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Revisión

Role of metabolic endotoxemia in insulin resistance and obesity. The Buffering Efficiency Hypothesis

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Abstract

Recent studies have shown that elevated concentrations of plasma lipopolysaccharide (LPS) constitute a metabolic mechanism enough for triggering insulin resistance, obesity and type 2 diabetes in animal models, and that high fat diets lead to increased plasma LPS concentrations through changes in the gut flora. We review here the LPS effects in metabolic processes *in vitro*. In humans, an altered innate immune system has also been associated with metabolic disorders such as insulin resistance, high endotoxemia markers (LBP, sCD14) and low LPS-neutralizing proteins (adiponectin, bactericidal permeability-increasing protein, α-defensins and lactoferrin). In fact, insulin resistance is well known to be associated with inflammation, with a decrease in innate immune efficiency and a reduction in the production of antimicrobial proteins. In this revision, we propose a new view according to which buffering efficiency of the innate immune system could prevent LPS-induced metabolic diseases.

Keywords: metabolic endotoxemia, endotoxemia markers, LPS-neutralizing proteins, insulin resistance, obesity.

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Abbreviations:

ADIPOQ: Adiponectin; AGEs: Advanced Glycation End-Products; BPI: Bactericidal/increasing protein permeability; CD14, CD14 molecule; DEFA1-3: Human α-Defensins; FABP4: Fatty Acid Binding Protein 4; GPD1: Glycerol-3-phosphate Dehydrogenase 1 (soluble); GPI-linked protein CD14: Glycosylphosphatidylinositol-linked protein CD14; IKKβ: Inhibitor of Nuclear Factor kappa B Kinase beta subunit; IL-6: Interleukin-6; IL-1β: Interleukin 1-β; LBP: Lipopolysaccharide Binding Protein; LPL: Lipoprotein Lipase; LPS: Bacterial Lipopolysaccharide or Endotoxin; LTF: Lactoferrin; MD-2: Myeloid Differentiation Protein-2; NF-κB: Nuclear Factor kappa-B DNA binding subunit; PAMP: Pathogen associated Molecular Patterns; PKB: RAC-alpha serine/threonine-protein kinase; PI3K: Phosphatidylinositol 3-kinase; sTNFR1: Soluble tumor Necrosis Factor Receptor 1; sTNFR2: Soluble tumor Necrosis Factor Receptor 2; PPARγ: Peroxisome proliferator activated receptor gamma; TLR4: Toll-like Receptor 4;TNF-α: Tumor Necrosis Factor α.

Introduction

Obesity is well known to be associated with a cluster of metabolic diseases, such as dyslipidemia, hypertension, insulin resistance, type 2 diabetes and atherosclerosis.[1] Adipose tissue has an essential role as energy storage depot and for secreting adipokines influencing diverse tissue targets such as brain, liver, muscle, β cells, gonads, lymphoid organs, and systemic vasculature.[2,3] Expression analysis of macrophage and non-macrophage cell populations isolated from adipose tissue demonstrates that adipose tissue macrophages are responsible for secretion of almost all of pro-inflammatory cytokines.[4] In recent years, it has become evident that alterations in the function of the innate immune system are intrinsically linked to metabolic pathways in humans.[5-7] Central to metabolic diseases is insulin resistance associated with a low-grade inflammatory status.[8-11] The mechanisms through which proinflammatory cytokines, like tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and interleukin 1-beta (IL-1β) interact with cellular insulin signal transduction cascades have been better understood in the last few years.[12-17] *In vivo*, a direct correlation between increased circulating proinflammatory cytokines and insulin resistance has been well-demonstrated.[10,18]

The origin of this increased inflammatory activity in obesity and type 2 diabetes is virtually unknown. Immune system homeostasis is challenged by continuous external insults, like saturated fatty acid-rich diets,[19] pathogen associated molecular patterns (PAMP) like lipopolysaccharide (LPS)[20] and advanced glycation end-products (AGEs),[21] burden of infection[22] and oxidative stress.[23] These continuous insults could result in a chronic low level of inflammation associated with insulin resistance.

This revision is focused in the effects of metabolic concentrations of plasma LPS in insulin resistance and other

metabolic disorders associated with obesity, and the possible role of an inefficient innate immune system responding to this challenge.

Endotoxemia effects on obesity and insulin resistance

LPS is an important structural component of the outer membrane of Gram-negative bacteria. LPS consists of three parts: a lipid A, an oligosaccharide core, and an O side chain.[24] LPS is one of the best studied and most potent immunostimulatory components of bacteria which induces toxicity through increased signaling, triggering systemic inflammation.[25] LPS leads to a strong stimulatory release of several cytokines that are key inducers of insulin resistance which is a putative factor for the triggering of metabolic disorders. Lipid A is the main pathogen associated molecular pattern of LPS and is acylated with saturated fatty acids. Removal of these fatty acids results in complete loss of endotoxic activity.[26] LPS stimulation of mammalian cells occurs through series of interactions with several proteins including the LPS binding protein (LBP), CD14, MD-2 and TLR4.[27] LBP is a soluble shuttle protein which directly binds to LPS and facilitates the association between LPS and CD14.[28] CD14 is a glycosylphosphatidylinositol-anchored protein, which also exists in a soluble form. CD14 facilitates the transfer of LPS to the TLR4/MD-2 receptor complex and modulates LPS recognition.[29] MD-2 is a soluble protein that associates non-covalently with TLR4 but can directly form a complex with LPS in the absence TLR4. MD-2 is associated with the extracellular domain of TLR4 and augments TLR4-dependent LPS responses in vitro being essential for correct intracellular distribution and LPS-recognition of TLR4.[30] The TLR4 receptor complex, recruits the adaptor protein, myeloid differentiation factor-88 (MyD88). MyD88 in turn recruits interleukin-1 receptor–associated kinase (IRAK) and, by activating IKKβ and NF-κB, ultimately induces the expression of numerous inflammatory mediators.[31] Furthermore, TLR4 can be activated with saturated free fatty acids, stimulating NF-κB signaling and expression of inflammatory cytokine genes, such as TNF-α and IL-6 in adipocytes and macrophages.[20,32,33]

A recent article has demonstrated that metabolic concentrations of plasma LPS are a sufficient molecular event to trigger insulin resistance, obesity and type 2 diabetes.[34] This process was called metabolic endotoxemia, defined as the association between metabolic concentration of circulating endotoxin (LPS) and inflammation- and high fat diet-induced metabolic diseases. Metabolic concentration is the minimum LPS concentration to produce metabolic disorders, but not enough to produce acute endotoxemia. Interestingly, metabolic concentrations of plasma LPS were increased by a high fat diet. The same authors reported new interactions between a high fat diet and the microbial gut flora. They found that high fat feeding changes microbial gut flora (decreasing *Bifidobacterium* spp.) and increases intestinal permeability, leading to increased LPS absorption, endotoxemia, inflammation and metabolic disorders.[35] Selective increases of *Bifidobacterium* spp. in gut flora improved high fat diet-induced diabetes in mice and this was associated with decreased concentration of circulating LPS.[36]

In vitro, LPS induces nuclear factor-kappaB- and MAPK-dependent proinflammatory cytokine/chemokine expression primarily in preadipocytes. These changes are associated with a decrease in adipogenic gene expression, lower ligand-induced activation of peroxisome proliferator activated receptor (PPAR)-γ and decreased insulin-stimulated glucose uptake in adipocytes.[37] Persistent LPS stimulus impaired adipocyte differentiation and decreased the expression of lipogenic enzymes (FABP4, LPL), of different adipokines (adiponectin, resistin, visfatin, leptin) and of PPAR-γ.[38] Thus, LPS stimulus lead to adipose tissue insulin resistance and to adipocyte dysfunction usually found in the systemic metabolic disorders linked to obesity.

These findings in animal models are mirrored by different observations regarding endotoxemia markers and LPS-neutralizing proteins in humans, summarized below (table 1).

Table 1. Endotoxemia markers and LPS-neutralizing proteins

Endotoxemia markers	LPS-neutralizing proteins
• Soluble CD14 (sCD14)	• Adiponectin (ADIPOQ)
• Lipopolysaccharide binding protein (LBP)	• Bactericidal/permeability increasing protein (BPI)
	• Human α-defensins (DEFA1-3)
	• Lactoferrin (LTF)

Endotoxemia markers and LPS-neutralizing proteins

Endotoxemia markers

Endotoxemia markers are molecules of innate immune system which are produced by leukocytes (macrophages and neutrophils), adipose tissue, liver, kidney, lung, thymus, small intestine and mammary tissue in response to LPS stimuli. This review is focused in the main endotoxemia markers soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP), two phase acute reactants that are released in response to LPS.

Soluble CD14 (sCD14)

The earliest cell-mediated events following endotoxin release appear to involve the glycosylphosphatidyl-inositol anchored protein CD14. Different lines of evidence support a central role for CD14 in LPS-mediated responses. Specific monoclonal antibodies (mAb) against CD14 inhibit the ability of LPS to stimulate monocytes.[39] Transfection of CD14 into the 70Z/3 pre-B cell line enhances the responsiveness of these cells to LPS by more than 1000-fold.[40] CD14 also exists in a soluble form (sCD14),[41] and the levels are significantly raised in septic patients.[42]

The physiological role of sCD14 is not yet completely understood. sCD14 has been shown to inhibit the LPS-induced TNFα production in whole blood and monocytes,[43,44] and in a mouse model of endotoxin shock, sCD14 has been shown to inhibit lethality as well.[45] However, contrary to this inhibiting effect of sCD14 on LPS effects, sCD14 facilitates the activation of endothelial cells that do not express membrane CD14.[46,47] Troelstra et al reported that the effect of sCD14 on neutrophil response to LPS was a balance between activation and inhibition, depending on the concentration of circulating LBP in serum.[48] However, sCD14 could play a key role as intermediate in the neutralization of LPS under physiological conditions. sCD14 accelerates the transfer between LPS micelles and lipoproteins by acting as a carrier. sCD14 also enhances the release of monocyte-bound LPS, transferring LPS into plasma and into lipoproteins and, thus, decreasing cellular responses to LPS, such as induction of TNF-α and interleukin 6 synthesis.[49,50]

Recently, a direct relationship between sCD14 and endothelial function in type 2 diabetic subjects has been found in opposite to the inverse association of these parameters in non-diabetic subjects.[51] Furthermore, sCD14 was significantly and inversely associated with insulin resistance, waist to hip ratio, systolic and diastolic blood pressure and inflammatory markers (sTNFR1 and sTNFR2), once fasting triglycerides and smoking was controlled.[52] sCD14 could also be a marker of hepatic insulin resistance and dysfunction. In fact, decreased serum sCD14 concentration was associated with higher circulating alanine aminotransferase levels.[53] These apparently protective associations of sCD14 with metabolic parameters (insulin sensitivity, blood pressure, hepatic injury) are supported by the anti-inflammatory activities of sCD14, neutralizing LPS effects in *in vitro* models.

Interestingly, genetic variations that lead to lower serum concentration of sCD14 were associated with insulin resistance and the presence of increased inflammatory markers.[52]

Lipopolysaccharide Binding Protein (LBP)

Lipopolysaccharide Binding Protein (LBP) is an important LPS marker. LBP is a 65-kDa protein present in blood at high concentrations (approximately 2-20 μg/mL).[54] LBP is an acute-phase reactant, predominantly derived from the liver, and its plasma levels rise dramatically after inflammatory challenge, including bacterial sepsis.[54] Although the molecular structure of LBP is not entirely known, LBP clearly binds LPS (and LPS substructures, such as lipid IVa) through the recognition of lipid A.[55] The plasma protein LBP dramatically accelerates binding of LPS monomers from aggregates to CD14,[56] thereby enhancing the sensitivity of cells to LPS. Furthermore LBP acts as a lipid transfer protein, a function in correlation with its sequence homology to lipid transferases (phospholipid transfer protein and cholesterol ester transfer protein). LBP co-purifies with HDL particles and additional studies have shown that LBP can transfer LPS to lipoproteins,[57] neutralizing LPS effects.[58]

Serum LBP reflects the serum endotoxin (LPS) concentration and is negatively associated with insulin sensitivity.[59] Interestingly, serum LBP concentrations are increased in patients with type 2 diabetes.[59]

LPS-neutralizing proteins

Several naturally occurring proteins possess the capacity to bind to bacteria-associated LPS, resulting in reduction of bacterial viability. These LPS-neutralizing proteins are adiponectin, bactericidal/permeability-increasing protein (BPI), human α-defensins and lactoferrin.

Adiponectin (ADIPOQ)

Adiponectin (ADIPOQ) is almost exclusively produced by adipocytes and abundantly present in serum, where it circulates in two higher-order forms: a low-molecular weight dimers or trimers and a larger high-molecular weight complex of 12-18 subunits.[60] ADIPOQ is known to affect LPS-mediated inflammatory events. It inhibits LPS-induced NF-κB activation and IL-6 production, and increases PPARγ2 expression in adipocytes, while in macrophages it suppresses both LPS-induced TNFα and IL-6 production. Peake et al suggested that adiponectin may have anti-inflammatory potential by directly binding to LPS.[61]

It is well known that adiponectin expression is reduced in obesity and insulin resistance states. Plasma levels of adiponectin have also been reported to be significantly reduced in obese/diabetic mice and humans, and in patients with cardiovascular diseases, hypertension or metabolic syndrome.[62] A direct insulin-sensitizing effect of adiponectin *in vivo* has been extensively reported. The main mechanism of action of adiponectin in insulin-sensitizing actions are mediated through a reduction of tissue triglycerides content and activation of PPARα[63] and AMP kinase,[64,65] leading to the up-regulation of insulin signaling. However, the anti-inflammatory effects of adiponectin and LPS neutralizing action cannot be forgotten, as two indirect ways to improve insulin sensitivity. Single nucleotide polymorphisms studies also support the role of adiponectin as a factor influencing the susceptibility to insulin resistance and type 2 diabetes.[66,67]

Bactericidal/increasing protein permeability (BPI)

Bactericidal/increasing permeability protein (BPI) is located in the azurophilic granules of neutrophils. BPI is an approximately 55 kDa cationic protein with selectivity towards Gram-negative bacteria, most likely due to its strong affinity for LPS.[68] Besides its bactericidal activity, BPI also neutralizes the cytotoxic effects of LPS. Most of the antibacterial and LPS binding activity of holo-BPI is found in 20-25 kDa N-terminal fragments/regions/domains of the protein.[69] N-Terminal fragments/regions of BPI also inhibit LPS induced E-selectin expression and reduce NF-κB activation in LPS-stimulated endothelial cells.[70] Furthermore, rBPI21, a recombinant 21 kDa protein/peptide corresponding to amino acids 1-193 of N-terminal human BPI in which a cysteine is replaced by an alanine at position 132), is bactericidal and binds to and neutralizes endotoxin.[71]

Plasma BPI concentration was directly correlated with insulin sensitivity and HDL-cholesterol concentrations, and inversely associated with metabolic parameters (waist-to-hip ratio, fasting triglycerides) and with serum LBP and LPS concentration. Genetic variations that lead to lower serum concentration of BPI were associated with insulin resistance and increased circulating inflammatory markers.[59]

Human α-defensins (DEFA1-3)

Human α-defensins are arginine-rich peptides, containing 29-35 amino acids. Their three disulfide bridges connect cysteines 1-6, 2-4 and 3-5. Human α-defensins are synthesized as 93-100 amino acid prepropeptides with a 19-amino acid signal peptide and a 41-51 amino acid anionic pro-segment. α-defensins are predominantly found in neutrophils (mainly DEFA1-3) and in small intestine Paneth cells. A stimulus-dependent release of pre-synthesized defensin-containing cytoplasmic granules contributes to the local antimicrobial response.[72] Recently, significant positive associations among plasma α-defensins (1-3) concentrations and non-atherogenic lipid profile and vascular function in apparently-healthy Caucasian men have been reported.[73]

Lactoferrin (LTF)

Lactoferrin is a pleiotropic glycoprotein of the innate immune system that is involved in LPS buffering. Lactoferrin is a monomeric, 80 kDa glycoprotein, with a single polypeptide chain of about 690 amino acid residues and two sialic acid molecules, that is produced by neutrophils and several epithelia types. Lactoferrin is folded into homologous N- and C-terminal lobes, each comprising two domains that enclose a conserved iron binding site. This protein is positively charged in N-terminal region (the first 60 aminoacids) of N-lobe at physiological pH because it is rich in arginine.[74] Lactoferrin is able to bind and buffer other pathogen associated molecular patterns in addition to LPS, viral DNA and RNA, CpG sequences, and soluble components of the extracellular matrix.[75] This ability is associated with lactoferrin anti-inflammatory activity, as demonstrated in several studies,[76,77] in which lactoferrin down-regulates pro-inflammatory cytokine production in cell lines acting via NF-κB,[78] decreasing the release of TNF-α and IL-6 in mice.

Circulating lactoferrin concentration was inversely associated with body mass index, waist-to-hip ratio, fasting triglycerides and fasting glucose, and directly associated

with HDL-cholesterol.[79] Furthermore, circulating lactoferrin concentration was associated with vascular function in obese subjects with altered glucose tolerance. Two non synonymous LTF gene polymorphisms that produce two aminoacid changes in the N-terminal region were associated with dyslipidemia according to glucose-tolerance status.[79]

The concentration of all these proteins and peptides with LPS-neutralizing effect were decreased in metabolic disorders associated with insulin resistance and obesity. Thus, the high LBP and endotoxin concentrations could be markers of an unbalance of the innate immune system.

Buffering Efficiency Hypothesis

The evidences reviewed here led us to propose the buffering efficiency hypothesis (figure 1). LPS is an important factor that might produce insulin resistance and obesity in humans. Chronic low-grade inflammation and associated insulin resistance might be viewed in the context of an unbalanced innate immune system. A decreased production of anti-LPS proteins and peptides were associated with insulin resistance, obesity, vascular dysfunction, hepatic dysfunction and dyslipidemia. A partial lost in the buffering efficiency of LPS could increase its negative effects on metabolism. Furthermore, insulin resistance might result in a decreased concentration of those proteins that buffer LPS. It is well known that adiponectin production by the adipose tissue is decreased under insulin resistance and inflammatory conditions. Neutrophils also lose antimicrobial efficiency in insulin resistant conditions, decreasing the production of lactoferrin, BPI and other antimicrobial proteins. Neutrophil activity may be restored by controlling hyperglycemia using insulin.[80,81] Stegenga et al reported that hyperglycemia impaired neutrophil degranulation in humans after intravenous endotoxin administration.[82]

Impairment of neutrophil function was associated with a poor metabolic profile in subjects with type 2 diabetes, including a decreased neutrophil deformability and a high production of reactive oxygen species and proinflammatory cytokines.[83-85] High PKB (a major downstream PI3K effector) activity was found to promote neutrophil and monocyte development/proliferation.[86] In this sense, insulin resistance and a low grade inflammatory state potentiate each other, producing a vicious cycle, strengthened by an unbalanced innate immune system.

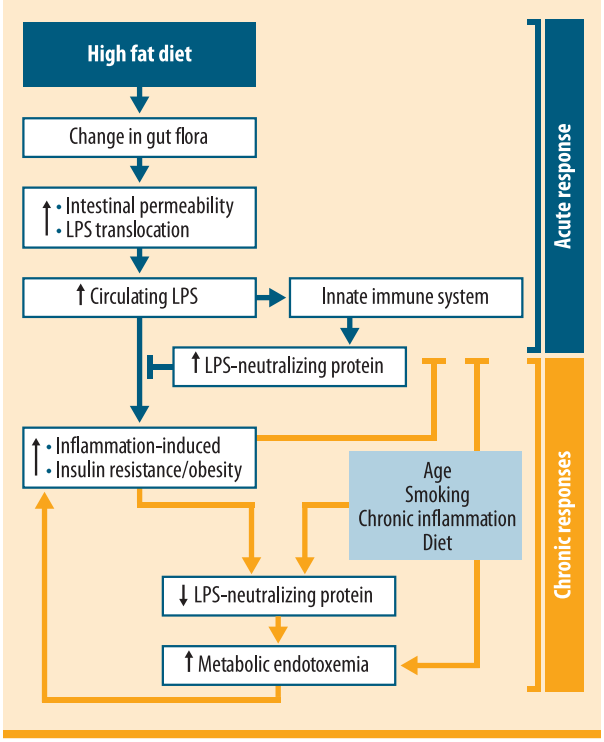

Figure 1. The effects of chronic activation of the innate immune system by external insults in LPS-neutralizing proteins and metabolic disorders

Conclusions

There is now strong evidence that type 2 diabetes and the metabolic syndrome are associated with a low-grade inflammatory state associated with an unbalanced innate immune system. This low-grade inflammatory state is triggered by a continuous exposure to external insults such as reactive oxygen species (ROS), fatty acids, AGEs and LPS. Metabolic concentrations of plasma LPS are associated with insulin resistance and obesity in animal models, and this increase in plasma LPS could be caused by intestinal translocation of LPS from Gram-negative bacteria present in gut flora. High fat diet could contribute to this increased LPS translocation from intestine into the bloodstream.

LPS stimulates the release of antimicrobial proteins (by neutrophils and by epithelial cells from the lung, liver and adipose tissue) which also protect from other injuries such as reactive oxygen species and AGEs. A higher efficiency (response and release) in this process could allow a greater neutralizing effect of LPS. On the other hand, insulin action is an important factor in the devel-

opment and maintenance of neutrophil function and efficiency. Thus, a continuous exposure to metabolic concentrations of plasma LPS could begin a vicious cycle, weakening the innate immune system and increasing proinflammatory cytokines and ROS due to a partial inefficiency of the innate immune system. As a consequence, the decreased protection in front of LPS challenge would increase metabolic disturbances. According to the buffering efficiency hypothesis, an increased efficiency of the innate immune system attenuates metabolic endotoxemia, and decreases thereby the negative effects of LPS on insulin sensitivity and metabolism.

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Declaration of potential conflicts of interest
No disclosures.

Practical considerations

- Elevated circulating endotoxemia markers (LBP) and decreased LPS-neutralizing proteins (BPI and LTF) could reflect metabolic endotoxemia associated with insulin resistance.
- These markers may help to predict the development of type 2 diabetes and cardiovascular disorders. Also, an increased efficiency of the innate immune system is associated with an insulin-sensitive profile in healthy subjects.
- External administration of LPS neutralizing peptides (synthetic cationic peptides) may be helpful to stop the inflammation-insulin resistance vicious cycle and to recover the efficiency of the innate immune system.

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