed by a 15-question survey given before and 1 week after the course. Change in practice behavior was accessed by a 10-question survey administered 2 months after the course. *Results:* Dietitians were the predominant discipline group attending the course. Sixty-three percent of those surveyed practiced hospital nutrition, 19% outpatient nutrition, and 18% an equal mix. Forty-eight percent indicated that they write parenteral nutrition (PN) orders and 51% write enteral nutrition (EN) orders; of these, 62% indicated they are comfortable writing PN orders and 81% are comfortable writing EN orders. Twenty-three percent indicated that they manage home PN and EN patients. Twenty-six percent stated they were certified in nutrition support. Seventy-eight percent of the participants responded to survey 2; the median correct response rates were 51% pre- and 76% postcourse. Seventy percent responded to survey 3; the median positive clinical practice behavior change was 69%. *Conclusion:* This live CME course improved knowledge, and a majority of attendants reported changing their nutrition practice after this course.

The Mayo School of Continuing Medical Education held its first live continuing medical education (CME) nutrition course in 1990. This course has been cosponsored with the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) since 1996. In April 2007, the 17th Annual *Advances and Controversies in Clinical Nutrition* course was completed in Savannah, Georgia. Over the past 17 years, an estimated 3000 clinicians have been educated as part of this course.

In this report, we describe how the curriculum was constructed, and present results of prospectively collected outcome data used to improve the course as it was developed. Pre- and postcourse surveys were used to determine the degree of knowledge improvement and behavior change after completing the course. To the best of our knowledge, this is the first report that describes how outcome data were collected for a live CME nutrition course, and to show improved knowledge and practice behavior after practitioners attended a nutrition course.

Materials and Methods

Curriculum and Course Design

During a 4-month period before this course, a program-planning committee communicated ideas for educational objectives (Table 1), content, and program design *via* e-mail and teleconferences. Topics were selected according to the course directors' knowledge of needs assessments from previous years' courses, other educational nutrition programs in which they had been involved, recently published topics, and common nutrition health concerns in daily clinical practice (Table 2). The curriculum consisted of 20- to 30-minute didactic lectures and small-group workshops. The course was advertised in multiple mailing lists that targeted dietitians, pharmacists, physicians, and nurses in the United States, Puerto Rico, and Canada.

Course faculty was selected according to their field of expertise, teaching experience, and speaking skills. Twenty-five faculty members participated in this 3-and-a-half-day course that offered 16.75 category 1 credits for physicians, dietitians, pharmacists, and nurses. The education curriculum included clinical case presentations, workshops,

Correspondence: James S. Scolapio, MD, Professor of Medicine and Associate Dean of CME, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224. Electronic mail may be sent to scolapio.james@mayo.edu.

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Table 1
Education objectives

1. Identify methods to feed patients with acute pancreatitis.
2. Recognize potential use of pre- and probiotics.
3. Use strategies to improve safety outcomes on patient discharge with enteral nutrition (EN).
4. Tabulate accurate parenteral nutrition (PN) calculations and adjustments.
5. Analyze dietary modifications in patients with renal stones.
6. Apply new information in the management of obesity.
7. Calculate glucose levels in hospitalized patients.

didactic lectures, and panel discussions. An audience response system (ARS) was used to elicit participant interaction during the course. ARS is a computer-based technology in which participants can enter their responses to questions *via* a remote keypad located at their desk. The results for the group are then instantly tabulated and projected onto a screen for immediate participant or learner feedback.

This study was approved by the Mayo Clinic Institutional Review Board.

Precourse and Postcourse Surveys

Two precourse surveys were e-mailed to the first 100 participants registered 3 weeks before the course using a Zoomerang survey tool (Market Tools Inc., San Francisco, CA). The first survey had 22 questions that assessed the participant's nutrition experience at their institutions and their specific educational needs (Table 3). Specific questions were used to determine the method in which they learn best, as well as the participants' specific educational goals for attending this course.

The second precourse survey included 15 questions used to determine each participant's nutrition knowledge (Table 4); these questions were developed from the educational objectives and planned topics of the course. Results were reviewed by the course directors and shared with faculty members before the course. Course content was adjusted according to the survey results to address identified deficiencies. For example, participants indicated that they wanted more information on nutrition certification. Brochures were obtained from A.S.P.E.N. and distributed to the participants. Participants also indicated that they wanted up-to-date information on specific topics. Each faculty member was asked to include an "update" slide in their presentations that highlighted recent information related to their specific topic. One week after attending the course, the same 15-question survey was e-mailed to participants to determine the change in their knowledge.

Each participant was asked to complete a course evaluation on the last day of the course. This evaluation ranked the quality of each faculty presentation and the overall course content. The following scoring system was used: 1 (very poor), 2 (poor), 3 (average), 4 (good), and 5 (excellent).

Two months after completing the course, a 10-question survey was e-mailed to each participant to determine whether they had changed their nutrition practice behavior as a result of attending this course (Table 5). Behavior changes were determined by participants' personal feedback and were not objectively measured.

Results

Nutrition Experience and Education Preferences

Eighty-three of the 100 participants surveyed responded to survey 1. Of these participants, 58 were dietitians, 11 physicians, 7 pharmacists, 6 nurses, and 1 physician assistant. Sixty-two percent practiced predominantly inpatient nutrition, 19% practiced predominantly outpatient nutrition, and 19% an equal mix of inpatient and outpatient nutrition. Forty-nine percent had a nutrition support team at their institution run predominantly by dietitians. Thirty-five percent indicated that they had a physician leader as part of their nutrition support team. Forty-one percent of the participants indicated that they were active members of the nutrition support team. Forty-nine percent indicated that they write orders for parenteral nutrition (PN) and 54% for enteral nutrition (EN). Of these, 63% indi-

Table 2
Course topics

1. Nutrition support of pancreatitis
2. Pre- and probiotics: application to intestinal disease
3. Fructose intolerance: gastrointestinal (GI) symptoms
4. Nutrition support of inflammatory bowel disease and short bowel
5. Nutrition support of liver transplant patients and fatty liver disease
6. Transition of enteral nutrition from hospital to home: National Patient Safety Guidelines
7. Neuropsychiatry disorders caused by cobalamin deficiency
8. Protein delivery in cirrhotics
9. Parenteral nutrition (PN) calculations and tailoring*
10. Dietary management of renal stones
11. Information technology and nutrition
12. Nutritional rickets
13. Food allergy
14. Drug-nutrient interactions
15. Dispensing from physician order to bedside: National Patient Safety Guidelines
16. Infants with poor weight gain
17. Childhood obesity
18. Ethics of feeding during end of life
19. Dietary, behavioral, and surgical management of obesity
20. Metabolic syndrome
21. Dietary management of diabetes

*Workshop topic.

Table 3
Precourse survey 1 (nutrition experience)

1. What is your discipline?
2. Do you practice predominantly inpatient or outpatient nutrition?
3. What is your primary area of nutrition practice in the outpatient clinic?
4. Do you have a nutrition support team at your hospital?
5. If yes to question #4, which department runs your team?
6. Are you an active member of your nutrition support team?
7. Do you have a physician leader as part of your team?
8. Do "you" write orders for parenteral nutrition administration at your hospital?
9. Do "you" write orders for enteral nutrition administration at your institution?
10. Do you believe that, at this moment, you are comfortable calculating a parenteral nutrition formula and safely administering it to a patient?
11. Do you believe that, at this moment, you are comfortable calculating an enteral formula and safely administering it to a patient?
12. Are you certified in nutrition support, diabetes, or obesity?
13. Do you directly manage any home parenteral nutrition (HPN) patients?
14. Do you directly manage any home enterally tube-fed patients?
15. Are you or have you been involved in any nutrition research projects?
16. Are you directly involved in the nutrition management of patients with obesity?
17. Are you directly involved in the nutrition management of any pediatric patients?
18. Are you directly involved in the nutrition management of patients with diabetes?
19. Do you believe at this moment you are comfortable with the nutrition management of patients with diabetes?
20. Do believe at this moment you are comfortable with the nutrition management of patients with obesity?
21. What are your educational goals/needs of this course (ie, what would you most like to learn)?
22. How do you believe you learn best at an education course (ie, podium lectures, reading syllabus material on your own, small group discussions, one-on-one communication with the teacher, panel discussions, other)?

cated they were comfortable calculating a PN formula and safely administering it to a patient, and 84% indicated that they were comfortable calculating an EN formula and safely administering it to a patient. Twenty-three percent directly manage home PN patients and 24% manage home EN patients. Sixty-four percent were directly involved in the nutrition management of patients with obesity, 32% with pediatric patients, and 75% with patients with diabetes. Seventy-six percent stated they were comfortable in the nutrition management of patients with diabetes and 71% in the management of patients with obesity. Twenty-eight percent indicated they were certified in nutrition support, diabetes, or obesity. See data listed in Table 6.

Eighty-four percent indicated they learn best from podium lectures, 42% from panel discussions, and 36% from small group discussions. Participants' education goals included, but were not exclusive of, nutrition as it relates to gastrointestinal illness, PN and EN calculations, current updates and developments in clinical nutrition, current treatments for obesity and diabetes treatments, and preparation for nutrition certification.

Knowledge Evaluation

The knowledge surveys were taken before and after attending the course. The survey response rate was 77% before and 78% after the course. Median correct response rates were 51% before the course and 76% 1 week after the course (Table 4).

Course Evaluation

One hundred thirty-five participants (78%) of the 172 actual attendees completed faculty and topic evaluations on the last day of the course. Of the 172 attendees, there were 105 registered dietitians, 23 physicians, 14 pharmacists, 12 nurses, and 18 other disciplines. Course topics were the primary reason why participants indicated they elected to attend this course. The overall course rating was 4.81 (5.0-point scale). The mean faculty evaluation scores for presentations were 4.55 (range, 3.80–4.86). The mean topic pertinence score was 4.59 (range, 4.11–4.76). The participants indicated that the educational objectives of the course were 89% accomplished or fully met. Ninety-nine percent of the participants indicated that they thought the audience response system was good to excellent, and 95% indicated they had a good to excellent opportunity to ask the faculty questions. Eighty-four percent of the participants indicated that they had a 75% or greater confidence that they would take what they had learned from this course and apply it their clinical practice. More focus on the nutrition recertification examination and PN calculations was a predominant suggestion for future topics.

Changes in Nutrition Practice Behavior

Seventy participants (70%) responded to the survey monitoring practice behavior that was sent 2 months after completion of the course. This survey was sent to the original 100 registrants (refer to Table 5). After attending this course, 60% had recommended or ordered EN instead of PN in a patient with acute pancreatitis. Sixty-nine percent indicated that they considered or recommended probiotics in patients with diarrhea. Seventy-three percent indicated that they were now more comfortable in the calculation and adjustment of a PN formula, and 67% thought their potential errors in PN prescribing had decreased. Eighty-two percent indicated they

Table 4
Precourse and postcourse knowledge assessment survey

	Precourse (% correct)	Postcourse (% correct)
1. Clinical studies have shown that glutamine increases nutrient absorption in patients with short bowel syndrome. (False)	35	35
2. Fructose has a low glycemic index. (True)	42	82
3. What is the minimum time Medicare requires for HPN use? (90 days)	51	76
4. Probiotics have been demonstrated to have strain-specific benefits in which of the following conditions? (pediatric diarrhea, antibiotic-induced diarrhea, pouchitis)	60	92
5. Enteral nutrition improves survival compared with parenteral nutrition in those patients with severe acute pancreatitis. (False)	39	33
6. Well-controlled, randomized trials have shown that weight loss is an effective therapy for nonalcoholic fatty liver disease. (False)	29	39
7. Deficiency of which trace element can result in cardiomyopathy? (Selenium)	54	86
8. Aromatic amino acids have been shown to decrease the severity of hepatic encephalopathy. (False)	81	83
9. Which of the following drugs are known to reduce serum folic acid levels? (Dilantin and sulfasalazine)	79	88
10. Which of the following diet measures is useful for patients with calcium oxalate renal stones and enteric hyperoxaluria? (Increased calcium intake)	29	76
11. The concentration of vitamin D in breast milk of unsupplemented mothers is approximately . . . (25 IU/L)	49	80
12. If a patient weighs 70 kg, how many calories would you administer in parenteral nutrition? (1750)	86	89
13. Which BMI is an indication for obesity surgery in patients "without" obesity-related comorbid illness? (40)	74	75
14. There are strong data to suggest that increased intake of whole grains and dietary fiber reduces the risk of diabetes. (False)	40	46
15. Lap banding will typically result in the same degree of weight loss as Roux-en-Y gastric bypass surgery. (False)	67	76

The same 15 questions were used in the precourse and postcourse knowledge surveys. Answers to each question are listed parenthetically. Percentages indicate the proportion of participants who provided correct answers.
BMI, body mass index; HPN, home parenteral nutrition.

thought their dietary instructions for renal stones had improved, and 63% indicated they are now better at keeping serum glucose levels of their hospitalized patients in the acceptable range. Eight-two percent indicated that they now consider fructose intolerance as a cause of bloating and diarrhea in their patients. Seventy-one percent indicated they are now more comfortable discussing "end-of-life feeding" with patients, families, and colleagues.

Examples of improved practice behavior are shown by the following responses: "I was able to inform a physician who ordered a low-calcium diet for a patient with kidney stones that it was not needed," "I've suggested gastric banding as an alternative to surgical gastric bypass in an obese patient," "I have definitely been recommending better glucose control especially in intensive care unit (ICU) patients," "I'm more precise in estimating my patients' caloric and protein needs," "I'm not restricting protein in liver patients as much as I used to," and "I'm more aggressive in using EN in patients with severe acute pancreatitis."

Discussion

Although live CME courses have become a very popular method for nutrition clinicians to stay current with advances in their specialty and maintain licensure requirements, few courses actively collect outcome data of participants' knowledge and practice behavior changes after the education event. Although one may assume that knowledge increases and practice behavior improves after a CME course, these data have never been reported, to the best of our knowledge. We hope that this manuscript provides the methods needed to collect learner outcome data and the importance of this information.

In the 1970s, the primary focus of nutrition CME courses was that of compounding and administration of PN. Continuing medical education courses have now shifted their focus in more recent years to obesity topics, given the current obesity epidemic in the United States, and the controversies and indications of specialized enteral formulas in specific disease states.[1] The article by Kahn[1] provides examples of CME programs that are available in the United States specific to nutrition.

Table 5
Nutrition practice behavior survey post course

1. As a result of attending this course, have you recommended or ordered enteral nutrition *vs* parenteral nutrition (PN) in patients with acute pancreatitis?
2. As a result of attending this course, do you now consider or have you recommended "pre- or probiotics" in a patient with diarrhea?
3. As a result of attending this course, do you feel more comfortable in the calculation and adjustment of a patient's PN formulas?
4. As a result of attending this course, do you believe your potential errors in PN prescribing have decreased?
5. As a result of attending this course, do you believe your dietary instructions to patients with renal stones improved?
6. As result of attending this course, are you now better at keeping serum glucose levels of your hospitalized patients in the acceptable range?
7. As a result of attending this course, do you now consider fructose intolerance as a cause of bloating and diarrhea in your patients?
8. As a result of attending this course, do you now consider and are you more comfortable discussing "end-of-life feeding" with patients, their families, and colleagues?
9. As a result of attending this course, have you applied new information that you have learned in the management of an obese patient?
10. Please give an example of how your practice behavior has changed after attending this course.

As shown in our data, dietitians are the predominant nutrition healthcare providers that attend nutrition CME courses. This fact is also reflected in the membership of A.S.P.E.N. In the authors' opinion, fewer physicians are being trained in nutrition and fewer are attending nutrition CME courses compared with dietitians and pharmacists. Reasons stated by gastroenterology trainees for not pursing specialization in nutrition include too few mentors, poor exposure, it is considered an allied health specialty, is not endoscopy focused, and poor financial reimbursement.[2,3] We suspect that most hospitals are also deficient in providing the necessary nutrition education for their employees. Therefore, in most instances, nutrition clinicians must obtain their ongoing education by attending live CME courses or through journal reading. Although we acknowledge that journal reading can be a great source of education and perhaps may be more cost-effective for the participant, we believe that the live interaction between learner and teacher is invaluable.[4] Studies would suggest that multimedia teaching methods enhance learning more so than 1-dimensional teaching.[5,6] As shown in our data, participants indicated that they learn best in a variety of ways, including traditional podium lectures, small group discussions, and panel question-and-answer sessions. Our results would also indicate that the participants found the keypad ARS to be very helpful. Using this teaching method, participants were required to think and process educational information before responding. The ARS also enabled the participants to see how their responses compared with those of other course participants. The moderator of each session could then focus the discussion phase of the course on the recognized knowledge deficiency identified by the ARS. Although we do not have data to support that the ARS directly increases knowledge retention or behavior change, we would suggest by virtue of adding another piece of interactive teaching that it may.[5,6]

Table 6
Nutrition experience of attendees

Inpatient predominant	62%
Outpatient predominant	19%
Combined inpatient and outpatient	19%
Nutrition support team (yes)	49%
Active team member (yes)	41%
Physician leader on team (yes)	35%
Write parenteral (PN) orders	49%
Write enteral nutrition (EN)	54%
Comfortable writing PN orders (yes)	63%
Comfortable writing EN orders (yes)	84%
Manage home PN patients	23%
Manage home EN patients	24%
Manage obese patients	64%
Manage pediatric patients	32%
Manage diabetic patients	75%
Certified in nutrition support	28%

Eighty-three of the 100 participants surveyed responded to survey 1. Of these participants, 58 were dietitians, 11 were physicians, 7 were pharmacists, 6 were nurses, and 1 was a physician assistant.

The results of the course have also been written to show that a CME course can have positive outcome on a learner, and the importance and method for collecting this information. We believe the methods used in this study can be applied to other CME courses as well. The Accreditation Council for Continuing Medical Education (ACCME) encourages the evaluation and collection of level 1–4 outcomes.[7] Level 1 is the collection of course evaluations or participant feedback, including speaker evaluations. We suspect that most traditional CME courses collect level 1 data, but the actual application of this information may vary. Level 2 outcome refers to the evaluation of knowledge before and after attending an education course. We believe that most CME courses do not collect this information and course directors may assume the participant has learned what the lecturer has presented. This may not necessarily be the case. We would encourage that a set of questions be given to participants before attending a CME course, and then at some time interval after the course. The same questions or preferably different questions with the same concept can be administered to determine if there has been a change in knowledge. The time interval of when to

have participants answer the postlecture questions is not well established in the education literature.

Level 3 outcome refers to the participant's change in practice behavior after attending an education course. As highlighted in this paper, pre- and postcourse electronic survey data collection can help determine if the CME course actually resulted in improved practice behavior. An example of level 3 outcome gained from this course is that the participants reported being more aggressive in the control of serum glucose levels in hospitalized patients than before attending the course. It is difficult to objectively measure this outcome, and we therefore had to rely on each participant's opinion. Level 4 outcome data refers to improved patient outcomes as a result of a clinician attending a CME course. Although this is the ultimate goal of an education course, it is more difficult information to ascertain. Methods to ascertain this information may include patient surveys, chart reviews, or having participants provide specific examples of improved patient outcome that can be directly linked to knowledge they had acquired at a CME course. Future courses should explore creative ways to collect this information.

As shown in our Methods section, electronic survey tools are a valuable instrument to communicate with participants before and after a course. As we have illustrated, the education content of a course can be adjusted or tailored to fit the education needs or objectives of the real-time learner.[8] Traditionally, CME courses have relied on the previous year's course evaluations to plan the following year's course. Unless the same participants attend each year, what is being taught at a current year's course may not necessary reflect the participants' needs or knowledge deficiencies. A great opportunity to positively affect education can be missed if the needs of the learner are not known before the course.

In conclusion, CME courses are important in the ongoing education of nutrition clinicians, and it is imperative that course directors collect learner outcome data to determine if in fact learning and positive practice behavior changes are occurring as a direct result of the education curriculum. This manuscript provides methods that can be used to collect such information. Our course curriculum resulted in improved knowledge and practice behavior of our CME participants.

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Book Review

Nutrition in Promoting the Public's Health: Strategies, Principles, and Practice

Mildred Kaufman. Jones & Bartlett, Boston, MA, 2007, 617 pages, $74.95, ISBN 0-7367-2840-3

This extremely readable text is intended to introduce the concepts of public health nutrition to a student audience. Its 25 chapters cover needs assessment, program development, funding, project and personnel management, and policy initiatives in language that is easy to understand, with a minimum of jargon. It is dotted with tables and diagrams that are simple to comprehend, and each chapter is accompanied by a summary, a bibliography, and suggested readings.

One identifiable problem in this book is the lack of a glossary. Although jargon has been held to a minimum, the chapter authors use some terms that are not commonly known among healthcare workers who do not specialize in public health, such as *public charge*. Definitions of these terms would have been helpful either in a global glossary for the volume or in a short list for the chapter in which they appear.

The second difficulty I noticed is that the book assumes no knowledge base in anything—nutrition, civics, policy, research—perhaps because there is no identified position for public health nutrition in the curriculum sequence. This requires that the text take a superficial look at 2 dozen topics, rather than allowing any depth in any one. A text with chapters on subjects as wide-ranging as evidence-based practice, marketing, personnel management, and legislation makes it difficult for the reader to discover the take-home points for the book as a whole.

There were some areas that were underdeveloped, perhaps because of the lack of focus. Discussion of health disparities and food insecurity was extremely brief, and no attempt was made to describe the role of healthcare providers in alleviating the causes of these social problems.

However, with these caveats, I would recommend the text to professionals whose education did not include an introduction to population-focused health strategies. It is a wide-ranging description of the role of nutrition in population-based medicine.

Jane Harper, PhD(c), RN, APN
St. Anthony College of Nursing
Rockford, Illinois

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Invited Review–Articles about a specific clinical subject that review pertinent literature. These articles are well-referenced and designed to bring the reader up to date on the subject, and provide a scientific basis for practices in the area.

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Techniques and Procedures–"How-to-do-it" contributions by practitioners. This section should include descriptions of certain procedures, treatments, or other aspects of managing patients receiving nutrition support.

Pivotal Paper–A review of a previously published pivotal article. The first page of the original article will be reprinted as the second page of this article. The rest of the article should be comprised of 4 sections: *Prevailing Belief System* – What were the prevailing common beliefs and practices of the nutrition community at the time the original paper was published; *Unique Scientific Contribution* – What scientific information was imparted by this paper that changed thinking and practices; *Validation* – What studies have subsequently supported or contradicted the findings of this paper and how has the information held up over time; *Future Considerations* – Are the concepts established by this paper likely to be challenged in the future by new issues, developments, modifications or change in thinking.

Clinical Controversies–A review of management in an area that is in dispute. There should be at least 2 opposing views on management of a particular clinical problem, and each view should be defensible from the literature.

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Nutrition in Clinical Practice

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NCP Peer-reviewed, practical solutions in clinical nutrition

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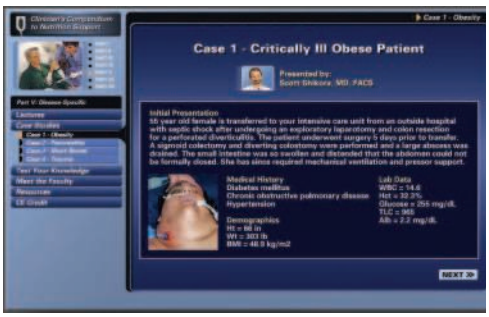

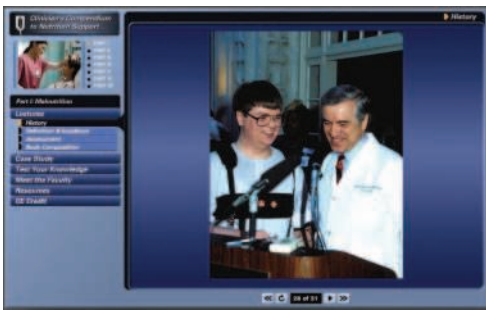

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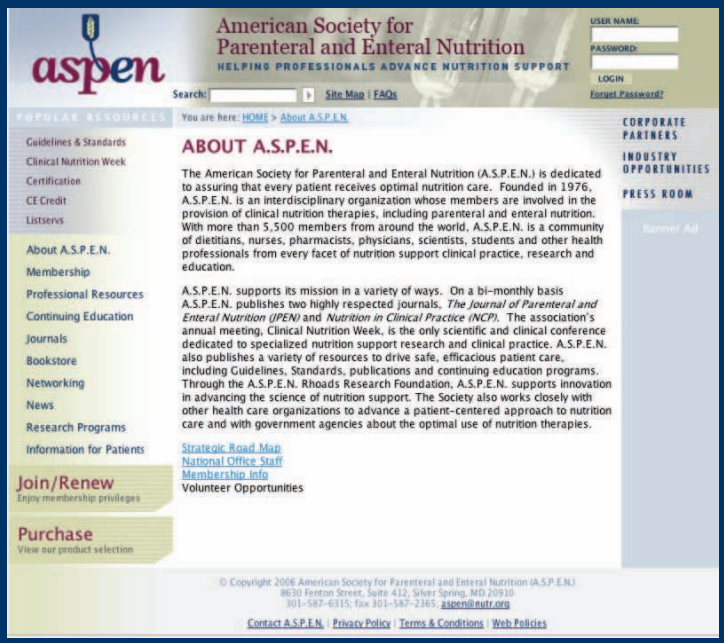

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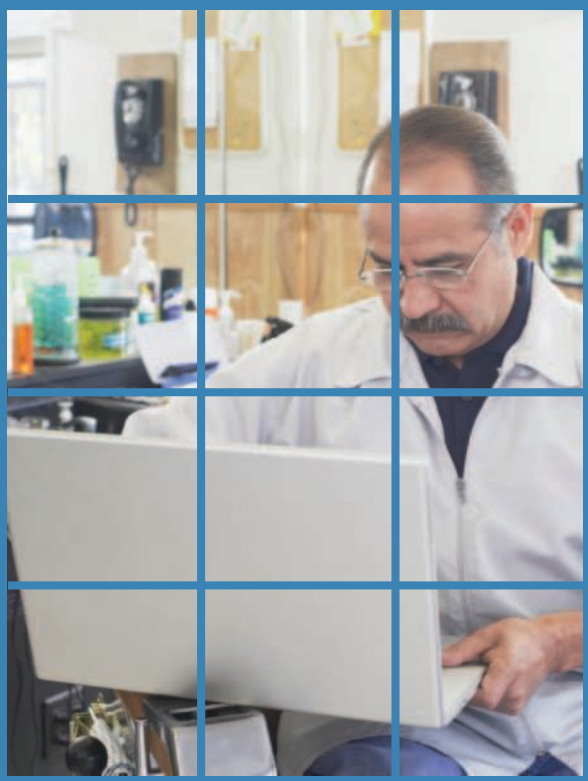

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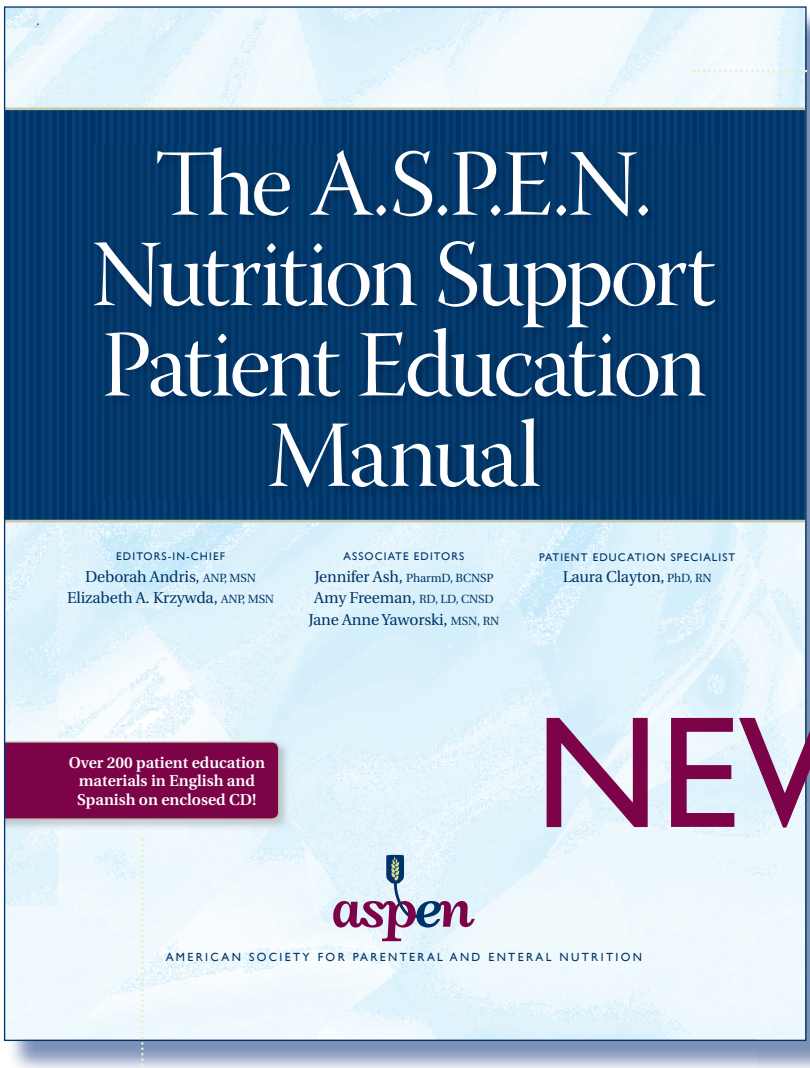

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Editors-in-Chief: Deborah Andris, ANP, MSN
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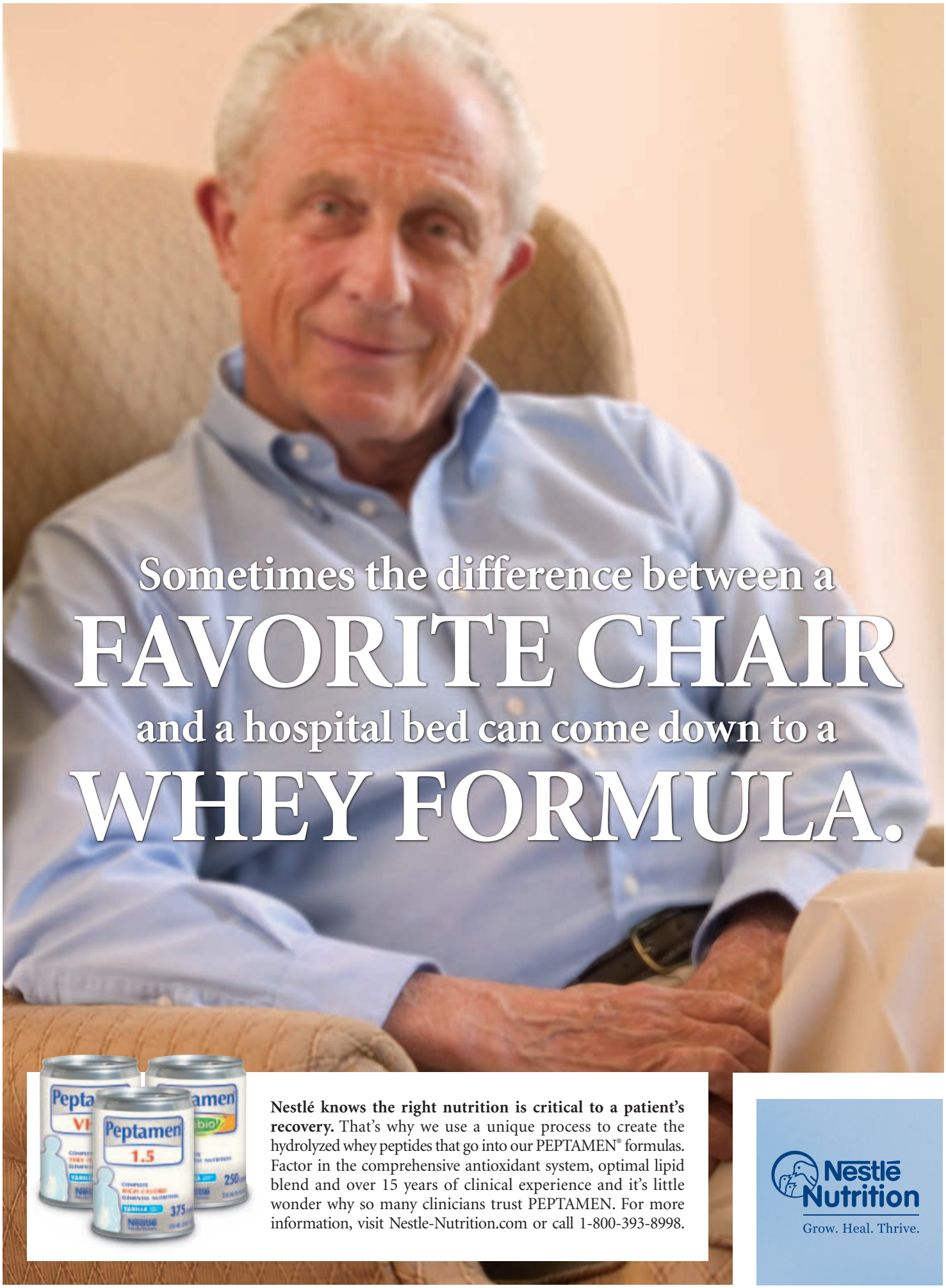

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