# Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: Involvement of the glucagon-like peptide 1 (GLP1) pathway.

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# Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: Involvement of the glucagon-like peptide 1 (GLP1) pathway.

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Running title: LPS enhances Glucagon-like peptide 1

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**Abstract.**

Lipopolysaccharides (LPS) of the cell wall of Gram (-) bacteria trigger inflammation, which is associated with marked changes in glucose metabolism. Hyperglycemia is frequently observed during bacterial infection and it is a marker of a poor clinical outcome in critically ill patients. The aim of the present study was to investigate the effect of an acute injection or continuous infusion of LPS on experimentally-induced hyperglycemia in wild-type and genetically-engineered mice. The acute injection of a single dose of LPS produced an increase in glucose disposal and glucose-stimulated insulin secretion (GSIS). Continuous infusion of LPS through mini-osmotic pumps was also associated with increased GSIS. Finally, manipulation of LPS detoxification by knocking out the plasma phospholipid transfer protein (PLTP) led to increased glucose disposal and GSIS. Overall, glucose tolerance and GSIS tests supported the hypothesis that mice treated with LPS develop glucose-induced hyperinsulinemia. The effects of LPS on glucose metabolism were significantly altered as a result of either the accumulation or antagonism of glucagon-like peptide 1 (GLP1). Complementary studies in wild-type and GLP1-R knockout mice further implicated the GLP1R-dependent pathway in mediating the LPS-mediated changes in glucose metabolism. Hence, enhanced GLP1 secretion and action underlies the development of glucose-mediated hyperinsulinemia associated with endotoxemia.

**Introduction**

Lipopolysaccharides (LPS) or endotoxins are components of the cell wall of Gram (-) bacteria. When LPS enters the bloodstream, it activates Toll-like receptor 4 (TLR4) located at

the surface of immune cells, leading to the release of pro-inflammatory cytokines and to inflammation (1). Excessive inflammatory response to LPS can be harmful and may lead to endotoxic shock and ultimately death. Because Gram (-) bacteria are present in large amounts in the gut lumen and the environment, bacterial translocation and the resulting endotoxemia can occur as a result of inflammatory bowel disease (2), a high-fat diet (3) or cigarette smoking (4). Whatever the severity of the insult, endotoxemia is accompanied by marked metabolic alterations, including changes in glucose metabolism, which is of particular importance. Early hyperglycemia is a common metabolic feature following exposure to bacterial endotoxins (5). It is known to contribute to poor immune function, oxidative stress, increased susceptibility to infectious complications, impaired recovery of organ failure, and endothelial and myocardial dysfunction (6; 7). In critically ill patients, hyperglycemia is not only a marker of illness severity but also a predictor of a poor clinical outcome (7). Although effective glucose control through aggressive insulin therapy has been reported to improve clinical outcome, it remains unclear to what extent insulin infusion may in fact be beneficial (8). Hyperglycemia, moderate to severe hypoglycemia and hyperinsulinemia have all been found to be associated with an increased risk of death in critically ill patients admitted to the intensive care unit (9; 10). In LPS-treated mice, a decrease in both inflammation and mortality was reported when the plasma glucose level was strictly maintained at normal values, suggesting that appropriate/tight glucose control is a major determinant of outcome (11). Whether abnormalities in glucose control may vary according to the endotoxemic insult is unknown, and the molecular mechanisms involved are poorly understood.

In the present study, the glucose and insulin responses to experimentally-induced hyperglycemia were investigated in LPS-treated mouse models. Endotoxins were administered through either the acute i.p. injection of a single dose of LPS, a continuous i.p. infusion of a low dose of LPS using mini-osmotic pumps, or the use of the phospholipid

transfer protein-knocked out (PLTP-KO) mouse model, which is known to display an impaired capacity to inactivate and detoxify LPS in the body (12). In all of the models used in the present study, we found that LPS increased GSIS, which has been shown to be due, at least in part, to an increase in the level and activity of glucagon-like peptide 1 (GLP1).

## Research design and methods

### Animals

Five to six-month-old WT mice (Charles River, L'Arbresle, France), GLP1R-KO mice (13) and PLTP-KO mice (a kind gift of Dr Jiang (14)) from a homogeneous C57BL6/J background were housed in a controlled environment. All of the animal experimental procedures below were validated by the local ethics committee of the University of Burgundy (protocol number 1209). Mice had free access to water and standard diet-A03-10-Rats and Mice Breeder Diet (SAFE, AUGY, France). The diet contained 21.4% protein, 5.7% mineral, 5.1% fat, 4% cellulose and mixture of vitamins and micro-nutrients.

**Surgical procedures and injections/infusions of LPS**. LPS were from *Escherichia coli* 055:B5 (Sigma, St. Louis, MO). The mice were either given intraperitoneal injections of LPS (0.5, 1, or 2mg/kg) or had a mini-osmotic pump implanted subcutaneously (Alzet® Model 2004; Alza, Palo Alto, CA) and linked to the peritoneal cavity by a catheter. The pumps were filled with either NaCl (0.9%) or LPS to infuse 300 or 1000µg.kg$^{-1}$.day$^{-1}$ for 4 weeks. After 4 weeks, plasma samples were drawn and frozen at -20°C. The mice were then killed and muscle tissue and the liver were immediately excised, immersed in liquid $N_2$ and stored at −80°C for further mRNA analyses.

**Glucose tolerance test.** After fasting overnight, the mice were fed with glucose by oral gavage (1.5-2g/kg). Blood was drawn from the tail vein at 0, 15, 30, 60, 90 and 120 min following the glucose load, and glucose concentration was determined with a glucose meter (One Touch Ultra®). Oral glucose tolerance tests (OGTT) were performed either 6 hours after the acute i.p injection of LPS or at the end of the 28-day infusion period. In some experiments, Sitagliptin (Januvia®, Merck Sharp and Dohme-Chibret, France) (5mg/kg) or Exendin$^{9-39}$ (5 µg/mouse) was administered via oral gavage or i.p. injection, respectively, 30

minutes prior to the glucose gavage. NaCl (0.9%) was administered as a control. The area under the curve (AUC) (0-120) was calculated for each group of mice, and the plasma insulin concentration was measured 30 minutes before and 15 minutes after the glucose load. The insulinogenic index (i.e. an index of β-cell function) was calculated as the delta insulin to the delta glucose ratio ($\Delta I_{0-15}/\Delta G_{0-15,}$ pmol/mmol)

**Insulin tolerance test**. After 6h of fasting, mice were given an intraperitoneal injection of 0.5U/kg insulin (Humalog®), and blood glucose was measured as mentioned above.

**Plasma GLP1 concentration**. After fasting overnight, mice were given an injection of the DPP4 inhibitor sitagliptin (5mg/kg). One hour later, the mice were given an intraperitoneal injection of LPS (2mg/kg) or NaCl (0.9%) as the control. Six hours after the LPS or NaCl injection, the mice were given 2g/kg of glucose through oral gavage. Blood samples were collected in EDTA-containing tubes supplemented with the DPP4 inhibitor (Millipore, France), 30 minutes before and 15 minutes after the glucose load to assess the plasma GLP1 concentration.

**Real-time quantitative PCR**. Total RNA was isolated from tissues using Trizol reagent (Invitrogen, France). Total RNA (0.5µg) was reverse-transcribed using MMLV reverse-transcriptase (Invitrogen, France) and random primers at 42°C for 1 h. QPCRs were performed using ABI Prism 7900 (Applied Biosystems, France). Primer sequences for the targeted mouse genes are available upon request (jacques.grober@u-bourgogne.fr). The mRNA expression level of target genes was normalized to levels of β-actin mRNA and the results were expressed as relative expression levels. The data were quantified by the method of $2^{-\Delta\Delta Ct}$.

**Glucose uptake in muscle**. Muscles were isolated and preincubated for 10 min in Krebs-Henseleit buffer (pH 7.4) containing 2 mg/mL BSA, 2 mmol/L sodium pyruvate, and 20

mmol/L HEPES. The muscles were then incubated for 45 min in the absence or in the presence of 100 nmol/L insulin as previously described (15).

**Biochemical analyses**.

LPS concentrations were measured in total plasma, high-density lipoproteins (HDL) and the lipoprotein-free fraction (LFF) through the quantitation of 3-β-hydroxymyristate concentration according to the general procedure previously described (12). Plasma insulin and GLP1 concentration were determined by ELISA (ALPCO, Salem, USA), in accordance with the manufacturer's instructions. Muscle glycogen content was determined with the Glycogen synthase assay kit (Biodivision, Mountain View, USA), in accordance with the manufacturer's instructions. The concentrations of interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα,) were measured in mouse plasma by Milliplex® Map 5-Plex kit (Mouse Cytokine/Chemokine Magnetic Bead Panel) from Millipore (Millipore, Billerica, MA, USA) according to the manufacturer's protocol and using a LuminexR apparatus (Bio-Plex® 200, BioRad)

**Statistical analyses**. Results are presented as mean ± SEM. Statistical significance of differences was analyzed by the unpaired *t* test.

## Results

### Acute i.p injection of LPS enhances glucose-stimulated insulin secretion.

To evaluate the glucose and insulin responses during LPS-mediated inflammation, wild-type mice were given a single acute i.p injection of 2 mg/kg of LPS. Plasma β-hydroxymyristate content, reflecting plasma LPS level was markedly increased upon LPS injection (603.2 ±12.2 ng/mL in LPS-injected mice (n=8) versus 68.4 ± 16.2 ng/mL in NaCl controls (n=8); $p<0.001$). Six hours after the LPS injection, hyperglycemia was induced by gavage with a glucose solution (2g/kg). As shown in Figure 1A and B, the glucose tolerance curve was improved with the LPS injection, and glucose-stimulated insulin secretion (GSIS) increased (Fig 1C). Consistent results were obtained by using two lower doses of LPS. Whereas in fasting conditions, no significant differences were observed in insulin plasma levels upon LPS injection of either 0.5mg/kg or 1mg/kg of LPS (data not shown), again, and with either dose of LPS, GSIS after the glucose load was significantly enhanced as revealed by the insulin plasma levels at t=15min (766.9 ± 125.5 pmol/L in LPS-injected mice (n=10) versus 434 ± 37.49 pmol/L in NaCl controls (n=9), $p<0.05$ with the 0.5 mg/kg dose; 666.5 ± 89.39 pmol/L in LPS-injected mice (n=10) versus 373.8 ± 31.16 pmol/L in NaCl controls (n=10), $p<0.01$ with the 1 mg/kg dose). The function of pancreatic β-cells was evaluated by calculating the insulinogenic index (IGI) at the 15-min time point (*i.e.* in the very early phase of insulin secretion). As shown in Figure 1D, the acute i.p. injection of LPS increased the IGI.

### Continuous i.p. infusion of LPS increases glucose-stimulated insulin secretion

We next assessed the consequences of sustained LPS administration by implanting mice with mini-osmotic alzet® pumps that continuously delivered LPS or NaCl for 28 days (300µg/kg/day). This LPS dose was reported to mimic the metabolic endotoxemia induced by a high fat diet (3). Plasma β-hydroxymyristate content was modestly but significantly increased upon LPS infusion (LPS-infused mice (n=7): 51.9 ± 3.9 ng/mL NaCl controls

(n=4): 35.8 ± 1.3 ng/mL $p<0.05$). In contrast to the acute i.p. injection of LPS (Fig 1A), the continuous i.p. infusion of LPS did not improve the glycemic response during the OGTT (Fig 2A), as indicated by similar values for the area under the curve (Fig 2B). However, GSIS and IGI were increased with LPS treatment, although to a lesser extent than with acute LPS injection (Fig 2C,D vs. Fig 1C,D). Using a higher dose of LPS (1mg/kg/day) during continuous infusion led to the same enhancement of GSIS after a glucose load as shown by the insulin plasma levels at t=15min (LPS-infused mice (n=4): 892.5 ± 131.4 pmol/L; NaCl controls (n=4): 431.8 ± 10.1 pmol/L, $p<0.05$).

**Reduced LPS neutralization and detoxification in PLTP-KO mice is associated with amplification of glucose and insulin responses**

To further evaluate the impact of LPS on glucose and insulin responses, we took advantage of the PLTP-KO mouse model, which displays a reduced capacity to detoxify LPS (12). After acute i.p. LPS injection, plasma β-hydroxymyristate content was higher in PLTP-KO mice as compared to WT mice (371.5± 24.3 ng/mL in PLTP-KO mice (n=6) versus 298.7 ± 23.8 ng/mL in WT controls (n=6), $p<0.05$). In the absence of LPS, PLTP expression *per se* did not modify the glucose response to oral glucose challenge (Fig 3A and 3B, "NaCl"). In LPS-treated mice, glucose clearance was increased to a significantly greater extent in PLTP-KO mice than in wild-type mice (Fig 3A and 3B, "LPS"). Whereas GSIS was not dependent on PLTP expression in the absence of the LPS challenge (Fig 3C, NaCl), insulin levels were significantly greater in PLTP-KO mice after acute LPS injection (Fig 3C, LPS). Similarly, the IGI was significantly increased after acute i.p. injection of LPS in WT and in PLTP KO mice (Fig 3D).

We next assessed the consequences of chronic LPS infusion in WT and PLTP-KO mice. Continuous infusion of a low dose of LPS did not lead to significant differences in LPS plasma levels between WT and PLTP-KO mice after 28 days of LPS infusion (52.3± 2.6 ng/mL in PLTP-KO mice (n=8), versus 56.6 ± 3.5 ng/mL in WT mice (n=7), ns). However, and as PLTP is involved in LPS transfer between the plasma lipoprotein-free fraction (LFF) and plasma lipoproteins, we next determined whether this experimental set-up was effective to modulate LPS distribution between high density lipoproteins (HDL) and LFF as reported earlier with acute i.p. injection (12). In agreement with earlier observations, PLTP-KO mice that received a continuous infusion of LPS through the implanted mini-osmotic pump displayed a lower 3-β-hydroxymyristic acid content of the plasma HDL fraction (Fig 4A), a higher LPS content of the LFF fraction (Fig 4B) and a lower HDL to LFF LPS ratio (Fig 4C). In further support of the greater pro-inflammatory effect of LPS when infused in mice with a PLTP-deficient trait, liver *Tnf-α* mRNA levels were significantly higher in PLTP-KO than in WT mice (Fig. 4D). Compared with data before pump implantation (Fig 5A, 5B "-LPS"), glucose clearance was increased in LPS-infused WT mice, and the increase was even greater in PLTP-KO mice (Fig. 5A,B "+LPS"). Insulin levels and IGI both increased with LPS infusion, independently of PLTP expression (Fig 5C, D). In contrast, insulin sensitivity was higher in PLTP-KO mice (Fig 5E, F).

Because glucose clearance and insulin sensitivity in LPS-infused PLTP-KO mice were greater than those in LPS-infused WT mice, the ability of muscle to utilize glucose was investigated. As shown in Fig 6A, insulin-mediated glucose uptake was more efficient in PLTP-KO than in WT mice. Consistently, muscle *Glut 4* and *Glycogen synthase-1* mRNA levels were significantly higher in PLTP-KO than in WT mice (Fig. 6B and 6C, respectively). The glycogen content of muscle was also higher in PLTP-KO mice (Fig 6D).

**Accumulation of GLP1 magnified the LPS-mediated increase in GSIS**

As GLP1 was previously identified as a downstream target of inflammatory cytokines that also regulates the magnitude of GSIS (16; 17), we examined the GLP1 axis during an LPS challenge. Under fasting conditions, i.p injection of LPS significantly increased plasma GLP1 levels in WT mice (Fig. 7A left: 6.32 ±0.65 pmol/l in LPS-treated *vs.* 1.16 ±0.17 pmol/l in untreated WT mice; $p<0.01$). Furthermore, plasma GLP1 levels increased further after oral glucose loading in control WT mice, and the increase was significantly greater in LPS-treated WT mice (Fig 7A: 17.8 ±2.98 pmol in LPS-treated *vs.* 4.07± 0.17 pmol in control mice; $P<0.001$). Finally, LPS-induced increases in plasma insulin levels were observed both in fasting and after the glucose load (Fig. 7B), and a significant, positive correlation was found between plasma GLP1 and insulin levels (Fig 7C).

Next, we assessed whether the acute LPS-mediated attenuation of glycemic excursion could be further enhanced by increased levels of active GLP1 achieved through treatment with sitagliptin, a DPP4 inhibitor (18). Glycemic excursion in response to LPS was further reduced by the combination of LPS and sitagliptin (Fig 7D,E).

**Partial antagonism of the GLP1 receptor blocks the LPS effect on glycemic excursion**

To assess the importance of GLP1 as a transducer of the LPS-mediated glycemic response we assessed whether the administration of the GLP1R antagonist exendin$^{(9–39)}$ (Ex-9) could modify the glucoregulatory actions of LPS. Administration of Ex-9 substantially attenuated the LPS-mediated change in glucose disposal (Fig 7F,G ).

**LPS-mediated changes in insulin and glucose responses are blunted in GLP1R-KO mice**

To further investigate the role of the GLP1/GLP1R pathway in the LPS-mediated changes in glucose homeostasis, we assessed LPS action in GLP1R KO mice (19). As compared to NaCl-injected mice which displayed low levels of LPS (WT mice (n=11), 33.6 ± 3.5 ng/mL; GLP1R-KO mice (n=11), 43.9 ± 6.2 ng/mL ns), plasma LPS concentrations were markedly increased after acute i.p. injection of LPS and to a similar extent in WT and GLP1R-KO mice (WT mice (n=11), 362.0 ± 17.5 ng/mL; GLP1R-KO mice (n=11): 352.1 ± 25.2 ng/mL, ns). Interestingly, whereas acute i.p. injection of LPS led to the same LPS plasma level in WT and GLP1R-KO mice, the plasma levels of IL6 and TNFα (i.e. two main proinflammatory cytokine targets of LPS) displayed a more pronounced increase in GLP1R-KO as compared to WT mice (Fig 8A and 8B). The LPS-mediated change in glucose response in WT mice was completely absent in GLP1R KO mice (Fig 8C-8E). Furthermore, the LPS-mediated increase in GSIS observed in WT mice was not observed in GLP1GLP1R-KO mice (Fig. 8F and 8G), and enhanced β-cell function in response to LPS was completely lost in GLP1R-KO mice (Fig 8H).

**Discussion**

In the present study, the insulin response to experimental hyperglycemia was studied in mice with LPS-mediated inflammation. Our data support the hypothesis that LPS increases glucose-stimulated insulin secretion (GSIS), resulting in increased glucose clearance. Consistent observations were made with acute versus continuous LPS infusion and to a greater extent when the phospholipid transfer protein (PLTP)-mediated detoxification pathway of LPS did not operate. The LPS-mediated changes in glucose homeostasis were found to require a functional GLP1R-dependent pathway.

Sepsis is known to impair whole body glucose disposal (20), resulting in hyperglycemia, often associated with metabolic adaptation and increased insulin secretion. However, bacterial infection or LPS administration does not always lead to consistent changes in glucose disposal or insulin secretion (21-28). For instance, the prevention of hyperglycemia by depleting glycogen storage did not abrogate the increase in serum insulin levels in endotoxemic animals (29). This indicates that the relationship between endotoxemia and glucose homeostasis is rather complex, and one hypothesis holds that the effect of LPS might be dependent on the metabolic context and the experimental conditions used (including in particular the magnitude and duration of the endotoxemic challenge). In the present study, glucose and insulin responses were first investigated in mice given a single intraperitoneal injection of LPS. Six hours after the LPS injection, GSIS significantly increased, and consistent observations were made with distinct LPS doses. GSIS increase was associated with increased glucose disposal. In contrast, in an earlier study, a decrease in glucose disposal was reported 48h after the LPS injection (30). These contrasting observations might be partly related to temporal differences. In support of this hypothesis, plasma LPS concentrations were reported to peak nine hours after a single intraperitoneal injection of LPS in mice, but returned to barely detectable levels after 48 hours (12). In keeping with the above, increased insulin sensitivity was observed 2

hours after LPS injection in humans (31; 32), whereas insulin resistance was observed 24 hours after the initial LPS challenge (33).

The hypothesis of a differential impact of transient versus continuous LPS on glucose metabolism was addressed in the present study by comparing a single injection of LPS with a continuous infusion achieved using mini-osmotic pumps. After 28 days of continuous infusion of LPS, GSIS was found to be significantly increased, and again consistent observations were made with distinct LPS doses. However, there were no changes in glucose disposal. Finally, to further explore the effect of experimental endotoxemia on glucose and insulin responses *in vivo,* we studied PLTP-deficient mice in which the binding of LPS to plasma high density lipoproteins is delayed. LPS-lipoprotein complexes are known to be less pyrogenic and less active in inducing the release of proinflammatory cytokines than is the case for LPS in the plasma lipoprotein-free fraction (34-36), and the PLTP-deficient trait is therefore associated with impaired neutralization and clearance of LPS (12). Interestingly, using both acute injection or continuous intraperitoneal infusion, endotoxemia was consistently associated with greatly increased GSIS and glucose disposal in PLTP-deficient mice. These observations support the hypothesis that GSIS is an adaptive response to endotoxemia.

The underlying mechanism of the LPS-mediated changes in glucose metabolism has been a matter of intense research and speculation. On the one hand, LPS itself might exert a direct insulin-like activity (37-39). Alternatively, LPS might act indirectly through the stimulation of pancreatic insulin secretion, and pancreas excised from LPS-treated animals displays increases in both basal insulin secretion (40; 41) and GSIS (40-42). However, neither perfusion of excised pancreas with LPS (41), nor LPS treatment of cultured pancreatic □-cells (expressing TLR4 (43)) were able to directly increase insulin secretion (44) suggesting a more complex pathway with intermediate mediators. IL-1, which has been shown to increase

insulin secretion in endotoxemic rats (45), and IL-6, which has been shown to promote hypoglycemia during acute LPS-induced inflammation (46) and to enhance insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells (17) are possible glucoregulatory targets of LPS. Although IL-6 secretion is known to be highly inducible by LPS, a LPS challenge was not used in the studies cited above. This led us to investigate whether increased production of GLP1 (a potent inducer of insulin secretion (16)) might underlie the LPS-mediated increase in GSIS. Our data clearly show that LPS injection increases plasma levels of GLP1, under both fasting and glucose-stimulated conditions. Moreover, increased levels of GLP1 correlated positively with increased GSIS and were associated with higher glucose disposal during an oral glucose tolerance test. Second, the glucose response after treatment with LPS was of lower magnitude when GLP1 degradation was prevented with sitagliptin (18). Third, exendin$^{(9–39)}$, an antagonist of the GLP1 receptor, prevented the LPS-mediated change in glucose homeostasis. Finally, the LPS-increased GSIS and glucose disposal was totally blunted in GLP1 receptor-knockout mice. Collectively, these findings argue for an important role for GLP1 as a downstream metabolic target of a subset of LPS actions. Indeed, GLP1 has been shown to enhance glucose uptake, and to increase the conversion of glucose into glycogen (47) in muscle, thus contributing to an overall increase in insulin sensitivity (48). Similar findings were observed in the present study when PLTP-KO mice were continuously infused with LPS. In humans, GLP1 administration has been associated with increased β-cell function as revealed by the increase in the IGI (49). In the present study, the higher IGI after LPS administration was completely abrogated in GLP1R-KO mice which as compared to WT mice displayed a more pronounced increase in plasma levels of IL6 and TNFα after acute LPS injection.

The present study adds to the recently recognized enteroendocrine pathway through which the proinflammatory mediator IL6 (well-known as an LPS target) can contribute to increased

insulin secretion. Because IL6 increases responsiveness to glucose through the increased production of GLP1 by intestinal L cells, IL6 results in increased insulin secretion and production by pancreatic beta cells(17). It is possible that the same molecular mechanism may apply to both the IL6- and the LPS-mediated effects (45 and present study, respectively). It was suggested that IL6 increased GLP1 production through increased proglucagon transcription and PC1/3 expression (17). In keeping with this hypothesis, LPS sensitizes adenyl cyclase activity (50) and increases the production of cAMP (51), which is known to be involved in the regulation of proglucagon gene expression (52). In addition, the proglucagon protein precursor is known to undergo posttranslational processing through the action of two main prohormone convertases (PC), PC1/3 and PC2 (53), Given that both PC1/3 and PC2 are induced when immune cells are exposed to LPS (54; 55), a role for enhanced PC1/3 activity cannot be excluded. Alternatively, neural pathways may also be involved, as sensory nerves have been shown to be involved in the glucose metabolic response to endotoxins (56), and the expression of the neuropeptide calcitonin gene-related (CGRP), which is thought to play a role in the regulation of GLP1 secretion (57), is up-regulated by LPS (58). Finally, a direct effect of the LPS challenge on TLR4 signaling might make a significant contribution to the LPS-induced production of GLP1. Indeed, it has recently been shown that enteroendocrine cells expressed functional TLR4 (59), and hypoglycemia as well as GSIS were reported to be blunted in TLR4-deficient mice (30).

Cumulative evidence in favor of a link between endotoxemia, enteroendocrine cells, and glucose metabolism was recently brought in patients with inflammatory bowel diseases. In Crohn's disease with abnormally elevated endotoxemia (2), enteroendocrine cell activity was reported to be enhanced (60). It was associated with higher expression and circulating levels of GLP1 (60; 61). In addition, DPP4 expression and activity are reduced in patients with Crohn's disease, thus making a putative contribution to increased GLP1 expression levels and

hyperinsulinemia of IBD (62; 63). Observations of the present study come in direct support of sustained endotoxemia as a significant contributor to enhanced insulin secretion, which constitute one main trait of inflammatory bowel diseases and a protective factor on relapse rate (61; 64).

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## Figure Legends

**Figure 1: Acute i.p. injection of LPS enhances GSIS**
**A.** OGTT (2g/kg) performed 6 hours after injection of NaCl (Open circle) (n=6) or LPS 2mg/kg (Closed squares) (n=6) in WT mice. **B**. AUC, area under curves of glucose response expressed as arbitrary units (a.u.). **C.** Plasma insulin levels measured before and 15min after an oral glucose load (2g/kg) in NaCl-injected mice (n=10) and LPS-injected mice (2mg/kg) (n=10). **D.** Insulinogenic index. White bars: NaCl-injected mice; Black bars: LPS-injected mice. Body mass: NaCl:29.2 ± 0.6 g, LPS : 30.0 ± 1.6 g (ns). Values are means ± SEM. *****p<0.05, **p<0.01, ***p<0.001, b,c p<0.001 vs Fasting, d p<0.05 vs NaCl glucose load.

**Figure 2: Continuous i.p. infusion of LPS enhances GSIS.**
WT mice were i.p. infused for 28 days with NaCl or LPS (300µg/kg/day) using miniosmotic alzet®pumps. **A.** OGTT (2g/kg) performed 28 days after a NaCl (Open circle) (n=4) or LPS (Closed squares)(n=7) i.p. infusion in WT mice. **B.** AUC, area under curves of glucose response expressed as arbitrary units (a.u.). **C.** Plasma insulin levels measured before and 15min after an oral glucose load (2g/kg) in NaCl-infused mice (n=4) and LPS-infused mice (black bar) (n=7). **D.** Insulinogenic index. White bars: NaCl-infused mice; Black bars: LPS-infused mice, Body mass: NaCl:26.9± 0.3 g, LPS : 27.3 ± 0.5 g (ns). Values are means ± SEM. b,c p<0.001 vs Fasting, d p<0.05 vs NaCl glucose load. *p<0.05

**Figure 3: GSIS is increased to a greater degree in PLTP-KO mice after acute i.p injection of LPS**
**A.** OGTT (2g/kg) performed 6 hours after i.p injection of NaCl or LPS (2mg/kg) in WT (Open circle) (n=5) or PLTP-KO mice (Closed squares) (n=5). **B.** AUC, area under curves of glucose response expressed as arbitrary units (a.u.). **C.** Plasma insulin levels measured before and 15 min after a glucose load (2g/kg) in WT mice (n=10) and PLTP-KO mice (n=9). **D.** Insulinogenic index. White bars: WT mice; Black bars: PLTP-KO mice. Body mass: WT:30.8 ± 1.4 g, PLTP-KO: 28.0 ± 0.7 g (ns). Values are means ± SEM. *p<0.05, **p<0.01, ***p<0.001, b,f: p<0.001 vs WT NaCl; c,g: p<0.05 vs PLTP KO NaCl; d: p<0.05 vs WT LPS

**Figure 4: Enhanced inflammatory response in PLTP-KO mice i.p. infused with LPS**
β-hydroxymyristate content in High Density Lipoproteins (HDL) (**A)**, lipoprotein free fraction (LFF) **(B)** in WT mice (n=6) and PLTP-KO mice (n=6). **C.** HDL to LFF β-hydroxymyristate ratio. **D.** Liver Tnfα mRNA levels in WT mice (n=7) and PLTP-KO mice (n=8). White bars: WT mice; Black bars: PLTP-KO mice. Body mass: WT: 32.1 ± 0.5 g, PLTP-KO: 31.6 ± 0.6 g (ns). Values are means ± SEM. a: p<0.01 vs WT , b: p=0.09 vs WT.

**Figure 5 Glucose response following i.p infusion with LPS is increased to a greater degree in PLTP-KO mice.**

**A.** OGTT (2g/kg) performed before (-LPS) and at the end (+LPS) of the i.p infusion of LPS (300µg/kg/day) in WT mice (Open circle) (n=6-11) and PLTP-KO mice (Closed squares)(n=7-8) **B.** AUC, area under curves of glucose response expressed as arbitrary units (a.u). **C.** Plasma insulin levels 15 min after an oral glucose load (2g/kg) in WT mice (n=15) and PLTP-KO mice (n=15-16). **D.** Insulinogenic index. **E.** insulin tolerance test at the end of the i.p. infusion of LPS (Open circle: WT mice (n=14); Closed squares: PLTP-KO mice (n=14)). **F.** AUC, area under curves of glucose response expressed as arbitrary units (a.u.). White bars: WT mice; Black bars: PLTP-KO mice. Values are means ±SEM. *p<0.05, **p<0.01; b,f: p>0.001 vs WT (-LPS); c: p<0.001 vs PLTP KO (-LPS); d: p<0.05 vs WT (+LPS); g,j: p<0.05 vs PLTP KO (-LPS); "i: p<0.05 vs WT (-LPS)

**Figure 6: Muscle glucose uptake is increased in PLTP-KO mice i.p infused with LPS**

**A.** Insulin mediated glucose uptake in WT mice (n=7) and in PLTP-KO mice (n=6). **B.** Muscle Glut4 mRNA levels in WT mice (n=7) and in PLTP –KO mice (n=8)**. C.** Muscle Glycogen synthase 1 mRNA levels in WT mice (n=7) and in PLTP-KO mice (n=8). **D**. Glycogen content in muscle in WT mice (n=6) and in PLTP-KO mice (n=8). White bars; WT mice; Black bars: PLTP-KO mice. Values are means ± SEM. *p<0.05

**Figure 7: GLP-1 pathway is involved in LPS-induced increase in GSIS**

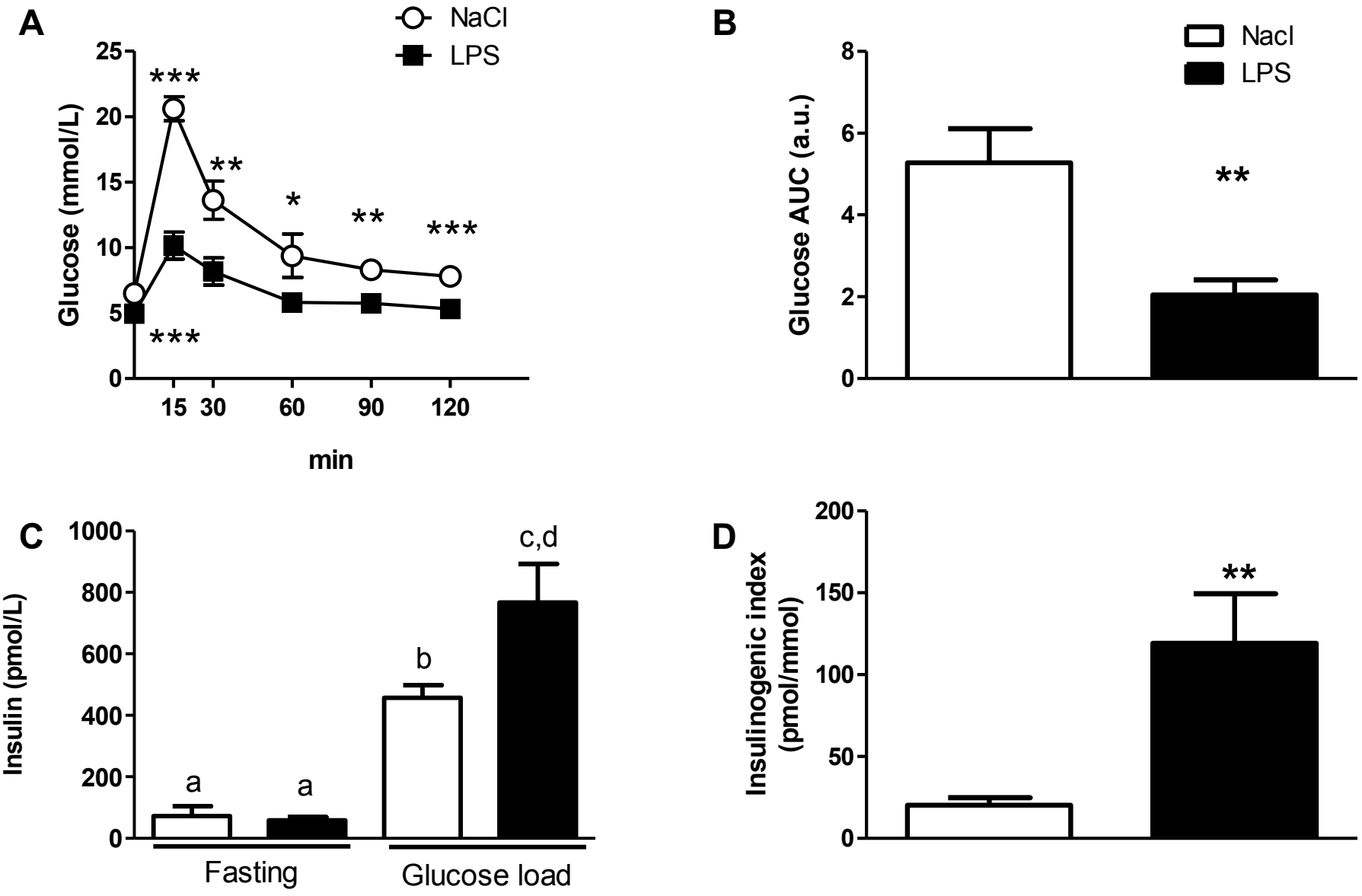
**Upper**: LPS induces GLP-1 and insulin secretion **A.** Plasma GLP-1 levels 6 hours after i.p injection of NaCl (n=8) or LPS (n=18), before and after a glucose load (2g/kg) **B.** Plasma Insulin levels before and after an oral glucose load (2g/kg) in NaCl-injected mice (n=8) and LPS-injected mice (n=18) (White bars; NaCl-injected mice; Black bars: LPS injected mice). **C.** Plasma GLP-1 *vs* Insulin levels Spearman correlation. **Middle**: Sitagliptin enhances LPS-improved glucose disposal. **D.** OGTT (2g/kg) in LPS-injected mice (2mg/kg) (Open circle) (n=4) or LPS (2mg/kg)+ Sitagliptin(5mg/kg) injected mice (Closed squares) (n=5). Body mass: LPS: 23.5 ± 0.4 g, LPS + sitagliptin: 23.5 ± 0.4 g (ns). **E.** AUC, area under curves of glucose responses expressed as arbitrary units (a.u). **Lower:** Exendin9 blocks LPS-improved glucose disposal **F.** OGTT (1.5g/kg) in LPS injected-mice (Open circle) (n=6) or LPS (2mg/kg) + Exendin9 (5µg) (Closed squares) (n=6). Body mass: LPS: 19.6 ± 0.3 g, LPS + Exendin9: 19.9 ± 0.2 g (ns). **G.** AUC, area under curves of glucose responses expressed as arbitrary units (a.u). Values are means ±SEM. *p<0.05, **p<0.01, ***p<0.001

**Figure 8: LPS-induced increases in GSIS and glucose disposal are blunted in GLP-1R KO mice**

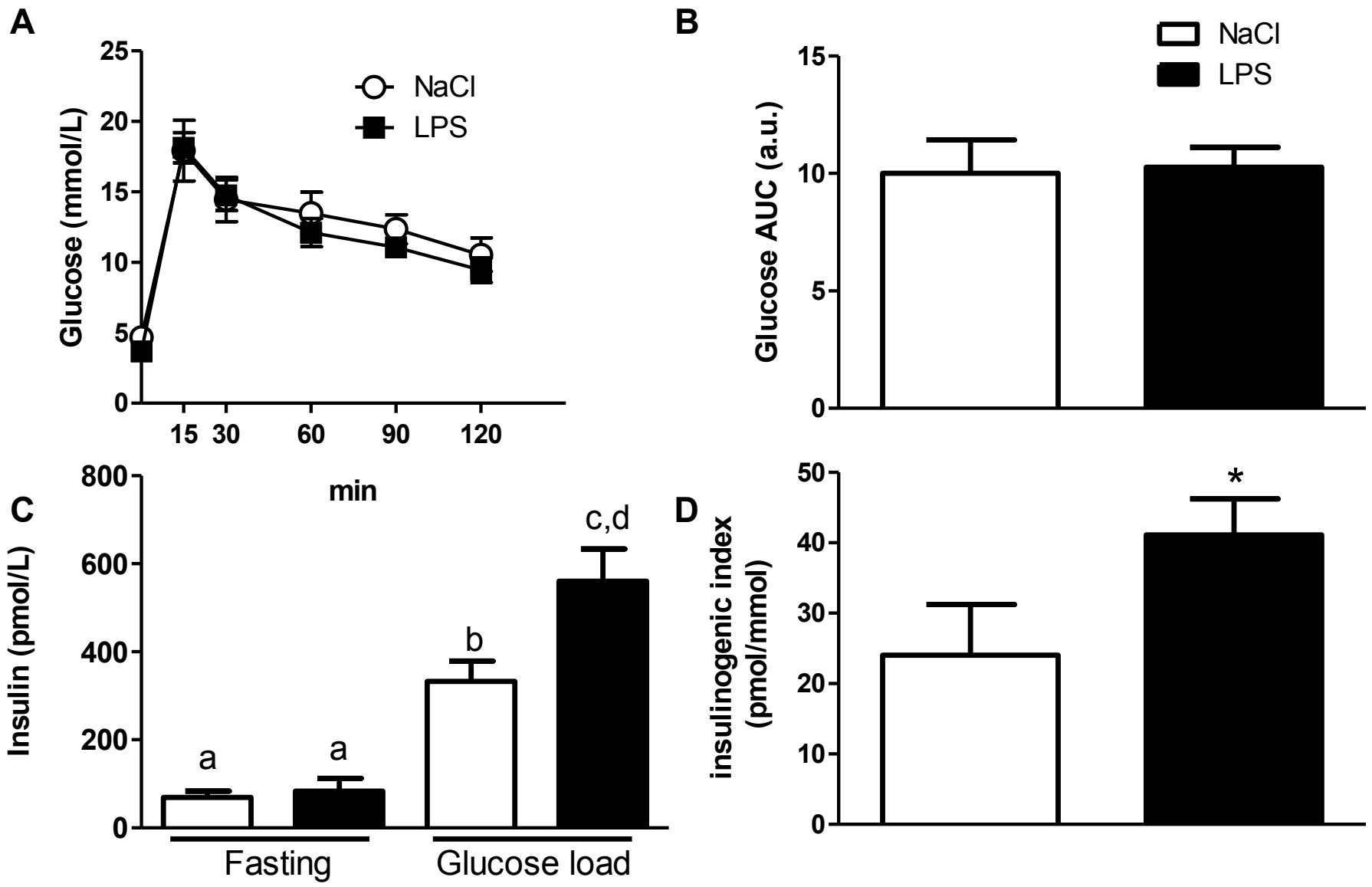
Plasma levels of cytokines in WT mice (White bars) and GLP1-R KO mice (Black bars) as measured 6 hours after acute injection of NaCl or LPS (2mg/kg). **A.** Interleurkin 6 (IL6). **B.** Tumor necrosis factor alpha (TNF$\alpha$). **C.** OGTT (2g/kg) in NaCl-injected WT mice (Open circle)(n=11) or LPS-injected WT mice (2mg/kg) (Closed squares) (n=11). Body mass: NaCl:24.5 ± 0.9 g, LPS: 24.0 ± 0.5 g (ns). **D.** OGTT (2g/kg) in NaCl-injected GLP-1R KO mice (Open circle) (n=11) and LPS-injected GLP-1R KO mice (Closed squares) (n=10). Body mass: NaCl: 21.7 ± 0.2 g, LPS: 21.0 ± 0.3 g (ns). **E.** AUC, area under curve of glucose responses expressed as arbitrary units. **F.** Plasma insulin levels 15min after an oral glucose load (2g/kg) in NaCl-injected WT mice (n=12) and LPS-injected WT mice (n=11). **G.** Plasma insulin levels 15min after an oral glucose load (2g/kg) in NaCl-injected GLP-1R KO mice (n=10) mice and LPS-injected GLP-1R KO mice (n=8). **H.** insulinogenic index. White bars: NaCl-injected mice,

Black bars: LPS-injected mice (2mg/kg). Values are means ± SEM. (*p<0.05, **p<0.01, ***p<0.001)

# FIGURE 1


A
NaCl
LPS
Glucose (mmol/L)
25
20
15
10
5
0
***
**
*
**
***
***
15
30
60
90
120
min
B
Nacl
LPS
Glucose AUC (a.u.)
8
6
4
2
0
**
C
Insulin (pmol/L)
1000
800
600
400
200
0
a
a
b
c,d
Fasting
Glucose load
D
Insulinogenic index (pmol/mmol)
200
150
100
50
0
**

# FIGURE 2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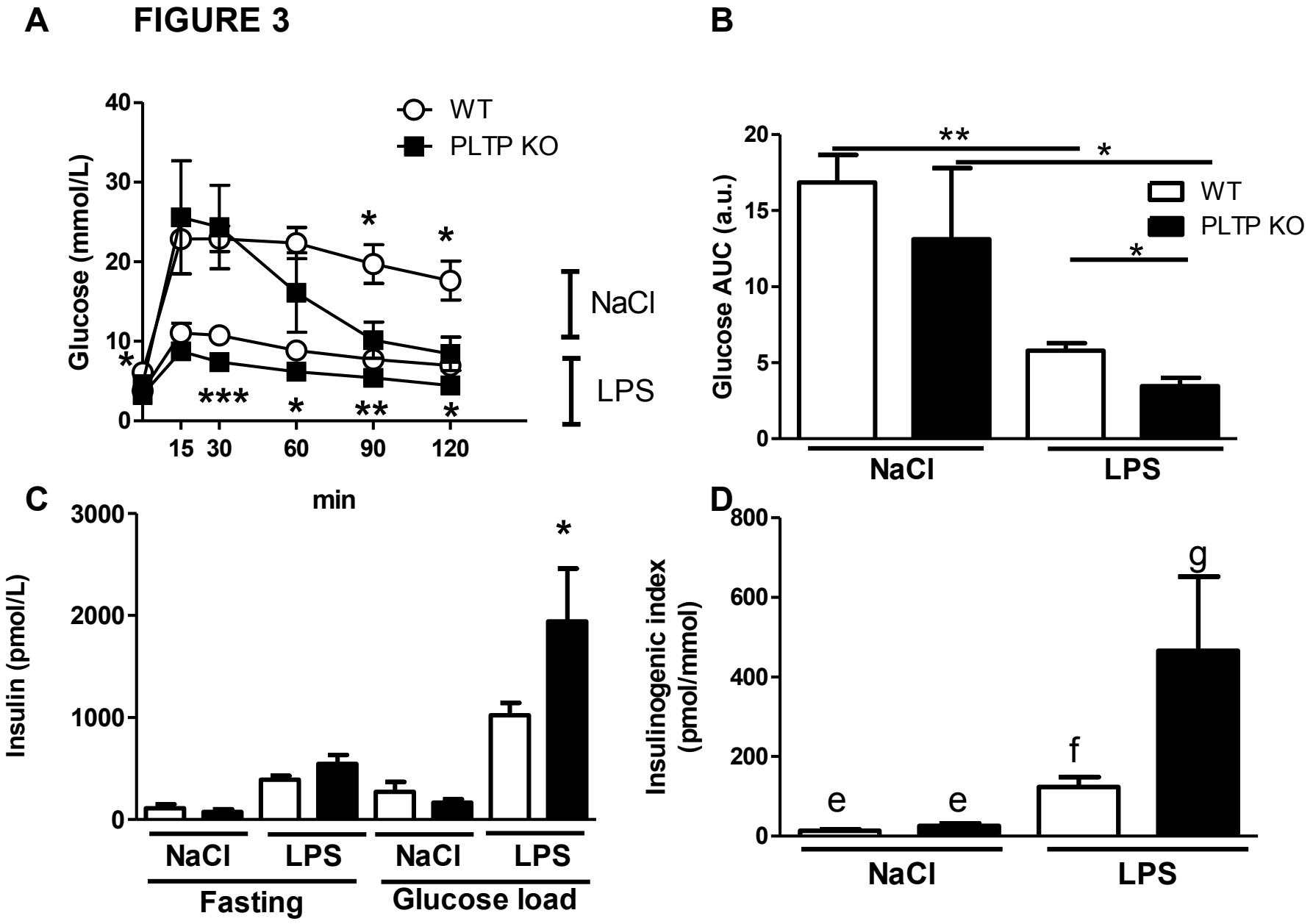
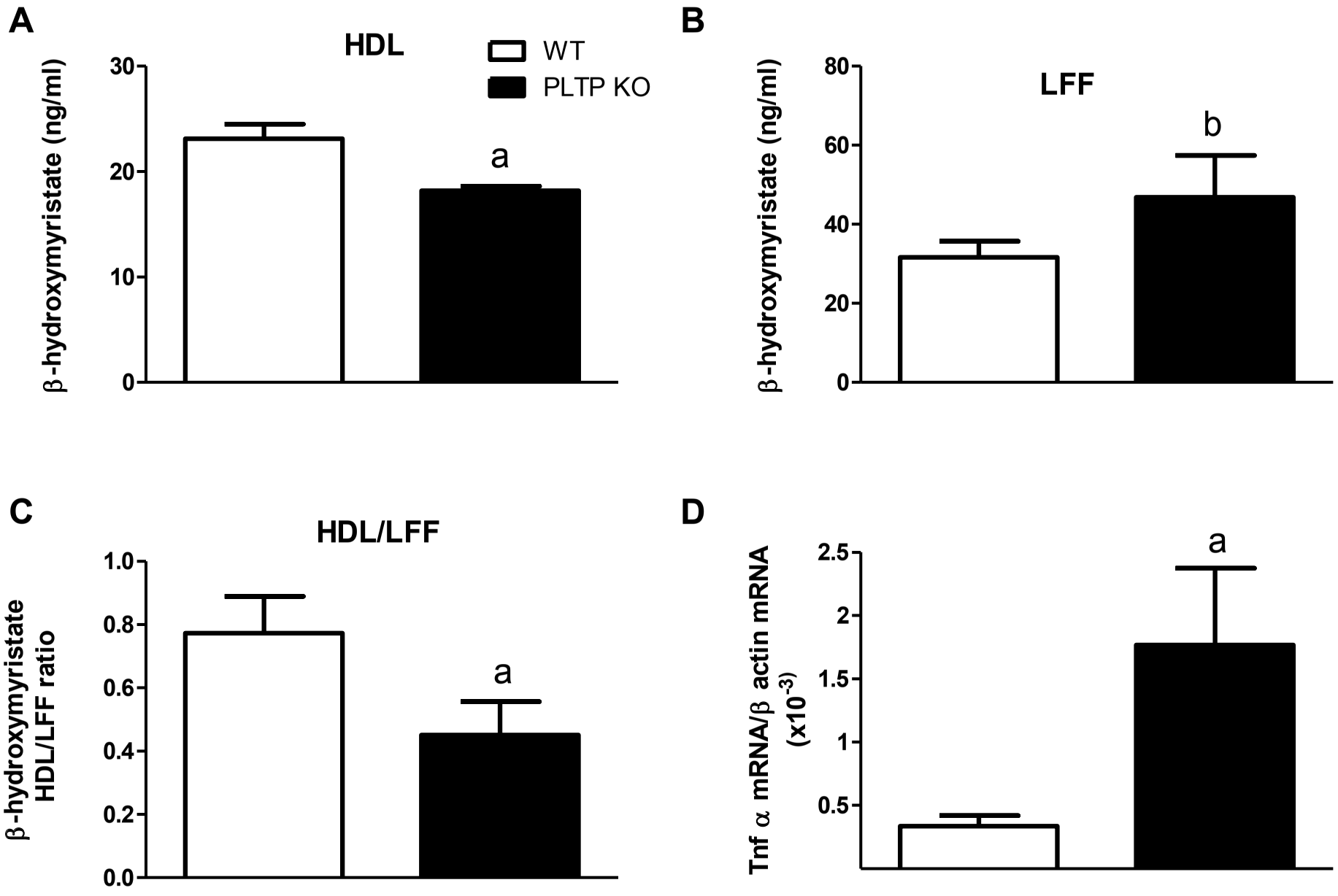
Glucose (mmol/L)
25
20
15
10
5
0
NaCl
LPS
15 30 60 90 120
min
B
Glucose AUC (a.u.)
15
10
5
0
NaCl
LPS
C
Insulin (pmol/L)
800
600
400
200
0
a
a
b
c,d
Fasting
Glucose load
D
insulinogenic index (pmol/mmol)
50
40
30
20
10
0
*

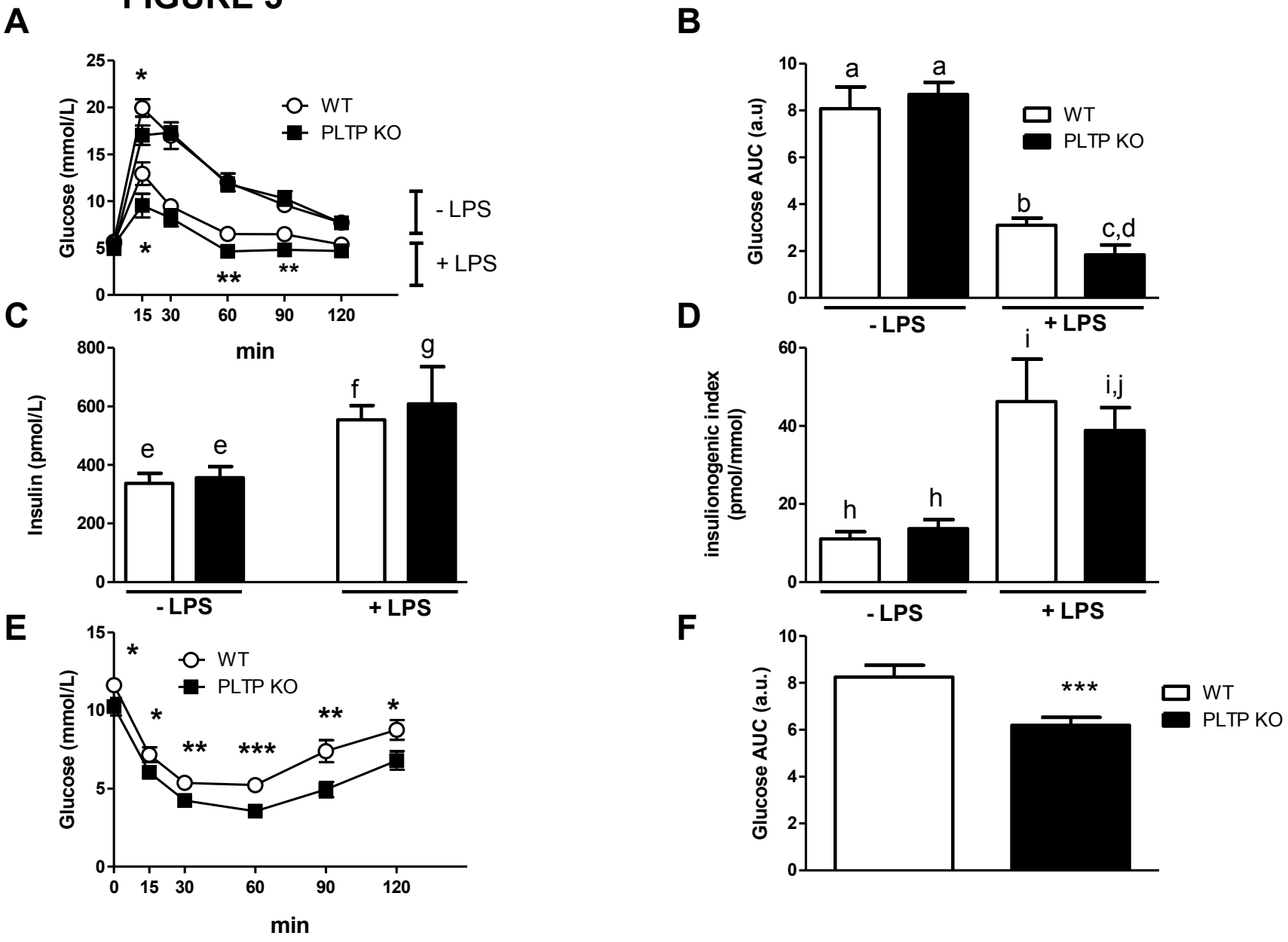
# FIGURE 3


A
B
C
D
WT
PLTP KO
Glucose (mmol/L)
min
NaCl
LPS
Glucose AUC (a.u.)
Insulin (pmol/L)
Insulinogenic index (pmol/mmol)
Fasting
Glucose load
e
f
g

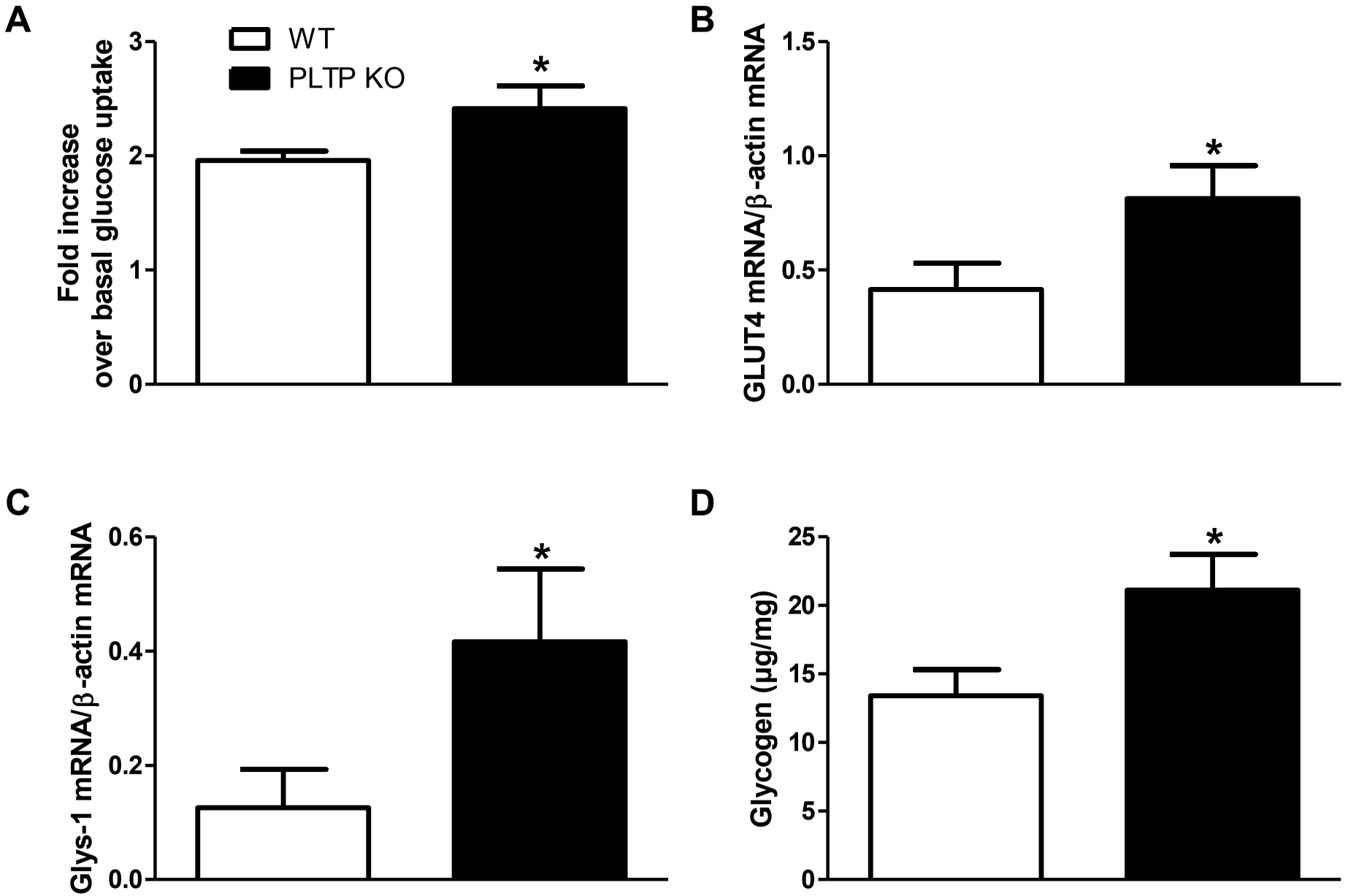
# FIGURE 4


A
HDL
WT
PLTP KO
β-hydroxymyristate (ng/ml)
30
20
10
0
a
B
LFF
β-hydroxymyristate (ng/ml)
80
60
40
20
0
b
C
HDL/LFF
β-hydroxymyristate HDL/LFF ratio
1.0
0.8
0.6
0.4
0.2
0.0
a
D
Tnf α mRNA/β actin mRNA (x10-3)
2.5
2
1.5
1
0.5
a

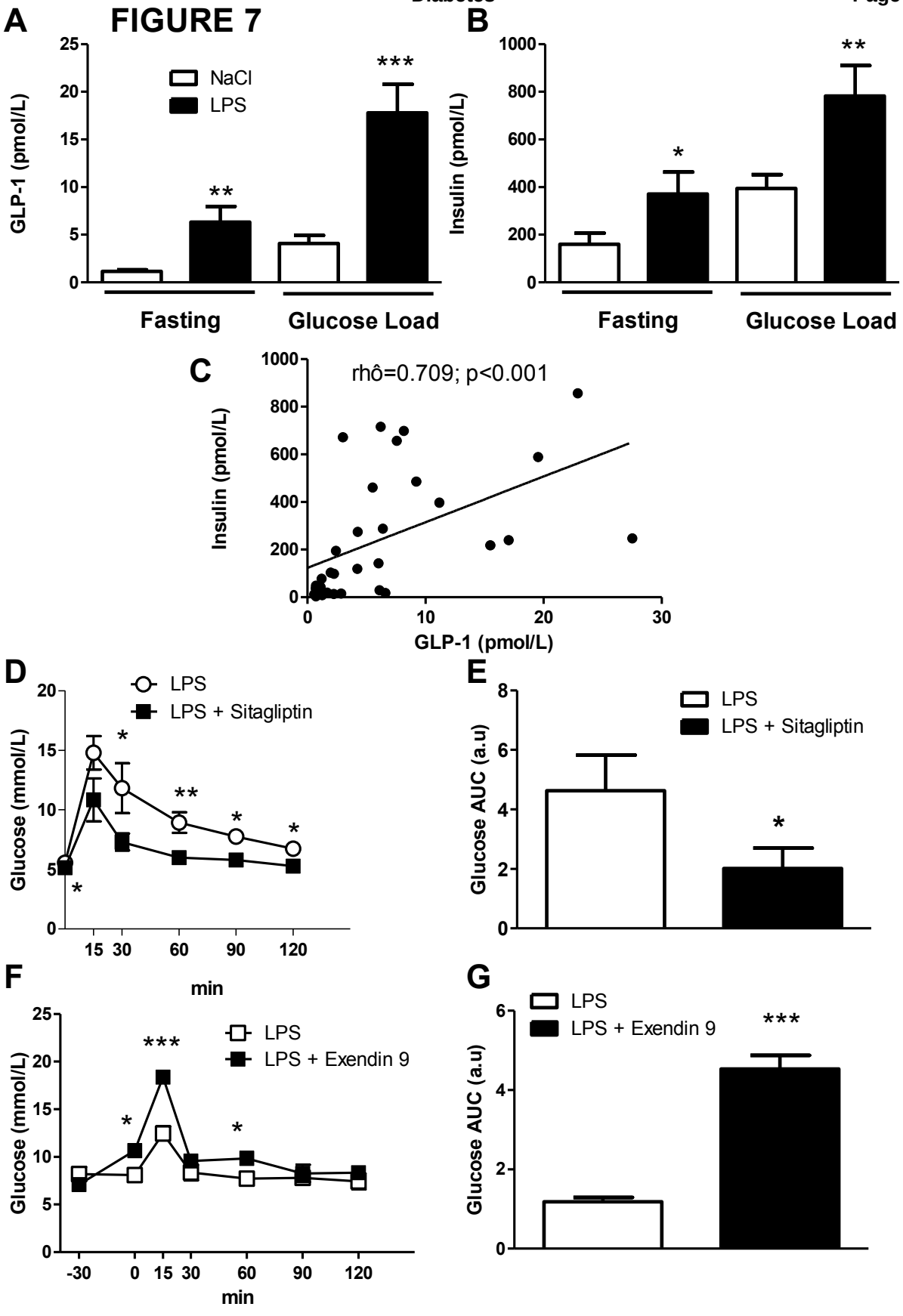
# FIGURE 5


A
Glucose (mmol/L)
WT
PLTP KO
- LPS
+ LPS
min
B
Glucose AUC (a.u)
C
Insulin (pmol/L)
D
insulinogenic index (pmol/mmol)
E
Glucose (mmol/L)
F
Glucose AUC (a.u.)

# FIGURE 6


A
WT
PLTP KO
Fold increase over basal glucose uptake
3
2
1
0
*
B
GLUT4 mRNA/β-actin mRNA
1.5
1.0
0.5
0.0
*
C
Glys-1 mRNA/β-actin mRNA
0.6
0.4
0.2
0.0
*
D
Glycogen (µg/mg)
25
20
15
10
5
0
*

# FIGURE 7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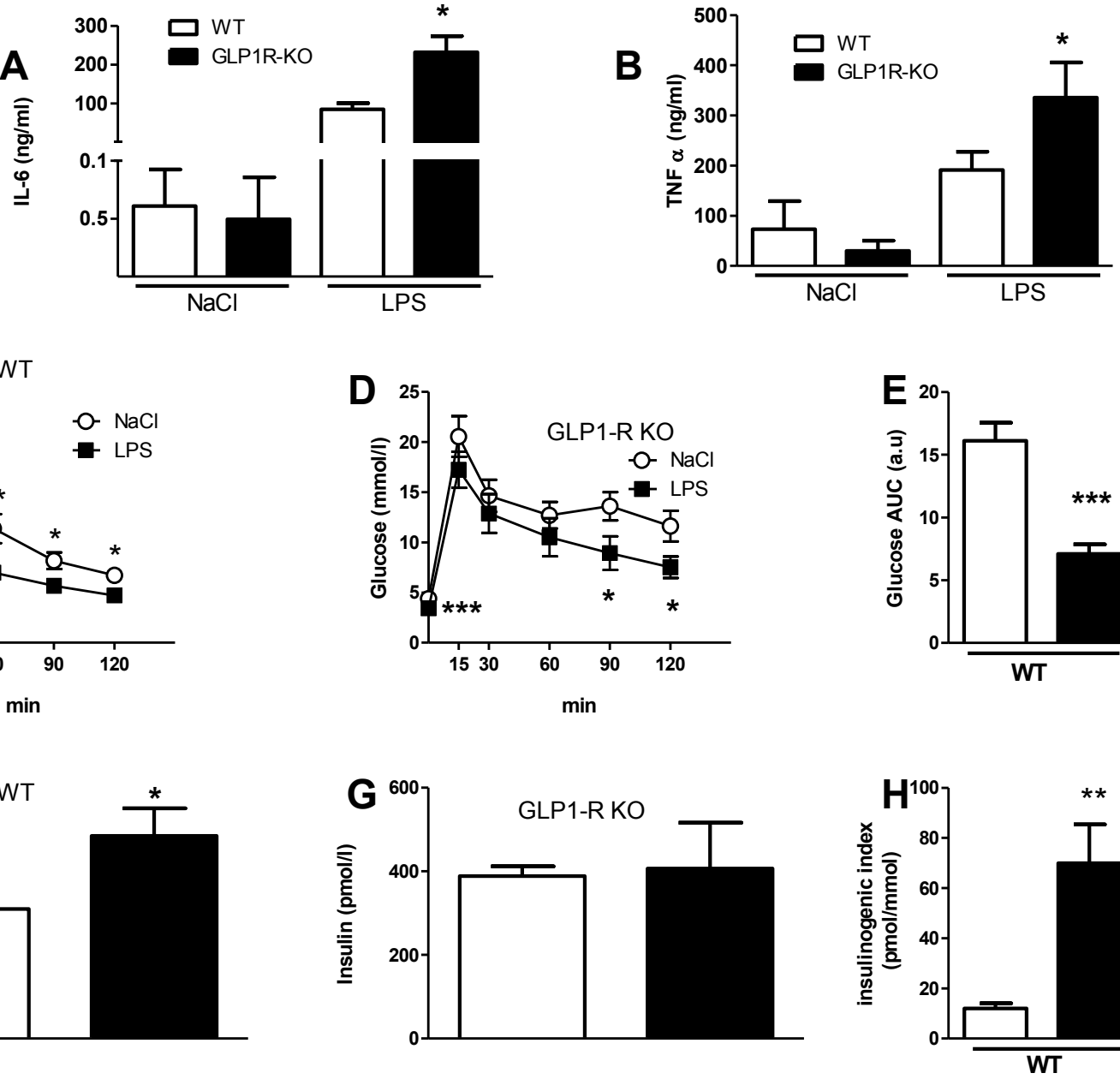
GLP-1 (pmol/L)
NaCl
LPS
Fasting
Glucose Load
B
Insulin (pmol/L)
Fasting
Glucose Load
C
rhô=0.709; p<0.001
Insulin (pmol/L)
GLP-1 (pmol/L)
D
LPS
LPS + Sitagliptin
Glucose (mmol/L)
min
E
LPS
LPS + Sitagliptin
Glucose AUC (a.u)
F
LPS
LPS + Exendin 9
Glucose (mmol/L)
min
G
LPS
LPS + Exendin 9
Glucose AUC (a.u)

# FIGURE 8


A
IL-6 (ng/ml)
300
200
100
0.1
0.5
WT
GLP1R-KO
*
NaCl
LPS
B
TNF α (ng/ml)
500
400
300
200
100
0
WT
GLP1R-KO
*
NaCl
LPS
C
WT
Glucose (mmol/L)
40
30
20
10
0
NaCl
LPS
***
***
**
*
*
*
15 30 60 90 120
min
D
GLP1-R KO
Glucose (mmol/l)
25
20
15
10
5
0
NaCl
LPS
***
*
*
15 30 60 90 120
min
E
Glucose AUC (a.u)
20
15
10
5
0
NaCl
LPS
***
WT
GLP1-R KO
F
WT
Insulin (pmol/l)
600
400
200
0
*
G
GLP1-R KO
Insulin (pmol/l)
600
400
200
0
H
insulinogenic index (pmol/mmol)
100
80
60
40
20
0
**
WT
GLP1-R KO