Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Acute low-dose endotoxin treatment results in improved whole-body glucose homeostasis in mice

Joseph R. Stevens[a], Ryan P. McMillan[a,c], Justin T. Resendes[a], Shannon K. Lloyd[a], Mostafa M. Ali[a], Madlyn I. Frisard[a], Stefan Hargett[b], Susanna R. Keller[b], Matthew W. Hulver[a,c,*]

[a] Department of Human Nutrition, Foods, and Exercise, Virginia Tech, Blacksburg, VA
[b] Department of Medicine/Division of Endocrinology, University of Virginia, Charlottesville, VA
[c] Metabolic Phenotyping Core, Virginia Tech, Blacksburg, VA

ARTICLE INFO

Article history:
Received 4 April 2016
Accepted 7 December 2016

Keywords:
LPS
Endotoxin
Skeletal muscle
Glucose homeostasis
Metabolic endotoxemia

ABSTRACT

Background. Obese individuals present with an increased inflammatory tone as compared to healthy, normal-weight individuals, which is associated with insulin resistance. One factor hypothesized to contribute to increased inflammation in obese and diabetic states is elevated blood endotoxin levels, a condition known as metabolic endotoxemia. In non-obese and insulin sensitive individuals, circulating endotoxin concentrations fluctuate over the course of the day with elevations in the post-prandial state that return to baseline levels in the post-absorptive state. Evidence suggests that high-fat feeding alters these fluctuations causing endotoxin levels to remain high throughout the day. The effects of alterations in endotoxin levels on glucose metabolism are not clearly understood.

Purpose/Procedures. The goal of this study was to determine the effects of both short-term and long-term increases in endotoxin (lipopolysaccharide, LPS) of a low magnitude on the glucose tolerance and insulin signaling in a human primary cell line as well as the effects of short-term endotoxin treatments on glucose homeostasis in a C57/Bl6 mouse model. First, we tested the hypothesis that short-term low-dose endotoxin treatments would augment insulin signaling and glycogen synthesis while long-term treatments would be disruptive in the cell culture model. Second, we examined if these short-term low dose treatments of endotoxin would contribute to similar improvements in whole-body glucose homeostasis in a mouse model.

Main findings. Contrary to our initial hypothesis, short-term endotoxin treatment had no effect on insulin signaling or glycogen synthesis, however long-term treatment indeed decreased glycogen synthesis ($P < .05$). Interestingly, short-term endotoxin treatment resulted in significant improvements in glucose homeostasis in the mouse model ($P < .01$); which is believed to be at least partly attributed to an inhibitory action of LPS on liver glucose production.

Conclusions. This research shows that low-magnitude, short-term changes in LPS can have significant effects on whole body glucose metabolism and this likely occurs through its direct actions on the liver. Additional studies are necessary to understand the mechanisms responsible for altered glucose metabolism in response to low magnitude changes in LPS levels.

* *Corresponding author at:* Virginia Tech, 295 West Campus Drive, Wallace Hall, Room 338, Blacksburg, VA 24061. Tel.: +1 540 231 7354; fax: +1 540 231 3916.
E-mail address: hulvermw@vt.edu (M.W. Hulver).

http://dx.doi.org/10.1016/j.metabol.2016.12.008
0026-0495/

1. Introduction

Inflammation is a key mediator of the relationship between obesity and Type 2 diabetes mellitus [1,2]. Normal-weight individuals exhibit an immune response to various instigators such as sickness, injury, or even high-fat meals that is resolved following removal of the instigator [3–7]. Obese individuals show a chronically heightened immune response that can negatively affect insulin signaling processes and result in insulin resistance [8–11].

A mechanism thought to contribute to this chronically elevated inflammatory response is known as metabolic endotoxemia [8,12,13]. Metabolic endotoxemia was first coined by Cani et al. [8] to describe the chronic elevation of blood endotoxin levels that is seen with obesity and prolonged high-fat feeding in rodents. Endotoxin has been shown to activate toll-like receptor 4 (TLR4) and has been well-classified as able to initiate an inflammatory signaling response [14,15]. Research from both mouse models and humans has attributed chronic increases of endotoxin to the development of metabolic diseases including obesity and insulin resistance [8,13,16]. Endotoxin levels respond to feeding patterns, with the lowest levels during fasting periods and reaching a peak during the postprandial period following a meal [6,8]. Studies in which rodents were fed a high-fat diet for 4 weeks, exhibited sustained increases in circulating endotoxin levels in comparison to animals fed a control diet [8]. The latter represents a vital connection between the diet and the consequent changes in endotoxin levels.

These chronic elevations in endotoxin are thought to contribute to activation of inflammatory processes, which have long been associated with detrimental roles in glucose metabolism primarily through inhibitory actions on insulin signaling [17–19]. In the context of metabolic endotoxemia, endotoxin levels 10–50 times lower than those seen in septic situations are sufficient to cause metabolic derangements when elevated chronically and are attributed to an enhanced inflammatory response [20]. Blood endotoxin levels that are associated with metabolic endotoxemia are difficult to define, and are best described as a relative increase compared to healthy, non-obese states. Much less is known about whether the short-term low-magnitude changes in endotoxin levels following a meal are capable of causing an inflammatory response and what role an inflammatory response would have on glucose metabolism. Previous studies aimed at determining the effects of short term endotoxin treatments on glucose metabolism have shown severe hypoglycemia, due in large part to increased glucose uptake along with inhibition of hepatic glucose production [21–24]. These studies used extremely high doses of endotoxin (4–5 mg/kg), many at septicemic levels. More recent studies using lower doses of endotoxin (.01–.1 μg/kg) administered acutely have shown decreases in muscle glucose uptake that were independent of changes in insulin signaling [25,26]. In disagreement with these findings, previous work from our laboratory has suggested a unique role for these short-term increases in endotoxin of a low magnitude, to cause significant enhancements in glucose metabolism in skeletal muscle cells [27]. This work showed that short-term treatments with low doses of endotoxin were capable of causing significant increases in glucose uptake and oxidation as well as lactate production.

The objective of these studies was to determine the effects of low-magnitude short-term changes in endotoxin on glucose metabolism through the use of acute and chronic treatments in both cell culture and a mouse model. Our approach was to use a dose of endotoxin that is both physiologically relevant to the changes seen in humans in the post-prandial state and may help better understand the mechanisms through which endotoxins regulate glucose homeostasis. These studies used much lower dosages than those used in most of the previous studies of this nature [16,18,28,29]. Additionally, these studies sought to further clarify the discrepancy between our lab and others regarding the effect of these doses of endotoxin on glucose metabolism. Using this approach coupled with our previous findings, our hypotheses were that short-term low-magnitude increases in endotoxin levels would contribute to enhanced insulin stimulated glucose uptake in both cells and animals, whereas long-term treatments in cell culture would result in a blunted glucose tolerance and insulin signaling.

2. Methods and Materials

2.1. *Skeletal Muscle Cell Culture*

Studies were conducted using human primary skeletal muscle cells. A muscle biopsy was taken from vastus lateralis of a lean male subject via a modified Bergstrom needle technique. Procedures were approved by the Institutional Review Board at Virginia Polytechnic Institute and State University. The muscle was collected in ice cold growth medium which consisted of low glucose (5.5 mmol/L) Dulbecco's Modified Eagle's Medium (DMEM; HyClone, Logan, UT) containing 10% fetal bovine serum (Life Technologies, Grand Island, NY), 5% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO), 5% fetal bovine (Sigma-Aldrich, St. Louis, MO), 0.5% gentamicin (Sigma-Aldrich, St. Louis, MO), 0.5% dexamethasone (Lonza, Walkersville, MD) and 0.5% recumbent human epidermal growth factor (Lonza). After collection, the tissue was minced into 1–2 mg fragments, added to HBSS (Invitrogen, Carlsbad, CA) and spun at 900 ×*g* for 3 min. The HBSS supernatant was discarded, fresh HBSS was added and the sample was spun again. This process was repeated as many times as needed to remove blood cells from the samples, determined by the light pink color. Then, 5 mL of a digestion mixture (2.5% trypsin, 10× liquid, Gibco, Grand Island, NY; 2% EDTA, Sigma-Aldrich; 1% collagenase type IV, Gibco; 1% BSA–RIA grade fraction V, Sigma-Aldrich) was added and the sample was trypsinized for 40 min at 37 °C, vortexing every 10 min, after which the trypsin was neutralized using 5 mL growth medium. The sample was then spun at 2100 ×*g* for 5 min; the medium was discarded and 3 mL of fresh growth medium was added before the sample was transferred to a collagen coated 50 mL tissue culture flask (Falcon, Franklin Lakes, NJ) and incubated overnight. At ~70% confluence, cells were transferred to a 250 mL tissue culture flask (Falcon), grown to ~70% confluence, trypsinized and stored in liquid N_2 in 1 mL aliquots. As experiments commenced, cells were thawed and grown to ~80% confluence (100 × 20 mm tissue culture dishes; Falcon, Corning, NY). At ~80% confluence, myocytes were differentiated into myotubes in low glucose (5.5 mmol/L) DMEM containing 2% horse serum (Invitrogen, Carlsbad, CA), BSA, fetal bovine serum, and gentamicin in the same concentrations listed above. At 7 days of differentiation, medium was changed to a serum-free