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Review

Metabolic endotoxemia with obesity: Is it real and is it relevant?

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ABSTRACT

Obesity is associated with metabolic derangements in multiple tissues, which contribute to the progression of insulin resistance and the metabolic syndrome. The underlying stimulus for these metabolic derangements in obesity are not fully elucidated, however recent evidence in rodents and humans suggests that systemic, low level elevations of gut derived endotoxin (lipopolysaccharide, LPS) may play an important role in obesity related, whole-body and tissue specific metabolic perturbations. LPS initiates a well-characterized signaling cascade that elicits many pro- and anti-inflammatory pathways when bound to its receptor, Toll-Like Receptor 4 (TLR4). Low-grade elevation in plasma LPS has been termed "metabolic endotoxemia" and this state is associated with a heightened pro-inflammatory and oxidant environment often observed in obesity. Given the role of inflammatory and oxidative stress in the etiology of obesity related cardio-metabolic disease risk, it has been suggested that metabolic endotoxemia may serve a key mediator of metabolic derangements observed in obesity. This review provides supporting evidence of mechanistic associations with cell and animal models, and provides complimentary evidence of the clinical relevance of metabolic endotoxemia in obesity as it relates to inflammation and metabolic derangements in humans. Discrepancies with endotoxin detection are considered, and an alternate method of reporting metabolic endotoxemia is recommended until a standardized measurement protocol is set forth.

1. Introduction

Endotoxins are large, heat stable lipopolysaccharides (LPS), which are the major glycolipid component of the outer membrane of gram-negative bacteria [1] that comprise approximately 70% of the total bacteria in the gut [2]. Endotoxin can enter the blood by either local or systemic infection by exogenous gram-negative bacteria, through paracellular absorption following bacterial cell lysis of endogenous gram-negative bacteria in the gut, and through transcellular (via chlyomicrons) transport of endogenous endotoxin following diurnal feeding patterns [3,4]. LPS contains a pathogen-associated molecular pattern, Lipid A, which initiates a signaling cascade resulting in activation of various pro-inflammatory pathways and increases oxidative stress upon binding to its pattern recognition receptor, Toll-like receptor 4 (TLR4) [4–8]. TLR4 resides on the cell surface of monocytes, other immune cells, and various other cell types (e.g., skeletal muscle, adipose tissue, and liver) [9–11].

Bacterial infections are the leading cause of sepsis, of which gram-negative bacterial infections account for of the 45–60% of cases [12,13]. In patients with sepsis, the concentration of circulating endotoxin is often elevated a hundred fold or higher compared to age-matched, healthy controls (e.g., 581 ± 49 vs. 5.1 ± 7.3 pg/mL, respectively) [14]. The elevation in endotoxin in bacterial infection (endotoxemia) results in mass overproduction of pro-inflammatory cytokines, which can lead to shock, cell damage, and potentially multiple organ failure [15]. Conversely, Cani et al.

Abbreviations: LPS, lipopolysaccharide; TLR4, Toll-Like Receptor 4; T2D, Type II Diabetes; GLP2, Glucagon Like Peptide 2; eCB, endocannabinoid; ROS, reactive oxygen species; CD, cluster of differentiation; IKK, IκB kinase; NAC, n-acetyl cysteine; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; LBP, LPS binding protein; Si, insulin sensitivity; HOMA-IR, homeostatic model assessment of insulin resistance; RYGB, Roux-en-Y gastric bypass surgery; LAL, Limulus amebocyte lysate; EU, endotoxin unit.

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[4] described "metabolic endotoxemia" as a condition of chronically elevated plasma LPS at levels 10–50 times lower than during septic conditions. Metabolic endotoxemia was observed in genetically obese (ob/ob) mice consuming normal chow, and could be induced in lean mice (C57bl6/J) consuming an obesogenic diet [4,16]. Diet induced elevations in endotoxin were related to increased fat deposition, heighten pro-inflammatory and oxidative pathways and insulin resistance [4,16], and these perturbations could be partially or completely abrogated with antibiotic treatment [4,16]. Thus, these findings were the first to indicate gut microbial endotoxin as a mediating source of a heightened pro-inflammatory milieu in obese rodent models [16].

Approximately 65% of US adults and >100 billion people worldwide are overweight or obese [17,18]. Accompanying obesity is a state of chronic low-grade inflammation, characterized by elevated systemic and local pro-inflammatory cytokines and acute phase proteins [19]. Heightened activation of the immune system in obesity plays a role in the development of the Type II diabetes (T2D), the metabolic syndrome, and cardiovascular disease [20,21]. Importantly, cardiovascular disease and T2D are the 1st and 7th leading cause of death in the United States, respectively [22,23].

Local adipose tissue inflammation and the secretion of a plethora of pro-inflammatory adipokines from visceral adipose tissue is indicated in the etiology of cardio-metabolic disease development during obesity [24]. However, the finding of Cani and colleagues, in association with recent evidence in humans, has lead to the hypothesis that metabolic endotoxemia may also participate in low-grade inflammation and the development of cardio-metabolic disease in obesity [25–27]. The present review will provide an overview of the evidence of the existence, consequences, and clinical relevance of metabolic endotoxemia in human obesity, and supports mechanistic associations with cell and rodent experiments. In addition, issues of endotoxin detection as it relates to measuring and reporting metabolic endotoxemia concentrations in the literature are discussed.

2. Metabolic endotoxemia and obesity: evidence in rodents

2.1. Metabolic endotoxemia and its involvement in obesity, inflammation, and insulin resistance

It is important to note that the influence of bacterial endotoxin on metabolism has been studied for nearly a century [28,29]. Initial studies explored the effects of lethal doses of endotoxin on metabolism, while later studies evolved to explore similar metabolic outcomes with sub-lethal concentrations of endotoxin or during bacterial sepsis [30–33]. More recently, the influence of chronically elevated plasma LPS, at levels 10–50 times lower than during septic conditions, on metabolism has been characterized and termed, "metabolic endotoxemia" [4].

In a series of studies, Cani et al. showed that chronic, modest elevations (~1.5 fold) in endotoxin could be induced in lean mice (C57bl6/J) consuming a high-fat (72% of total calories)/high energy diet for four weeks or in genetically obese (ob/ob) mice consuming normal chow [4,16]. In these studies, the diet induced elevations in endotoxin were related to increased fat deposition, systemic and tissue specific inflammation (e.g., liver, skeletal muscle, and adipose tissue) and insulin resistance [4,16].

The role of endotoxin as a mediator of adipose tissue development, systemic and local inflammatory processes and metabolic derangements was confirmed through low dose LPS (300 μg/kg/day) injection in lean mice on a normal chow diet [4]. Injection of 300 μg/kg/day of LPS in lean mice elicited similar derangements of diet induced obesity, however these mice developed slightly less glucose intolerance compared to mice consuming an obesogenic diet. Furthermore, lean mutants lacking the crucial LPS co-receptor, cluster of differentiation (CD) 14, were resistant to high fat diet induced weight gain, tissue specific inflammation, hepatic lipid deposition, and insulin resistance, therefore indicating TLR4 activation via LPS as a mediating event in high fat diet induced inflammation and metabolic derangements [4].

Follow-up studies in lean mice (C57bl6/J) showed that consuming either high-carbohydrate (37% of total calories)/high-energy or high-fat (72% of total calories)/high energy diets for four weeks lead to significant increases in circulating plasma endotoxin compared to mice consuming an isocaloric control diet for the same duration [34]. However, the increase in plasma endotoxin was significantly greater in the high-fat/high-energy diet group compared to the high-carbohydrate/high-energy group (~2.5 fold increase vs. ~1.5 fold increase, respectively) indicating that both dietary composition and energy intake influence the magnitude of elevation in circulating endotoxin.

2.1.1. The gut microbiota serves as the link between high fat feeding, endotoxin, and inflammation

Under normal physiological conditions, the gut microbiota promotes gut barrier function through a glucagon like peptide (GLP) 2 dependent mechanism [35]. However, a high fat diet can unfavorably alter the gut microbial composition, leading to increased intestinal permeability, as evidenced by less abundant and disorganized tight junction proteins, zonulin and occludin in the colon [35]. The gut microbiota dependent mechanisms mediating increased intestinal permeability are not fully elucidated, however a reduction in *Bifidobacterium*spp. and overactivation of the endocannabinoid (eCB) system seem to play an important role [16,35,36]. Furthermore, LPS, which is elevated due to increased intestinal permeability, can increase intestinal permeability and the peripheral eCB tone, thus completing a damaging positive feedback pathway [36]. Administration of antibiotics or prebiotics to genetically (ob/ob) or diet induced obese mouse models leads to a reduction in intestinal permeability and circulating plasma endotoxin [16,35,36]. Notably, selective modulation of the gut microbiota with prebiotics has been shown to decrease the mRNA expression of the eCB receptor, CB_1 in the colon; decrease the eCB agonists, anandamine and 2-aracidonoylglycerol; decrease the expression of eCB agonist inhibitor, fatty acid amide hydrolase; and increase the endogenous production of the intestinotrophic, GLP2 in mice administered a high fat diet [35,36]. Together the improvements in gut barrier function reduce circulating LPS, inflammation, and metabolic derangements; thus indicating that changes in the gut microbiota mediate metabolic endotoxemia and systemic and local inflammation.

2.2. Metabolic endotoxemia and altered adipose, liver and skeletal muscle metabolism

Endotoxin from LPS can initiate systemic and local inflammation and also result in reactive oxygen species (ROS) production upon binding with TLR4 and subsequent activation of NFκB [6–8]. TLR4 is abundant on immune cells, liver, adipose tissue, and skeletal muscle [9–11]. Collectively, these tissues play an important role in the regulation of glucose and lipid homeostasis, and it has been demonstrated that pro-inflammatory cytokines and ROS production interfere with normal metabolism in these tissues [37–40]. For instance, Cani et al. [35] have reported increased expression of pro-inflammatory (e.g., PAI-1 TNFa, IL6, IL-1), oxidative stress (NADPHox, iNOS) and macrophage infiltration markers (CD86) in liver tissue of genetically obese mice with metabolic endotoxemia. In addition, increased expression of pro-inflammatory markers have been observed in visceral and subcutaneous adipose tissue of lean