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Acute Low-Dose Endotoxin Treatment Results in Improved Whole-Body Glucose Homeostasis in Mice

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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.

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The authors declare no conflict of interest/ financial disclosure statement.

Author Contributions

J.R.S wrote and edited manuscript, performed experiments, analyzed data, and participated in the conception and design of experiments. M.W.H revised and edited manuscript, analyzed data, and conceived and designed studies. R.P.M edited manuscript, performed experiments, and analyzed data. S.R.K. conceived and designed glucose uptake assay and analyzed data. J.T.R., S.K.L., M.A. and S.H. performed experiments. M.I.F. edited manuscript.

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.

Keywords

LPS; Endotoxin; Glucose homeostasis; Metabolic Endotoxemia

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 are 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 (4–5mg/kg), many at septicemic levels. More recent studies using lower doses of endotoxin (.01–.1ug/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 than dosages 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 both in 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 media which consisted of low glucose (5.5mM) 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 $900 \times g$ 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, 5mL of a digestion mixture (2.5% trypsin, 10X 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 minutes

at 37°C, vortexing every 10 min, after which the trypsin was neutralized using 5 mL growth media. The sample was then spun at $2100 \times g$ for 5 min; the media was discarded and 3 mL of fresh growth media 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 N₂ in 1mL aliquots. As experiments commenced, cells were thawed and grown to ~80% confluence (100×20mm tissue culture dishes; Falcon, Corning, NY). At ~80% confluence, myocytes were differentiated into myotubes in low glucose (5.5 mM) DMEM containing 2% horse serum (Invitrogen, Carlsbad, CA), BSA, fetal bovine serum, and gentamicin in the same concentrations listed above. At 7 d of differentiation, media was changed to a serum-free media overnight and experiments were performed the following day.

LPS treatment in cell culture—Cell culture experiments were performed with 50 pg/ml of LPS (*E. coli* 0111:B4, catalog no. L2630; Sigma-Aldrich). All LPS preparations were made using ultra-pure, endotoxin-free water. Treatment time was either 2 hours or 24 hours, and 100 nM insulin (Sigma-Aldrich) was added to the plates 10 minutes before harvesting.

2.2. Glycogen Synthesis

Following treatment with LPS as described above cells were pre-incubated in Krebs-Ringer buffer for 5 hours, then washed with PBS and glycogen synthesis was determined by the addition of 1.25 μ Ci/mL of [U-¹⁴C]-glucose in low glucose DMEM. Following 3 hours of incubation, the plates were placed on ice and washed 3 times with ice-cold PBS. Cells were harvested in 30% KOH and placed in tubes containing 35 μ L of 60 mg/mL glycogen. Cells were vortexed and incubated for 20 minutes at 80°C. Cells were then placed on ice and 0.5 ml of 100% EtOH was added to each tube to precipitate glycogen. Tubes were centrifuged and the supernatant discarded. 70% EtOH was then added to the tubes, vortexed, and centrifuged again. Supernatant was removed and tubes were allowed to air-dry. Pellets were re-suspended in 0.5 ml dH₂O and vortexed until glycogen was completely dissolved. Glycogen synthesis was calculated based upon specific activity and expressed relative to protein content.

2.3. Animals Studies

Animal studies were performed under an approved protocol by the Institutional Animal Care and Use Committee at Virginia Polytechnic Institute and State University. Multiple studies were conducted using 12-week old male C57/Bl6 mice from the Jackson Laboratory (Bar Harbor, ME). Mice for all studies were maintained on a normal chow diet and a 12:12-h light-dark cycle.

2.4. Glucose Tolerance Assays

The first experiment assessed glucose clearance abilities via a glucose tolerance test in overnight-fasted (12–16hr), saline ($n=12$) and LPS-injected ($n=10$) mice. These mice were given an intraperitoneal injection of either saline ($n=12$) or low-dose (0.1 μ g/kg BW) LPS ($n=14$) followed 4 hours later by an intraperitoneal injection of glucose (2 g/kg BW). Blood glucose measurements were taken at baseline and then every 30 minutes until 120 minutes.

2.5. Assessment of Signaling Pathways in Skeletal Muscle

The second experiments occurred 2 weeks later and sought to assess non-insulin dependent tissue-specific effects of LPS on glucose clearance. In this experiment, the same animals used above were again fasted overnight and given an injection of saline or low-dose LPS and 4 hours post-injection were sacrificed and tissues were harvested for measurement of gene and protein expression of the inflammatory markers Tumor necrosis factor alpha (*Tnfa*), Interleukin 6 (*Il6*) and *Tlr4*, and glucose metabolism (Pyruvate carboxylase (*Pcx*), Phosphofructokinase (*Pfk*), *Glut2*, and Phosphoenolpyruvate carboxykinase (*Pepck*)). LPS from *Escherichia coli* 0111:B4 was used for all studies (catalog no. L2630; Sigma-Aldrich, St. Louis, MO). A second cohort of 12-week old C57/Bl6 mice was used to assess the insulin-dependent effects of LPS on glucose metabolism. These animals were fasted overnight for 12 hours and given an injection of saline or LPS as described above. Four hours following treatment, the animals were given an injection of glucose (2g/kg BW). In this case animals were sacrificed at 30 minutes post-injection and tissues and blood were harvested to assess insulin signaling markers (phosphorylated and total AKT, Glut4) in tissues as well as insulin levels (ALPCO Diagnostics, Salem, NH) in the blood.

2.6. Glucose disposal in adipose, skeletal muscle tissues, and glucose incorporation into glycogen in liver

Mice were fasted for 12 hours and then injected with either saline or low-dose (0.1 ug/kg BW) LPS intraperitoneally. After 4 hours, mice were injected intraperitoneally with 2-deoxy-D-[1, 2-³H] glucose (10 μ Ci) in either saline or glucose (2 g/kg BW) and blood was collected at 0, 15, 30, 60, and 120 minutes after the injections as previously described [30]. Blood was used for the determination of blood glucose and 2-deoxy-D-[1, 2-³H] glucose levels, and tissues were dissected and processed at 120 minutes for determination of tissue uptake. Insoluble material was pelleted from the tissue homogenates in water with perchloric acid treatment.

2.7. Pyruvate Challenge to Assess Gluconeogenesis

Mice were fasted for 12 hours and then injected with either saline or low-dose (0.1 ug/kg BW) LPS intraperitoneally. After 4 hours, mice were injected intraperitoneally with sodium pyruvate at 2 g/kg BW (Sigma). Blood glucose readings were taken in 30 minute increments out to 2 hours as previously described [31, 32].

2.8. RNA extraction and qRT-PCR

RNA was extracted using an RNeasy Mini Kit (Qiagen, Valencia, CA) and DNase I treatment (Qiagen, Valencia, CA), according to the manufacturer's instructions. qRT-PCR was performed using an ABI PRISM 7900 Sequence Detection System instrument and TaqMan Universal PCR Master Mix used according to the manufacturer's specifications (Applied Biosystems, Foster City, CA). Target gene expression in rodent skeletal muscle was normalized to β -actin RNA levels. Human skeletal muscle primary cell culture was normalized to cyclophilin B RNA levels. Primers and 5' FAM-labeled TaqMan probes were purchased as prevalidated assays (Applied Biosystems, Foster City, CA). Relative quantification of target genes was calculated using the 2^{-C_T} method. Derivation of the

2^{-C_T} equation has been described in Applied Biosystems User Bulletin no. 2 (P/N 4303859).

2.9. Western Blotting

Western analysis was performed using cell lysates harvested in Mammalian Cell Lysis Buffer (Sigma Aldrich) with the addition of protease and phosphatase inhibitors (Biorad). Proteins (30 μ g) were separated using a 10% Criterion-Tris-HCl gel (Bio-Rad, Hercules, CA) and subsequently transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). Blots were probed with primary antibodies against β -actin (1:1,000; Cell Signaling, Danvers, MA), Total AKT1/2 (1:1,000; Cell Signaling, Danvers, MA), P-AKT1/2 (Ser473) (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA), Total IRS-1 (1:1,000; Cell Signaling, Danvers, MA), and P-IRS-1 (Tyr612) (1:1,000; Cell Signaling, Danvers, MA) followed by anti-rabbit secondary antibodies (1:10,000; Jackson Immuno-Research Laboratories, West Grove, PA). Proteins were visualized using Super-Signal Chemiluminescent Substrate (Pierce, Rockville, IL) and a ChemiDoc XRS Imaging System (Bio-Rad, Hercules, CA). Phosphorylated protein expression is expressed as a value relative to the total form of the protein. All values are corrected to total protein loading using Image Lab software (Bio-Rad, Hercules, CA).

2.10. Western Blotting of membrane bound Glut4

Muscle tissue was homogenized in homogenization buffer consisting of 50 mM Hepes, 10 mM EDTA, 100 mM NaF, 50 mM Na pyrophosphate, and 10 mM Na orthovanadate. Protease and phosphatase inhibitor cocktail was added prior to homogenization at 10 μ l/ml final concentration. Homogenization was done in a 2 ml glass homogenizer tube until slurry appeared homogenous. Homogenate was centrifuged at $150k \times g$ for 1 hr at 4°C in a Beckman Ultracentrifuge. Following spin, supernatant was removed and collected as cytosolic fraction for later processing. The pellet was re-homogenized in homogenization buffer and 10% Triton x-100 for solubilization of membrane-bound proteins. Following a 30-min incubation period, homogenates were spun in micro-centrifuge at $16k \times g$ for 15 minutes and supernatant was harvested as membrane protein fraction. Protein fractions were separated on a 10% Criterion-Tris-HCl gel (Bio-Rad, Hercules, CA) and subsequently transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). Blots were probed with primary antibodies against Glucose transporter type 4 (Glut4) (1:1,000; Cell Signaling, Danvers, MA) followed by anti-mouse secondary antibody (1:10,000 Jackson Immuno-Research Laboratories, West Grove, PA).

2.11. Statistics

Data was analyzed using GraphPad Prism software. To compare saline versus LPS treatments and insulin versus non-insulin treatments in cell culture, protein and mRNA data were analyzed by two-way ANOVA with Tukey's post hoc analysis, where a significant F value was observed. To compare saline versus LPS groups in mouse studies, data were analyzed using an unpaired *t-test*. Data are presented as mean $\pm$ standard error of the mean (SEM). The level of significance was set *a priori* at $P < 0.05$.

3. Results

3.1. Acute and Chronic LPS treatments contribute to decreased glycogen synthesis independent of changes in insulin signaling in a Human Primary skeletal muscle cell line

Having previously identified a role for low-doses of LPS in contributing to significant improvements in glucose uptake and oxidation in mouse and human muscle cells [27], we sought to determine if these changes were occurring through influences on insulin signaling. Using acute (2 hour) and chronic (24 hour) treatments of LPS followed by a 10 minute insulin treatment we found that neither time frame of LPS treatment resulted in significant alterations in either insulin-stimulated phosphorylation of AKT (P-AKT Ser473) or IRS-1 (P-IRS1 Tyr612) protein, two main components of insulin signaling (Figure 1A–D). Because no changes were observed with insulin signaling between the two groups, we expected that our endpoint measure, glycogen synthesis would also show no changes. However, as demonstrated in Figure 2B, the 24 hour LPS treatment resulted in a significant ($p<0.05$) decrease in insulin-stimulated glycogen synthesis compared to the control group, while the 2 hour treatment showed no changes.

3.2. Acute low-dose LPS contributes to significant improvements in whole-body glucose tolerance in animals

We injected animals with low dose (0.1 $\mu\text{g/kg}$) LPS followed by a glucose tolerance test 4 hours later. As shown in Figure 3A, LPS injected animals show significantly enhanced glucose tolerance compared to their saline injected counterparts. As shown in Figure 3B, LPS treatment resulted in a significantly lower area under the curve (AUC) ($p<0.001$) compared to the saline group. To determine if the improvement in glucose tolerance was due to an increase in insulin secretion, we again injected animals with low-dose LPS (0.1 $\mu\text{g/kg}$) followed 4 hours later with an injection of glucose at 2 g/kg . We sacrificed animals 30 minutes after glucose injection and harvested blood serum for assessment of insulin concentrations. As shown in Figure 4A, there no significant difference in these insulin concentrations.

3.3. Assessment of insulin signaling components in skeletal muscle of mice

Skeletal muscle was harvested from the same animals and protein content of insulin signaling markers P-AKT (ser473) and total-AKT were examined. There were significant ($p<0.05$) increases in both (Figure 4B–C) total and P-AKT. Increases in an insulin signaling component suggested a possible increase in its downstream effector, Glut4. We examined whether there were significant changes in Glut4 protein content at the plasma membrane (Figure 4D), as this would indicate an increase in glucose uptake into the muscle. However, protein expression of membrane bound Glut4 remained unchanged between the treatment groups. In agreement with this data, gene expression data from red and white skeletal muscle under basal (non-insulin stimulated) or insulin-stimulated conditions showed no major changes (Data not shown).

3.4. Acute low-dose LPS treatment in mice causes significant improvements in whole-body glucose tolerance independent of changes in uptake of 2-deoxyglucose into tissues

Because LPS treated animal's exhibit improved whole body glucose tolerance independent of insulin secretion or Glut4 dependent skeletal muscle uptake, we sought to identify the tissues primarily responsible for this effect. Within the glucose uptake assay employed, LPS treated animals ($n=5$) again showed improved glucose tolerance compared to saline treated control animals ($n=5$) (Figure 5.A). AUC analysis shows a heightened response to glucose at the 15 min (Figure 5.B) ($p<0.05$) and 30 min (Figure 5.C) ($p<0.05$) time points. Figure 6 represents the uptake of 2-DG into skeletal muscle (Figure 6.A–B), adipose tissues (Figure 6.C–D), and glycogen incorporation in the liver (Figure 6.E). None of the tissues examined showed significant differences between the saline and LPS treated animals suggesting that tissue uptake was not the primary mechanism responsible for improvements in glucose tolerance in the mice treated with low-dose LPS.

3.5. Animals given acute low-dose LPS treatments exhibit decreased fasting glucose and decreased gluconeogenesis

Because low-dose LPS contributes to a significant enhancement of glucose tolerance independent of insulin stimulated glucose uptake into the primary insulin responsive tissues, we next wanted to identify if the effect on glucose homeostasis was at least partially a byproduct of decreased gluconeogenesis. Figure 7 shows fasting blood glucose levels in animals 4 hours after LPS injection. Blood glucose levels are significantly ($p<0.05$) lower in the LPS treated group compared to control, suggesting LPS may have an inhibitory effect on endogenous glucose production. To test this theory of altered gluconeogenesis, we used a pyruvate challenge assay. Animals were fasted overnight and then given an injection of low-dose LPS followed 4 hours later by an injection of sodium pyruvate (2 g/kg BW). Blood glucose measures were taken at baseline (time 0) and every 30 minutes out to 2 hours. Figure 8.A shows significantly lower blood glucose levels in the LPS treated versus saline treated group. Figure 8.B represents the AUC. These results suggest that LPS is contributing to a decrease in gluconeogenesis, and this, at least in part, explains the significant improvement in glucose tolerance in LPS treated animals.

3.6. Expression of genes controlling inflammatory and gluconeogenic processes in mice

To determine potential mechanisms underlying the effect of LPS on gluconeogenesis as well as whole-body glucose control, we examined the gene expression levels of various liver enzymes involved in these processes as well as the expression levels of genes that contribute to inflammation. We measured these under both basal and insulin-stimulated conditions. Figure 9 shows that under basal conditions there were significant alterations in gene expression within the liver. Interestingly, gene expression of Phosphoenolpyruvate carboxy kinase (*Pepck*) (Figure 9.A), a key enzyme in gluconeogenesis, was unaltered by LPS treatment. However, other markers of glucose flux in the liver were significantly decreased, including phosphofructokinase (*Pfk*) gene expression (Figure 9.C) ($p<0.01$) a rate-limiting enzyme involved in glycolysis, and *Glut2* ($p<0.001$), the primary transporter controlling the movement of glucose into and out of the liver. These changes were in concert with increased

gene expression of (Figure 9.E) *Tnfa* ($p<0.05$), signifying an increased inflammatory response; however, *Il6* mRNA expression showed a significant ($p<0.01$) decrease in the liver.

Under insulin stimulated conditions, there were similar alterations in gene expression (Figure 10). In this case, pyruvate carboxylase (*Pcx*) (Figure 10.A), a gluconeogenic enzyme, showed a decreased gene expression ($p<0.05$) that was not supported by similar changes in *Pepck* gene expression (Figures 10.C). *Glut2* gene expression (Figure 10.D) was significantly decreased ($p<0.05$) in these animals while PFK (Figure 10.B) was unaltered with LPS treatment under insulin-stimulated conditions. As with non-insulin stimulated conditions, *Tnfa* expression (Figure 10.F) was again significantly ($p<0.05$) increased with LPS treatment while *Il6* was unchanged (Figure 10.E).

4. Discussion

Changes in circulating LPS levels have been shown to modulate glucose metabolism [16, 19, 23, 24, 27, 33, 34]. The particular effect of LPS on glucose homeostasis can vary greatly depending on both the duration and intensity of the increase in LPS. LPS concentrations in the blood increase following meals and do so to a higher degree following high-fat meals [6, 8, 20, 35]. Chronically elevated LPS levels have long been shown to have deleterious effects on insulin signaling and glucose homeostasis [8, 12, 16, 19, 27]. Less is known about the effects of modest, short-term elevations in LPS on glucose flux. Research using septic (very high) levels of LPS cause hyperglycemia in the short-term and hypoglycemia in the long-term [36, 37] but these levels of LPS are not physiologically relevant to metabolic disease. Studies using acute low-dose LPS treatments that would be considered more physiologically relevant, in the context of obesity and type 2 diabetes, have resulted in improvements in glucose clearance [23, 38, 39]; however, other work has argued that low-doses are sufficient to initiate an inflammatory response that is deleterious to glucose homeostasis [25, 26, 40] and can cause hypoglycemia. These results show a disparity in what is defined as a “low-dose” or a “high dose” and these differences make it difficult to discern the exact role of LPS on glucose metabolism. The goal of the present studies was to provide a more thorough understanding of the effects of a short-term, low magnitude change in LPS that has been seldom used in previous studies and its effects on glucose metabolism.

Herein, we show a role for low magnitude short-term changes in LPS in contributing to significant alterations in glucose metabolism in a C57/Bl6 mouse. These studies used a low dose LPS treatment (0.1µg/kg) that resulted in significant improvements in glucose tolerance (Figure 3A) that were independent of changes in insulin secretion (Figure 4A), insulin signaling, nor glucose uptake into the primary insulin dependent tissues of skeletal muscle, white adipose and liver.

These findings represent a lack of consensus with previous studies using similar and higher dosages of LPS over a similar time course. Certain of these studies have shown an enhancement in glucose tolerance attributed primarily to higher insulin levels [38, 41], whilst others have shown when using a similar acute dose of endotoxin in mice decreases in muscle glucose uptake and whole-body glucose clearance [25, 26]. The current study found enhancements in glucose tolerance without differences in tissue specific uptake of into any

tissues. This disagreement in findings could largely be explained by differences in methodology as Otero et al. infused the LPS via a catheter [25] whereas our studies were done using I.P. injections and could contribute to different levels of LPS reaching the periphery. The dosage of endotoxin used in the current studies is similar to that used in previous studies from our laboratory and offers further proof of physiologically relevant dosages of LPS having significant effects on glucose metabolism.

We attribute the effect of LPS on glucose tolerance, at least partially to a decrease in endogenous glucose production. This is supported by the finding that fasting blood glucose levels in the LPS treated animals were significantly lower than saline treated animals (Figure 7), and that, LPS treated animals show decreases in endogenous glucose production when subjected to a pyruvate challenge (Figure 8). Along with the decrease in gluconeogenesis, the LPS treatment caused changes in the expression of genes in the liver involved in glucose handling under both insulin and non-insulin stimulated conditions. This includes a decrease in mRNA levels of Pyruvate carboxylase (gluconeogenesis) under insulin- stimulated conditions and Phosphofructokinase (glycolysis) under non-insulin-stimulated conditions. There were also decreases in the expression of the glucose transporter 2 under both conditions (Figures 9 and 10). These results differ slightly from the observations of others that have used much higher doses of LPS to suppress gluconeogenesis and showed increases in glycolytic genes such as Phosphofructokinase-1 and decreases in gluconeogenesis genes Fructose 2, 6 bisphosphate, Phosphoenolpyruvate carboxy kinase, and Glucose 6 phosphate. [21, 22, 42, 43]. Our studies showed decreases in glycolytic and gluconeogenic genes with no change in *Pepck*. These differences are explained by the use of a much higher dose of LPS than in the present study (4–20mg/kg vs. 1ug/kg). These data suggest that low-dose LPS of the magnitude used in the current studies may be sufficient to cause the suppression of gluconeogenesis independent of the concomitant changes in expression of key enzymes. Further studies should seek to investigate the activity of these enzymes in addition to their relative expression levels.

While the significant decreases in gluconeogenesis caused by low-dose LPS treatment could possibly explain the significant changes in whole-body glucose metabolism, there remain other mechanisms that could contribute to this effect. Of particular interest is the role of the immune cell and immune cell modulators. Low-doses of LPS, similar to those used here have been shown to activate immune cells [44–47], particularly macrophages, causing significant increases in the activity and uptake of glucose [48]. Activation of macrophages and other immune cells was not measured in the current study and represents an important limitation of the study as this could partially explain the increase in glucose clearance with these animals. Gene expression of inflammatory markers in the liver of LPS treated animals suggests a heightened inflammatory response in the liver under basal and insulin-stimulated conditions (Figures 9 and 10). Importantly, expression of *Tnfa* was increased under both insulin and non-insulin stimulated conditions. *Tnfa* has a well-characterized role in inhibiting gluconeogenesis through activation of the Nuclear factor kappa-B and the subsequent inhibition of Glucose 6 phosphatase and *Pepck* [49, 50]. The Nuclear factor kappa-B wasn't measured in the current study and could partially explain the suppression of gluconeogenesis.

The primary strength of our study was the systematic approach we used to interrogate the mechanism of improved glucose tolerance using low-dose LPS concentrations synonymous with metabolic endotoxemia. Primary weaknesses of our study design include the uncertainty of blood LPS concentration that resulted from i.p. delivery, and that we did not assess whole-body insulin sensitivity in response to low levels of LPS, which would have allowed for a more specific and perhaps more accurate portrayal of whole body glucose metabolism. Furthermore, assessment of insulin signaling under conditions other than a glucose tolerance test (e.g., insulin tolerance test) may have provided for a more robust investigation of LPS-mediated effects insulin stimulated glucose metabolism.

In summary, research regarding the role of low magnitude, short-term changes in LPS such as those used in the current study on mediating changes in glucose metabolism has been largely non-existent in animal models. Most work offering insight into the relationship between changes in LPS levels and subsequent changes in glucose metabolism has been done using very-high doses, characteristic of conditions such as septicemia. More recent work using similar doses has shown vastly different results that could be attributed to methodology. The present 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. From a translational perspective, this work furthers our understanding of the physiological role of acute and low magnitude changes in blood endotoxin levels on glucose homeostasis. However, it remains unclear how these observations in rodents would translate to humans. To this end, future studies that interrogate whole-body glucose clearance and skeletal muscle insulin signaling during the postprandial state, and how this may differ in insulin sensitive vs. insulin insensitive humans relative to circulating endotoxin levels may prove telling in this regard. An ideal approach would be to assess insulin sensitivity in humans using the hyperinsulinemic-euglycemic clamp under conditions of experimentally induced metabolic endotoxemia, however such studies may be problematic due to subject burden.

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References

1. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European cytokine network*. 2006; 17:4–12. [PubMed: 16613757]
2. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, et al. IKK-beta links inflammation to obesity-induced insulin resistance. *Nature medicine*. 2005; 11:191–198.
3. Oberholzer A, Oberholzer C, Moldawer LL. Cytokine signaling--regulation of the immune response in normal and critically ill states. *Critical care medicine*. 2000; 28:N3–N12. [PubMed: 10807312]
4. Vors C, Pineau G, Drai J, Meugnier E, Pesenti S, Laville M, et al. Postprandial Endotoxemia Linked With Chylomicrons and Lipopolysaccharides Handling in Obese Versus Lean Men: A Lipid Dose-Effect Trial. *The Journal of clinical endocrinology and metabolism*. 2015; 100:3427–3435. [PubMed: 26151336]

5. Al-Disi DA, Al-Daghri NM, Khan N, Alfadda AA, Sallam RM, Alsaif M, et al. Postprandial Effect of a High-Fat Meal on Endotoxemia in Arab Women with and without Insulin-Resistance-Related Diseases. *Nutrients*. 2015; 7:6375–6389. [PubMed: 26247966]
6. Anderson AS, Haynie KR, McMillan RP, Osterberg KL, Boutagy NE, Frisard MI, et al. Early skeletal muscle adaptations to short-term high-fat diet in humans before changes in insulin sensitivity. *Obesity*. 2015; 23:720–724. [PubMed: 25820254]
7. Schwander F, Kopf-Bolanz KA, Buri C, Portmann R, Egger L, Chollet M, et al. A dose-response strategy reveals differences between normal-weight and obese men in their metabolic and inflammatory responses to a high-fat meal. *The Journal of nutrition*. 2014; 144:1517–1523. [PubMed: 24812072]
8. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*. 2007; 56:1761–1772. [PubMed: 17456850]
9. Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *European journal of clinical investigation*. 2002; 32(Suppl 3):14–23. [PubMed: 12028371]
10. Wu Y, Wu T, Wu J, Zhao L, Li Q, Varghese Z, et al. Chronic inflammation exacerbates glucose metabolism disorders in C57BL/6J mice fed with high-fat diet. *The Journal of endocrinology*. 2013; 219:195–204. [PubMed: 24029730]
11. Khodabandehloo H, Gorgani-Firuzjaee S, Panahi G, Meshkani R. Molecular and cellular mechanisms linking inflammation to insulin resistance and beta-cell dysfunction. *Translational research : the journal of laboratory and clinical medicine*. 2016; 167:228–256. [PubMed: 26408801]
12. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008; 57:1470–1481. [PubMed: 18305141]
13. Moreno-Navarrete JM, Manco M, Ibanez J, Garcia-Fuentes E, Ortega F, Gorostiaga E, et al. Metabolic endotoxemia and saturated fat contribute to circulating NGAL concentrations in subjects with insulin resistance. *International journal of obesity*. 2010; 34:240–249. [PubMed: 19949414]
14. Khan KN, Kitajima M, Hiraki K, Fujishita A, Sekine I, Ishimaru T, et al. Toll-like receptors in innate immunity: role of bacterial endotoxin and toll-like receptor 4 in endometrium and endometriosis. *Gynecologic and obstetric investigation*. 2009; 68:40–52. [PubMed: 19365133]
15. Fitzgerald KA, Rowe DC, Golenbock DT. Endotoxin recognition and signal transduction by the TLR4/MD2-complex. *Microbes and infection / Institut Pasteur*. 2004; 6:1361–1367.
16. Mehta NN, McGillicuddy FC, Anderson PD, Hinkle CC, Shah R, Pruscino L, et al. Experimental endotoxemia induces adipose inflammation and insulin resistance in humans. *Diabetes*. 2010; 59:172–181. [PubMed: 19794059]
17. Pilon G, Charbonneau A, White PJ, Dallaire P, Perreault M, Kapur S, et al. Endotoxin mediated-iNOS induction causes insulin resistance via ONOO(–) induced tyrosine nitration of IRS-1 in skeletal muscle. *PLoS One*. 2010; 5:e15912. [PubMed: 21206533]
18. Tweedell A, Mulligan KX, Martel JE, Chueh F-Y, Santomango T, McGuinness OP. Metabolic response to endotoxin in vivo in the conscious mouse: role of interleukin-6. *Metabolism: clinical and experimental*. 2011; 60:92–98. [PubMed: 20102773]
19. Bandsma RH, Ackerley C, Koulajian K, Zhang L, van Zutphen T, van Dijk TH, et al. A low-protein diet combined with low-dose endotoxin leads to changes in glucose homeostasis in weanling rats. *American journal of physiology Endocrinology and metabolism*. 2015; 309:E466–E473. [PubMed: 26152763]
20. Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie*. 2016; 124:11–20. [PubMed: 26133659]
21. Ceppi ED, Knowles RG, Carpenter KM, Titheradge MA. Effect of treatment in vivo of rats with bacterial endotoxin on fructose 2,6-bisphosphate metabolism and L-pyruvate kinase activity and flux in isolated liver cells. *Biochem J*. 1992; 284:761–766. [PubMed: 1320377]

22. Knowles RG, McCabe JP, Beevers SJ, Pogson CI. The characteristics and site of inhibition of gluconeogenesis in rat liver cells by bacterial endotoxin. Stimulation of phosphofructokinase-1. *Biochem J.* 1987; 242:721–728. [PubMed: 2954543]
23. Lang CH, Spitzer JA. Glucose kinetics and development of endotoxin tolerance during long-term continuous endotoxin infusion. *Metabolism: clinical and experimental.* 1987; 36:469–474. [PubMed: 3574135]
24. Jones CG, Titheradge MA. The effect of treatment of the rat with bacterial endotoxin on gluconeogenesis and pyruvate metabolism in subsequently isolated hepatocytes. *The Biochemical journal.* 1993; 289(Pt 1):169–172. [PubMed: 8424754]
25. Otero YF, Mulligan KX, Barnes TM, Ford EA, Malabanan CM, Zong H, et al. Enhanced Glucose Transport, but not Phosphorylation Capacity, Ameliorates Lipopolysaccharide-Induced Impairments in Insulin-Stimulated Muscle Glucose Uptake. *Shock.* 2016; 45:677–685. [PubMed: 26682946]
26. Mulligan KX, Morris RT, Otero YF, Wasserman DH, McGuinness OP. Disassociation of muscle insulin signaling and insulin-stimulated glucose uptake during endotoxemia. *PLoS One.* 2012; 7:e30160. [PubMed: 22276152]
27. Frisard MI, McMillan RP, Marchand J, Wahlberg KA, Wu Y, Voelker KA, et al. Toll-like receptor 4 modulates skeletal muscle substrate metabolism. *American journal of physiology Endocrinology and metabolism.* 2010; 298:E988–E998. [PubMed: 20179247]
28. House LM 2nd, Morris RT, Barnes TM, Lantier L, Cyphert TJ, McGuinness OP, et al. Tissue inflammation and nitric oxide-mediated alterations in cardiovascular function are major determinants of endotoxin-induced insulin resistance. *Cardiovascular diabetology.* 2015; 14:56. [PubMed: 25986700]
29. Meinz H, Lacy DB, Ejiofor J, McGuinness OP. Alterations in hepatic gluconeogenic amino acid uptake and gluconeogenesis in the endotoxin treated conscious dog. *Shock.* 1998; 9:296–303. [PubMed: 9565259]
30. Lansey MN, Walker NN, Hargett SR, Stevens JR, Keller SR. Deletion of Rab GAP AS160 modifies glucose uptake and GLUT4 translocation in primary skeletal muscles and adipocytes and impairs glucose homeostasis. *American journal of physiology Endocrinology and metabolism.* 2012; 303:E1273–E1286. [PubMed: 23011063]
31. Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nature medicine.* 2007; 13:332–339.
32. Matsuzawa N, Takamura T, Kurita S, Misu H, Ota T, Ando H, et al. Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. *Hepatology.* 2007; 46:1392–1403. [PubMed: 17929294]
33. Cani PD, Osto M, Geurts L, Everard A. Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. *Gut microbes.* 2012; 3:279–288. [PubMed: 22572877]
34. Agwunobi AO, Reid C, Maycock P, Little RA, Carlson GL. Insulin resistance and substrate utilization in human endotoxemia. *The Journal of clinical endocrinology and metabolism.* 2000; 85:3770–3778. [PubMed: 11061537]
35. Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, et al. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes care.* 2009; 32:2281–2287. [PubMed: 19755625]
36. Vary TC, Drnevich D, Jurasinski C, Brennan WA Jr. Mechanisms regulating skeletal muscle glucose metabolism in sepsis. *Shock.* 1995; 3:403–410. [PubMed: 7656063]
37. Van Cromphaut SJ, Vanhorebeek I, Van den Berghe G. Glucose metabolism and insulin resistance in sepsis. *Current pharmaceutical design.* 2008; 14:1887–1899. [PubMed: 18691100]
38. Nguyen AT, Mandard S, Dray C, Deckert V, Valet P, Besnard P, et al. Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: involvement of the GLP-1 pathway. *Diabetes.* 2014; 63:471–482. [PubMed: 24186868]

39. van der Crabben SN, Blumer RM, Stegenga ME, Ackermans MT, Endert E, Tanck MW, et al. Early endotoxemia increases peripheral and hepatic insulin sensitivity in healthy humans. *The Journal of clinical endocrinology and metabolism*. 2009; 94:463–468. [PubMed: 18984669]
40. Nohr MK, Dudele A, Poulsen MM, Ebbesen LH, Radko Y, Christensen LP, et al. LPS-Enhanced Glucose-Stimulated Insulin Secretion Is Normalized by Resveratrol. *PLoS One*. 2016; 11:e0146840. [PubMed: 26751381]
41. Kahles F, Meyer C, Mollmann J, Diebold S, Findeisen HM, Lebherz C, et al. GLP-1 secretion is increased by inflammatory stimuli in an IL-6-dependent manner, leading to hyperinsulinemia and blood glucose lowering. *Diabetes*. 2014; 63:3221–3229. [PubMed: 24947356]
42. Maitra SR, Gestring ML, El-Maghrabi MR, Lang CH, Henry MC. Endotoxin-induced alterations in hepatic glucose-6-phosphatase activity and gene expression. *Molecular and cellular biochemistry*. 1999; 196:79–83. [PubMed: 10448905]
43. Wang LF, Yang GQ, Yang S, Yang GY, Li M, Zhu HS, et al. Alteration of factors associated with hepatic gluconeogenesis in response to acute lipopolysaccharide in dairy goat. *Journal of animal science*. 2015; 93:2767–2777. [PubMed: 26115264]
44. Baker B, Maitra U, Li L. Molecular mechanisms responsible for low-grade cellular stress induced by super low dose lipopolysaccharide (INM2P.421). *The Journal of Immunology*. 2014; 192:56.4.
45. Hirohashi N, Morrison DC. Low-dose lipopolysaccharide (LPS) pretreatment of mouse macrophages modulates LPS-dependent interleukin-6 production in vitro. *Infection and immunity*. 1996; 64:1011–1015. [PubMed: 8641750]
46. Morris MC, Gilliam EA, Button J, Li L. Dynamic modulation of innate immune response by varying dosages of lipopolysaccharide (LPS) in human monocytic cells. *The Journal of biological chemistry*. 2014; 289:21584–21590. [PubMed: 24970893]
47. Fukuzumi M, Shinomiya H, Shimizu Y, Ohishi K, Utsumi S. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. *Infection and immunity*. 1996; 64:108–112. [PubMed: 8557327]
48. Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, et al. Feeding our immune system: impact on metabolism. *Clinical & developmental immunology*. 2008; 2008:639803. [PubMed: 18350123]
49. Grempler R, Kienitz A, Werner T, Meyer M, Barthel A, Ailett F, et al. Tumour necrosis factor alpha decreases glucose-6-phosphatase gene expression by activation of nuclear factor kappaB. *The Biochemical journal*. 2004; 382:471–479. [PubMed: 15167811]
50. Metzger S, Nusair S, Planer D, Barash V, Pappo O, Shilyansky J, et al. Inhibition of hepatic gluconeogenesis and enhanced glucose uptake contribute to the development of hypoglycemia in mice bearing interleukin-1beta- secreting tumor. *Endocrinology*. 2004; 145:5150–5156. [PubMed: 15297440]

Figure 1. Protein content of insulin-signaling markers is unaffected by either acute or chronic LPS treatments in a Human Primary cell line

Serum-starved cells were treated for either 2 hours (A and C) or 24 hours (B and D) with low-dose LPS followed by a 10-minute insulin treatment. Cells were then harvested and P-AKT and P-IRS1 protein expression were measured via western blotting during both 2 hour (A and C) and 24 hour (B and D). Data are presented as mean $\pm$ SEM. *, $p < 0.05$.

Figure 2. Chronic LPS treatments contribute to decreases in insulin-stimulated glycogen synthesis in a Human Primary skeletal muscle cell line

Serum-starved cells were treated with LPS for either 2 or 24 hours then incubated for 3 hours with [U- 14 C]-glucose. Cells were then washed and harvested and glycogen synthesis (A and B) was measured. (C) Represents fold change in response to insulin between control and LPS treatments in the 2 hour and 24 hour groups. Data are presented as mean $\pm$ SEM. Different from control. *, $p < 0.05$.

Figure 3. Mice injected with low-dose LPS exhibit improved glucose tolerance compared with WT littermates

C57/Bl6J mice were injected with low dose (0.1 ug/kg) LPS ($n=10$) or saline ($n=12$) followed 4 hours later by GTT (A). (B) Represents area under curve for GTT graph. Data are presented as mean $\pm$ SEM. (Significantly decreased from control. ***, $p<0.01$.)

Figure 4. Serum insulin levels and plasma membrane Glut4 protein are unchanged while phosphorylated and total-AKT protein expression levels are up-regulated following LPS treatment

(A) Serum insulin levels were measured in blood from LPS ($n=14$) or saline ($n=13$) injected animals. (B–D) Protein expression levels of P-AKT, Total-AKT, and plasma membrane bound Glut4. (E) Corresponding western blots of these proteins. Data are presented as mean $\pm$ SEM. Different from control. *, $p<0.05$.

Figure 5. LPS-injected animal's exhibit enhanced glucose tolerance during glucose uptake assay compared to WT littermates

(A) Glucose tolerance curve of saline (n=5) or LPS (n=5) injected animals, 4hours post-injection. (B) Blood glucose levels of animals 15 (B) and 30 (C) minutes after glucose injection. Data are presented as mean $\pm$ SEM. *P .05.

Figure 6. Uptake of 2-Deoxyglucose into muscle and adipose tissues and incorporation into liver glycogen is unaffected by LPS treatment

Uptake of 2-DG 4 hours following LPS (n=5) or saline (n=5) injection in (A) quadriceps, (B) gastrocnemius, (C) white adipose tissue, (D) brown adipose tissue, and (E) 2-DG incorporation into liver glycogen. Data are presented as mean $\pm$ SEM. *, $p < 0.05$.

Figure 7. Fasting blood glucose levels are significantly lower in LPS versus saline treated animals
Fasting blood glucose levels from LPS ($n=10$) or saline ($n=12$) treated animals 4 hours after injection. Data are presented as mean $\pm$ SEM. *, $p < 0.05$.

Figure 8. LPS treated animals exhibit decreased endogenous glucose production following pyruvate challenge

Overnight fasted animals were injected with LPS (n=6) or saline (n=6) then 4 hours later injected with sodium pyruvate to assess their abilities to perform gluconeogenesis. Data are presented as mean $\pm$ SEM. * p <0.05, ** p <0.01.

Figure 9. LPS treatment causes significant changes in the expression of genes involved in liver glucose flux and inflammation under non-insulin stimulated conditions

Expression of genes involved in gluconeogenesis (A and B), glycolysis (C and D), and inflammation (E and F) from liver of animals harvested 4hours after injection with LPS ($n=10$) or saline ($n=12$). Data presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 10. LPS treatment causes significant changes in the expression of genes involved in liver glucose flux and inflammation under insulin-stimulated conditions

Expression of genes involved in gluconeogenesis (A and B), glycolysis (C and D), and inflammation (E and F) from liver of animals harvested 4 hours after injection with LPS ($n=10$) or saline ($n=12$) and 30 minutes following injection of glucose. Data presented as mean $\pm$ SEM. * $p<0.05$.