Postprandial macrophage-derived IL-1 stimulates insulin, and both synergistically promote glucose disposal and inflammation

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Abstract: The deleterious effect of chronic activation of the IL-1 system on type 2 diabetes and other metabolic diseases is well documented. However, a possible physiological role for IL-1 in glucose metabolism has remained unexplored. Here we found that feeding induced a physiological increase in the number of peritoneal macrophages that secreted IL-1, in a glucose-dependent manner. Subsequently, IL-1 contributed to the postprandial stimulation of insulin secretion. Accordingly, lack of endogenous IL-1 signaling in mice during refeeding and obesity diminished the concentration of insulin in plasma. IL-1 and insulin increased the uptake of glucose into macrophages, and insulin reinforced a pro-inflammatory pattern via the insulin receptor, glucose metabolism, production of reactive oxygen species, and secretion of IL-1 mediated by the NLRP3 inflammasome. Postprandial inflammation might be limited by normalization of glycemia, since it was prevented by inhibition of the sodium-glucose cotransporter SGLT2. Our findings identify a physiological role for IL-1 and insulin in the regulation of both metabolism and immunity.

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Postprandial macrophage-derived IL-1β stimulates insulin and both synergistically promote glucose disposal and inflammation

Erez Dror¹, Elise Dalmas¹,⁶, Daniel T. Meier¹,⁶, Stephan Wueest², Julien Thévenet³, Constanze Thienel¹, Katharina Timper¹, Thierry M. Nordmann¹, Shuyang Traub³, Friederike Schulze¹, Flurin Item⁷, David Vallois⁴, Francois Pattou⁵, Julie Kerr-Conte³, Vanessa Lavallard⁵, Thierry Berney⁵, Bernard Thorens⁴, Daniel Konrad², Marianne Böni-Schnetzler¹,⁶, Marc Y. Donath¹,⁶

¹Clinic of Endocrinology, Diabetes and Metabolism University Hospital Basel, and Department Biomedicine, University of Basel, 4031 Basel, Switzerland
²Dept. of Pediatric Endocrinology and Diabetology and Children’s Research Center University Children's Hospital, Steinwiesstrasse 75, 8032 Zurich, Switzerland
³University Lille, Inserm, CHU Lille, U1190 Translational research for diabetes, European Genomic Institute for Diabetes, EGID, 59000 Lille, France
⁴Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland
⁵Cell Isolation and Transplantation Center, Department of Surgery, Geneva University Hospitals and University of Geneva School of Medicine, 1211 Geneva, Switzerland
⁶Equal contribution

Correspondence: marc.donath@usb.ch
Abstract

The deleterious role of chronic activation of the IL-1β system in type 2 diabetes and other metabolic diseases is well documented. However, a possible physiological role of IL-1β in glucose metabolism remained unexplored. Here we show that feeding induces a physiological increase in the number of peritoneal macrophages, which secrete IL-1β in a glucose-dependent manner. Subsequently, IL-1β contributes to postprandial stimulation of insulin secretion. Accordingly, lack of endogenous IL-1β in mice during refeeding and obesity reduced plasma insulin. IL-1β and insulin increased glucose uptake into macrophages, and insulin reinforced a pro-inflammatory pattern via the insulin receptor, glucose metabolism, reactive oxygen species production, and NLRP3 inflammasome-mediated IL-1β secretion. Post-prandial inflammation is limited by normalization of glycemia and can be prevented by inhibition of sodium-glucose cotransporter 2 (SGLT2). Our findings identify a physiological role for IL-1β and insulin in the regulation of both metabolism and immunity.
Introduction

Activation of the innate immune system is an initial response of the body to infections and injuries. The resulting inflammation aims at protecting against stressors and at restoring tissue and organism homeostasis. This process is largely driven by IL-1β, one of the first described cytokines\(^1\). However, prolonged activation of the immune system by over-nutrition may eventually promote the development of metabolic diseases\(^2-6\). A critical sensor of nutrient overload is the NLRP3 inflammasome, which processes pro-IL-1β to its active form in various metabolic disorders. This is the case for uric acid crystals which activate the NLRP3 inflammasome in gout\(^7\), for cholesterol crystals in atherogenesis\(^8\), and for glucose, fatty acids and islet amyloid polypeptide in type 2 diabetes\(^9-13\). Importantly, a causal link between IL-1β-induced inflammation and these metabolic diseases has been demonstrated by various genetic and pharmacological approaches in animal models\(^14-16\) and in clinical trials\(^17-21\). While these studies shed light on the pathological role of IL-1β in metabolism, a physiological function of IL-1β in metabolic control remains largely unexplored.

In response to pathogens and obesity, profound changes in the metabolism of immune cells take place\(^22-25\). Beyond supplying energy, nutrients can also act as signaling molecules that promote the activation of immune cells. Indeed, elevated glucose and other metabolites drive production of IL-1β by macrophages\(^14,26\).

Insulin-producing β cells have the highest IL-1 receptor expression of any tissue\(^27\) and the IL-1 receptor is the most abundantly expressed cell surface receptor on β cells\(^28\), pointing to a hitherto unappreciated physiological function. While the deleterious role of high-dose and long-term IL-1β exposure on islet function and mass is well documented\(^29,30\), a few mainly in vitro observations hint to a possible beneficial role of IL-1β in insulin secretion and β-cell survival\(^31,32\).
Parenteral glucose stimulation leads to a rapid first and second phase of insulin release. In contrast, the physiological slower resorption of a meal elicits a sustained increase of insulin that depends on the duration of food intake\textsuperscript{33}. Thereby the insulin response to enteral food intake cannot be solely accounted for by the associated changes in blood glucose. It is modulated by several insulin secretagogues including the incretin hormones GIP and GLP-1\textsuperscript{34}. These hormones promote insulin secretion only when glucose levels are elevated. While GIP and GLP-1 are the best described incretins, other incretin-like factors are thought to exist.

Several studies show that food intake transiently induces a mild inflammatory response\textsuperscript{35,36}. We hypothesized that IL-1\textsubscript{β} contributes to this postprandial inflammation, regulating whole body glucose homeostasis along with an innate immune response. Thereby, it may deliver the energy needed to activate the innate immune system in order to prevent the dissemination of microorganisms contained in the food. We show here that a postprandial rise in glucose leads to acute elevation of macrophage-derived IL-1\textsubscript{β}, which contributes to postprandial insulin secretion via the abundantly expressed IL-1 receptor on β cells. Insulin reinforces a pro-inflammatory state and stimulates macrophages to produce IL-1\textsubscript{β} via glucose metabolism, subsequent production of reactive oxygen species (ROS) which leads to activation of the inflammasome\textsuperscript{14,37}. Both, insulin and IL-1\textsubscript{β} regulate glucose disposal, whereby IL-1\textsubscript{β} preferentially stimulates glucose uptake into the immune cell compartment.
Results

Feeding stimulates intra-peritoneal macrophages to produce IL-1β.

In order to study the physiological involvement of IL-1β in insulin secretion we performed overnight fasting followed by refeeding experiments in wild-type and \( IL1b^{-/-} \) mice. Two hours after refeeding, circulating IL-1β concentration was increased in wild-type mice, while, as expected, IL-1β remained undetectable in \( IL1b^{-/-} \) mice (Fig. 1a; for validation of the assay see Supplementary Fig. 1a). Of note, IL-1β concentration in serum was already elevated 30 minutes after refeeding (not shown). Next we investigated the source of increased IL-1β. Following refeeding, expression of \( Il1b \) mRNA and of the IL-1β-dependent chemokines \( Cxcl1 \) and \( Ccl2 \) were increased in the omental fat (the main site from which macrophages migrate into the peritoneum\textsuperscript{38,39}; Fig. 1b) but not in the circulating leukocytes, liver, spleen, epididymal fat or subcutaneous fat (not shown). Furthermore, the number of stromal vascular cells isolated from the omentum was reduced (fasted: 41.8 ± 13.4 x 10\(^3\) cells/mg; refed: 8.3 ± 2.6 x 10\(^3\) cells/mg; mean ± s.e.m.). Accordingly, we observed a marked increase in the number of peritoneal cells (Fig. 1c), which displayed an increase in \( Il1b \) mRNA expression (Fig. 1d). Flow cytometry revealed that the majority of these cells were macrophages (Fig. 1e; for gating strategy see Supplementary Fig. 1b), a repartition that remained unchanged between fasting and refeeding (not shown). Ex vivo, spontaneous release of IL-1β by cultured peritoneal macrophages from fasted or refed mice was comparable (not shown). However, macrophages from refed mice released more IL-1β following stimulation with ATP (Fig. 1f). To validate the source of the increased IL-1β during refeeding, we generated \( Il1b^{fl/fl}Lyz2-Cre \) mice, which specifically lack IL-1β in the myeloid lineage (Supplementary fig. 1c). Two hours after refeeding, circulating IL-1β concentration was increased in wild-type mice but not in their \( Il1b^{fl/fl}Lyz2-Cre \) littermates (Fig 1g). We then tested whether the increase in serum glucose following feeding mediated the postprandial
increase in IL-1β. We treated mice with the SGLT2 inhibitor canagliflozin, which decreases glycemia via inhibition of renal glucose reabsorption. Treatment of mice with canagliflozin induced glycosuria and prevented postprandial increase in glucose and insulin (Supplementary Fig. 1d-f), along with a complete prevention of postprandial increase in circulating IL-1β (Fig. 1h). Similarly, injection of the non-metabolizable glucose analogue 2-deoxyglucose (2DG) prior to refeeding strongly reduced IL-1β release (Fig. 1i). Finally, we tested the role of bacterial products in the stimulation of postprandial IL-1β by treating mice with broad-spectrum antibiotics or lipopolysaccharide (LPS). Antibiotics-treated mice tended to have milder postprandial inflammation than untreated mice as detected by Il1b and Cxcl1 gene expression in the omental fat pad (Supplementary Fig. 1g, h) together with a mild decrease in postprandial circulating IL-1β (Supplementary Fig. 1i) despite similar food intake and no change in the number of peritoneal cells (Supplementary Fig. 1j-l). Ex vivo, peritoneal macrophages from antibiotic-treated mice released less IL-1β, even in the presence of ATP (Fig. 1j). However, priming with LPS prior to ATP stimulation restored IL-1β secretion (Fig. 1j). Accordingly, in vivo i.p. injection of LPS into fed mice increased circulating IL-1β along with insulin and decreased blood glucose (Fig. 1k). However, injection of LPS in genomic Il1b−/− mice failed to induce insulin (Fig. 1l). This suggests that there is a need for a microbial-related stimulus and energy supply (glucose) to induce postprandial release of IL-1β. Thus, feeding increases the number of intra-peritoneal macrophages, which are primed by bacterial products to produce and release IL-1β in response to glucose.

Postprandial macrophage IL-1β promotes insulin secretion.

Next, we examined the direct effect of elevated IL-1β after refeeding on insulin secretion. First, we measured postprandial circulating insulin in littermate Il1b−/− or wild-type refed mice. Il1b−/− mice had reduced insulin secretion (Fig. 2a) and elevated blood glucose (Supplementary Fig. 2a) after refeeding compared to wild-type mice, despite comparable
food intake (not shown). Since the number of peritoneal cells was increased upon refeeding (Fig. 1c), and macrophages are the most abundant immune cells in the peritoneal cavity (Fig. 1e), we depleted macrophages in wild-type mice by i.p. injection of clodronate (Supplementary Fig. 2b) prior to performing fasting-refeeding experiments. Depletion of macrophages resulted in a marked decreased of postprandial insulin in the circulation (Fig. 2b). Similarly, postprandial insulin was lower in Il1bfl/flLyz2-Cre mice compared to their wild-type littermates (Fig. 2c). In addition, acute blockade of IL-1 with its natural antagonist IL-1Ra prior to refeeding resulted in slightly decreased circulating insulin in wild-type mice (Fig. 2d) and elevated blood glucose (Supplementary Fig. 2c). Since obesity is associated with inflammation and chronically elevated IL-1β and insulin, we investigated the effect of acute (1 hour before refeeding) IL-1 antagonism in diet-induced obese (DIO) mice to substantiate the ability of endogenous IL-1β to regulate insulin. Blocking the elevated circulating levels of IL-1β (Fig. 2e) with IL-1Ra lowered fasting insulin levels in DIO mice (Fig. 2f) without changing insulin sensitivity or hepatic glucose production as determined by a hyperinsulinemic-euglycemic clamp (Fig. 2g, h). As a second model, genetically obese db/db mice were injected with IL-1Ra, which also decreased insulin levels (Fig. 2i). Therefore, postprandial IL-1β derived from myeloid cells promotes insulin secretion.

**Acute exposure to IL-1β induces insulin secretion.**

To directly test the effect of IL-1β on insulin secretion in vivo, we performed acute injections of IL-1β in mice followed by i.p. glucose tolerance test (GTT). IL-1β led to a marked elevation in insulin secretion (Fig. 3a) and improved glucose tolerance (Fig. 3b). Insulin was also increased and glycemia improved by IL-1β administration in the absence of a glucose bolus but to a much lesser extent (Fig. 3c, d; note the different scale in the y-axis compared to 3a & b). This glucose-dependent potentiation of insulin secretion is reminiscent of effects elicited by the incretin hormones GLP-1 and GIP. However, circulating active GLP-1
concentrations remained unchanged following IL-1β injections (Supplementary Fig. 3a). Furthermore, the effect of IL-1β on insulin secretion and glucose tolerance was identical in Glp1r\(^{-/-}\)/Gipr\(^{-/-}\) double knock-out mice compared to their wild-type littermates (Supplementary Fig. 3b, c) and treatment of mice with the GLP-1 inhibitor Exendin fragment 9-39 did not reduce the IL-1β effect on insulin secretion (not shown). Therefore, IL-1β did not promote glucose-induced insulin secretion via incretin hormones. Since insulin secretion could be increased as a result of IL-1β-mediated peripheral insulin resistance, we determined if insulin sensitivity changes upon an acute IL-1β injection using insulin tolerance tests and hyperinsulinemic-euglycemic clamp studies. Acute injection of IL-1β had no effect on insulin sensitivity or hepatic glucose production (Fig. 3e, Supplementary Fig. 3d). IL-1β also improved glucose tolerance and strongly increased insulin secretion in DIO mice (Fig. 3f, g) and in genetically obese db/db mice (Fig. 3h, i). In contrast to wild-type mice, interleukin 1 receptor-associated kinase 4 (Ira4\(^{-/-}\)) deficient mice injected with IL-1β before an i.p. GTT showed no improvement in insulin secretion and no change in glycemia (Supplementary Fig. 3e, f) demonstrating that the observed effects of IL-1β are mediated by the IL-1 receptor signal transduction pathways. Of note, i.p. injection of 0.1 μg/kg of IL-1β resulted in circulating IL-1β concentrations similar to those obtained upon refeeding and also induced insulin secretion (Fig. 3j, k). Next we tested whether LPS has similar effects. Acute i.p. LPS injection improved glucose tolerance and increased insulin secretion in both wild-type and DIO mice (Fig. 4a, b). Importantly, IL-1Ra treatment blocked LPS-induced insulin secretion (Fig. 4c). Type 1 IL-1 receptor mRNA (Il1r1) was expressed at a much higher level in isolated endocrine cells than in islet resident immune or endothelial cells (Fig. 4d). Specific immunostaining of mouse pancreatic tissue sections revealed the presence of IL-1R1 in a subpopulation of β cells (Fig. 4e). Previous in vitro studies\(^{32}\) demonstrated that very low concentrations of IL-1β increased glucose-stimulated insulin secretion from islets. We confirmed this effect in mouse and human islets, and with the human β-cell line ENDOC.
(Supplementary Fig. 3g-i), suggesting a direct β-cell effect. To determine whether acute administration of IL-1β, in vivo, acts directly on the islet, we used streptozotocin (STZ) to eliminate β cells from recipient mice and transplanted them with islets from wild-type or Il1r1−/− donor mice. The effect of IL-1β on insulin secretion and glucose tolerance was lost in Il1r1−/− transplanted mice (Fig. 4f, g). These results suggest that systemic IL-1β potentiates glucose-induced insulin secretion via islet IL-1R1, and this is independent of the incretins GLP-1 and GIP or of changes in insulin resistance.

Insulin stimulates IL-1β secretion of macrophages

Since IL-1β enhanced insulin secretion during refeeding, we investigated the possible synergistic contribution of insulin and IL-1β to the stimulation of the immune system. First we investigated by flow cytometry the expression of the insulin receptor (InsR) on resident macrophages isolated from several tissues. We found that peritoneal macrophages had the highest insulin receptor expression compared to other resident macrophages (Fig 5a). Moreover, we found that the InsR was upregulated in peritoneal macrophages from DIO mice (Fig. 5b) and in the pro-inflammatory M1 compared to naive M0 macrophages, whereas it was mildly downregulated in anti-inflammatory M2 macrophages (Fig. 5c, Supplementary Fig. 4a). Accordingly, insulin induced AKT phosphorylation in naive M0, to a greater extent in M1 and not in M2 macrophages (Fig. 5d, Supplementary Fig.4b). The insulin effect on AKT phosphorylation was confirmed using a quantitative multiplex assay (Fig. 5e). In contrast to the PI3K activation pathway, insulin had no impact on MAPK signaling (Fig. 5f). Insulin also stimulated upregulation of the glucose transporter Slc2a1 (encodes GLUT1, the isoform that is mainly expressed in immune cells25; Fig. 5g) and the expression of hexokinase 2 (Hk2), the rate-limiting enzyme in glycolysis (Fig. 5h), in M1 macrophages. In line with this pattern, a 2 hour insulin treatment increased glycolytic activity in M1 macrophages, but not in naive or M2 macrophages (Fig. 5i, Supplementary
In accordance with the phosphorylation assay, the AKT signaling inhibitor LY294002 but not the MAPK signaling inhibitor U0126 blocked the effect of insulin on glycolytic activity in M1 macrophages (Fig. 5j). Further, insulin induced glucose uptake in naïve macrophages, and this effect was enhanced by IL-1β (Fig 5k). Similar to the pattern of insulin receptor expression and activation, insulin induced secretion of mature IL-1β preferentially in M1 macrophages in an NLRP3-dependent manner (Fig. 6a). Insulin also stimulated Tnf, Il6, and Cxcl1 expression and protein release in M1 macrophages but independently of NLRP3 activation (Supplementary Fig. 4d, e). This insulin effect on IL-1β appeared to be independent of cell death and survival, which remained unaffected (Supplementary Fig. 4f).

Similar to insulin-induced glycolysis, insulin stimulated IL-1β via the PI(3)K activation pathway and was blocked by LY294002 and downstream by the mTOR inhibitor rapamycin (Fig. 6b). We then tested the role of glucose metabolism in insulin-induced IL-1β secretion. First, the GLUT1 inhibitor fasentin blocked IL-1β secretion (Fig. 6c). In addition, insulin failed to induce IL-1β when cells were cultured with the non-metabolizable glucose analogue 2DG or with the mitochondria targeted ROS scavenger Mito-TEMPO (Fig. 6d). IL-1Ra was undetectable following short-term exposure (2 hours) to insulin (not shown) however prolonged (12 hours) exposure to insulin induced IL-1Ra in M0 and M1 macrophages indicating that the induction of IL-1β was later counterbalanced by IL-1Ra secretion (Fig. 6e, f). Similarly to our in vitro data, acute injection of insulin in mice increased amounts of circulating IL-1β and CXCL1 (Fig. 6g, Supplementary Fig. 4g). Overall, these data show that insulin induces inflammasome-mediated IL-1β secretion via enhanced glucose metabolism and mitochondrial ROS production. These effects are dependent on the AKT/PI(3)K pathway and on the activity status of the macrophages.

IL-1β shifts glucose uptake to immune cells
Next, we tested how IL-1β contributes to glucose disposal. Exposure of macrophages to IL-1β led to an increase in glucose uptake (Fig. 7a), while blocking endogenously produced IL-1 with IL-1Ra slightly decreased glucose uptake (Fig. 7b). We then examined the effect of IL-1β and insulin injection on glucose uptake in wild-type mice. To avoid glucose-stimulated insulin secretion, we only used trace amounts of non-metabolizable radiolabeled glucose. IL-1β stimulated glucose uptake in spleen and circulating leukocytes (Fig. 7c, d). IL-1β also increased glucose uptake in adipose tissue and in muscle, however to a lesser extent than insulin (Fig. 7e-g). This is potentially due to the mild stimulation of insulin secretion by IL-1β observed in the absence of a glucose bolus (Fig. 3c). To mimic the chronic elevation of IL-1β, we injected mice with IL-1β daily for 3 consecutive days and determined the number and glucose uptake of peritoneal cells. Similar to refed mice, IL-1β-injected mice had more peritoneal cells (Fig. 7h). Ex vivo, glucose uptake of macrophages from these mice was increased (Fig. 7i). To test the physiological relevance of IL-1β-induced glucose uptake, we investigated postprandial glucose uptake in immune cells of refed mice. Blockade of endogenous IL-1 with IL-1Ra decreased glucose uptake in peritoneal macrophages (Fig. 7j).

To further examine the contribution of immune cells to overall body glucose disposal, we first used T and B cell deficient (Rag2−/−) mice. A single injection of IL-1β prior to a GTT in Rag2−/− mice lowered glucose concentration as potently as in littermate control mice (Supplementary Fig. 5a). Since Rag2−/− mice have a compensatory increase in the number of macrophages, we additionally ablated macrophages in Rag2−/− mice with clodronate liposomes (Supplementary Fig. 5b). This diminished the profound beneficial effect of IL-1β on glucose disposal, despite increased insulin levels (Fig. 7k), suggesting that immune cells substantially contribute to IL-1β-induced glucose disposal. Thus, beside the stimulation of insulin, IL-1β directly regulates glycemia by promotion of glucose disposal preferentially in immune cells (Supplementary Fig. 6).
Discussion

In the present study we show that feeding induces a physiological elevation of macrophage-
derived IL-1β that promotes postprandial insulin secretion. This effect depends on bacterial
products, which primes macrophages to produce more pro-IL-1β, and on glucose that drives
the maturation of IL-1β. The production of IL-1β by M1, and to a lesser extent by M0,
macrophages is enhanced by insulin. Insulin upregulates functional insulin receptors,
signaling via the AKT/PI(3)K pathway, glucose uptake through the glucose transporter
GLUT1, glucose metabolism, and ROS production that activates the NLRP3
inflammasome
. Both insulin and IL-1β regulate whole body glucose disposal by
promoting glucose uptake in muscle and fat, and fuel the immune system by stimulating
glucose uptake into the immune cell compartment. Ablation of macrophages in T- and B-cell
deficient mice significantly reduced IL-1β-mediated glucose clearance. Self-amplification of
the system is limited by normalization of glycemia.

The number of macrophages in the peritoneum was increased upon refeeding along with
increased expression of inflammatory genes including Il1b in omental fat, which supports
active trafficking of macrophages into the peritoneal cavity. Therefore, feeding stimulates
immune surveillance, possibly to limit the dissemination of microorganisms contained in
food. Activation of the immune system requires energy and contributes substantially to
whole-body glucose consumption. We provide evidence that both, bacterial products and
glucose metabolism, are required to induce postprandial IL-1β secretion by peritoneal
macrophages. Indeed, we show that peritoneal cells from refed mice have elevated Il1b
mRNA levels. This might rather be promoted by translocation of ingested or intestinal
bacterial products than by the microbiota itself, since pretreatment of mice with
antibiotics before refeeding only mildly lowered IL-1β secretion. However, stimulation of IL-
1β release strongly depends on glucose uptake and metabolism. Indeed, we observed that
decreasing glycemia via SGLT2 inhibition or blocking glycolysis with 2DG prevented postprandial IL-1β in the circulation. This suggests that from all factors that could mediate this effect during feeding, such as mechanical stress of the digestive system, fiber, amino acid and fat intake, it is mainly glucose that drives IL-1β secretion. Our results also suggest a role for insulin in postprandial IL-1β secretion since insulin increased glucose uptake and metabolism in macrophages. Inhibition of glucose uptake with fasentin or 2DG blocked insulin-induced IL-1β secretion. Downstream, inhibiting mitochondrial ROS production, which activates the NLRP3 inflammasome\textsuperscript{14,37}, also prevented insulin from inducing IL-1β.

Previous \textit{in vitro} studies have shown that low concentrations of IL-1β mildly stimulate insulin secretion\textsuperscript{32}. Here we show that \textit{in vivo}, IL-1β injections strongly potentiate insulin secretion in the presence of a glucose bolus. However, the most natural way to stimulate insulin secretion is eating. We find that IL-1β-induced insulin release occurs in physiology by demonstrating that postprandially produced IL-1β increased insulin levels and decreased glycemia. This IL-1β effect is partly mediated by the highly expressed IL-1R1 on β-cells, since the effect of IL-1β on insulin secretion and glucose tolerance was blunted in diabetic mice transplanted with islets from \textit{Il1r1}\textsuperscript{−/−} mice.

The beneficial effects of postprandial IL-1β on glucose homeostasis are in apparent contrast to the glucose lowering effects of IL-1 antagonism in patients with type 2 diabetes\textsuperscript{17} and to the well-described deleterious effects of IL-1β on islet function and survival\textsuperscript{4}. Though IL-1β provokes β-cell demise\textsuperscript{30,44}, at low concentrations or upon short exposure, IL-1β paradoxically stimulates β-cell proliferation and decreases apoptosis\textsuperscript{32}. Therefore, IL-1β is not only detrimental for β-cells but has more complex biological functions\textsuperscript{45}. An explanation to reconcile the beneficial effects of IL-1 blockade in type 2 diabetes is the difference between acute and chronic effects and the concept of β-cell “rest”. Indeed, potassium...
channel openers, which decrease insulin secretion, ultimately improve insulin secretion in patients with type 2 diabetes\textsuperscript{46}. The benefit of IL-1 antagonism in patients with type 2 diabetes could result from β-cell rest, possibly in combination with inhibition of the toxic effects of IL-1β.

A further explanation why chronic upregulation of IL-1β leading to elevated insulin levels may become unfavorable for metabolism stems from our observation that insulin reinforces the inflammatory state of macrophages through enhanced glucose uptake and metabolism and increased InsR expression in macrophages of DIO mice. Indeed, activated macrophages play a crucial role in insulin resistance\textsuperscript{47}. In line with this, insulin receptor expression was also higher in macrophages from DIO mice, where macrophages have been shown to be pro-inflammatory\textsuperscript{6}. Thus, we propose that insulin, which is increased in early stages of type 2 diabetes, may drive and sustain the inflammatory state in macrophages and may therefore contribute to the chronic low-grade inflammation associated with metabolic diseases. Thereby, TNFα, IL-6 and CXCL1 may also add to the effect of IL-1β. In support, myeloid cell-restricted InsR deficient mice are protected against metabolic inflammation and insulin resistance\textsuperscript{48}.

The effect of insulin in macrophages was previously studied without taking into account the polarization status of the macrophages\textsuperscript{49}. Herein we show that insulin preferentially acts on pro-inflammatory M1 macrophages characterised by more InsRs, enhanced downstream AKT phosphorylation and glycolytic activity. Further insulin promotes the NLRP3 inflammasome and leads to macrophage-derived IL-1β release. Interestingly, peritoneal macrophages had the highest insulin receptor density, supporting their contribution to postprandial homeostasis.
SGLT2 inhibitors have recently been approved for the treatment of type 2 diabetes and the EMPA-REG outcome study revealed an impressive reduction in mortality. However the mechanisms leading to this protective effect are unclear. In the present study, we show that canagliflozin prevents postprandial IL-1β elevation in the circulation. This could be due to increased glucose excretion in the urine, which prevents an overload of glucose in tissues, thereby avoiding deleterious chronic effects of glucose-induced IL-1β.

Altogether, our findings show that IL-1β, a master regulator of inflammation, and insulin, a key hormone in glucose metabolism, promote each other. Both have potent effects on glucose homeostasis and on the activity of the immune system, supporting the emerging concept that inflammatory mediators play a role not only in the pathology of metabolism but are an integral part of its physiology. The physiological synergy between IL-1β and insulin on glucose disposal may be required to cope with the concomitant challenge by nutrients and microorganisms related to food intake.
358  **Accession Codes**

359  Not applicable

360

361  **Data Availability Statement**

362  The data that support the findings of this study are available from the corresponding author upon request.
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Author Contributions

E.Dr., M.B.S and M.Y.D designed the study and wrote the manuscript; E.Dr. performed and analyzed most of the experiments. M.B.S, E.Da, and D.T.M performed and analyzed experiments; C.T, K.T, T.N, S.T, F.S, and D.V helped with experiments. S.W, F.I, and D.K performed the clamps. J.T, F.P, J.K.C provided human islets and performed the islet transplantation experiments. V.L. and T.B provided human islets. B.T provided the \(Gipr^{-/-}\) mice; all co-authors helped with the manuscript. M.Y.D and M.B.S supervised the Research.

Competing Financial Interests Statement

M.Y.D. is listed as the inventor on a patent filed in 2003 for the use of an interleukin-1 receptor antagonist for the treatment of, or prophylaxis against, type 2 diabetes.
References


Figure legends

Figure 1

Feeding stimulates intra-peritoneal macrophages to produce IL-1β.

(a) Circulating IL-1β in wild-type (WT; n=22, 4 experiments) or Il1b<sup>−/−</sup> (n=3) mice before and after feeding (LLOD denotes the lowest level of detection). (b) Gene expression in omental fat isolated from fasted and refed mice (n=8, 3 experiments). (c) Number of peritoneal cells isolated per mouse (WT, fasted n=15 and refed n=17, 4 experiments). (d) Il1b gene expression in peritoneal cells isolated from fasted and refed mice (n=6). (e) Peritoneal cell composition following feeding determined by flow cytometry (pool of 4 refed mice). (f) IL-1β release of macrophages isolated from fasted (n=5) or refed (n=6) mice and stimulated with ATP. (g-i) Circulating IL-1β levels before (fasted) or after refeeding (refed) in Il1b<sup>fl/fl</sup>Lyz2-Cre and WT mice (g; n=16 and 15, respectively), in mice treated with or without the SGLT2 inhibitor canagliflozin (h; n=13 per group), and in mice treated with or without 2DG (i; saline n=9, 2DG n=10). (j) Ex vivo IL-1β secretion in unstimulated (basal) macrophages isolated from refed mice pretreated for a week with or without antibiotics (ABX, left) or stimulated with ATP (middle) alone or following LPS priming (right). (k) Circulating IL-1β, insulin, and blood glucose levels after intra-peritoneal (i.p.) injection of LPS (1 mg/kg) in fed mice (n=8).

(l) Circulating insulin levels in fed WT or Il1b<sup>−/−</sup> mice after i.p. injection of 1 mg/kg LPS or saline (WT; saline n=5, LPS n=7, Il1b<sup>−/−</sup>; n=7). *P < 0.05, **P < 0.01, ***P<0.001, ****P < 0.0001. Statistical significance (P) was determined by Student’s t test and in (b, g, h, l) by ANOVA. All error bars denote s.e.m.

Figure 2

Postprandial macrophage-derived IL-1β promotes insulin secretion.
(a) Circulating insulin concentration before (fasted) or after refeeding (refed) in Il1b−/− or WT mice (n=6 and 11, respectively). (b, c) Circulating insulin before (fasted) or after refeeding (refed) in (b) WT mice injected with liposomes containing clodronate or PBS (n=17 per group; 3 experiments), and in (c) Il1bfl/fl/Lyz2-Cre (n=16) and WT (n=15) mice. (d) Circulating insulin following an acute injection of saline or 10 mg/kg IL-1Ra in refed WT mice (n=26 and 23, respectively). (e) Basal circulating IL-1β levels in control (n=8) or DIO (n=10) mice. (f) Circulating insulin following acute injections of saline or 10 mg/kg IL-1Ra in DIO mice (n=15 and 13 respectively). Hyperinsulinemic-euglycemic clamp in DIO mice pre-injected with saline or with 10 mg/kg IL-1Ra (n=4 and 5, respectively): (g) Glucose infusion rate, (h) hepatic glucose production. (i) Circulating insulin levels following acute injections of saline or 10 mg/kg IL-1Ra in db/db mice (n=4 and 5, respectively). *P < 0.05, **P < 0.01. Statistical significance (P) was determined by Student’s t test and in (a-c) by ANOVA. All error bars denote s.e.m.

**Figure 3**

**Acute exposure to IL-1β induces insulin secretion without changing insulin sensitivity.**

(a, b) Concentrations of circulating insulin (a) and glucose (b) during an intra-peritoneal glucose tolerance test (i.p. GTT) in WT mice 18 minutes after a single injection with saline or 1 µg/kg IL-1β (n=5 and 6, respectively). (c, d) Concentrations of circulating insulin (c) and glucose (d) in 6 hour fasted WT mice injected with or without 1 µg/kg IL-1β (n=8 per group). (e) Glucose infusion rate and hepatic glucose production during a hyperinsulinemic-euglycemic clamp in WT mice pre-treated with 1 µg/kg IL-1β or saline (n=4 and 3, respectively). (f-i) Concentrations of insulin (f, h) and glucose (g, i) during an i.p. GTT in diet-induced obese (DIO) (f, g) and db/db (h, i) mice, pre-treated with 1 µg/kg IL-1β (n=12 per group). (j) Circulating IL-1β concentration and (k) insulin (fold of basal) 18 min after an i.p. injection of 0.1 or 1 µg/kg of IL-1β into WT mice (n=9). *P < 0.05, **P < 0.01, ***P<0.001,
****P < 0.0001. Statistical significance (P) was determined by Student’s t test and in (k) by ANOVA. All error bars denote s.e.m.

Figure 4

Systemic IL-1β potentiates glucose-induced insulin secretion via islet IL-1R1

(a) Circulating insulin and (b) glucose levels during an i.p. GTT in WT and DIO mice 3 hours after a single injection of LPS (1 mg/kg) or saline (n=5 per group). (c) Circulating insulin during an i.p. GTT in WT mice 3 hours after a single injection of saline or LPS (1 mg/kg) with or without IL-1Ra (10 mg/kg; n=5 per group). (d) Il1r1 mRNA expression in FACS sorted islet cells (n=6 experiments). (e) Double immunostaining of IL-1R1 and insulin in pancreatic tissue sections of WT and Il1r1-/- mice. Scale bar, 50 μm. (f) Circulating insulin and (g) glucose during an i.p. GTT in STZ-treated mice transplanted with WT islets or Il1r1-/- islets 18 minutes after a single injection with 1 μg/kg IL-1β (n=5 and 4). *P < 0.05, **P < 0.01, ***P<0.001, ****P < 0.0001. Statistical significance (P) was determined by Student’s t test and in (a-d) by ANOVA. All error bars denote s.e.m.

Figure 5

Insulin receptor expression and activation in peritoneal macrophages

(a) Relative mean fluorescence intensity (MFI) of insulin receptor (InsR) protein in different tissue resident macrophages (4 experiments, number of circles indicates number of mice per tissue). (b) Relative MFI of InsR protein in peritoneal macrophages from WT and DIO mice (n=3 mice). (c) Insr mRNA expression in naive (M0), pro-inflammatory M1 and alternative M2 polarized macrophages (n=12; 3 experiments). (d) A representative immunoblot of insulin-induced (s473) phospho-AKT in M0, M1 and M2 polarized macrophages (1 out of 3 experiments). Relative phosphorylation of (e) AKT (ser473) and (f) proteins in the MAPK signaling pathway (both assayed on the same samples; n=4 experiments). Gene expression
from M0 and M1 macrophages incubated with or without 1 μg/ml insulin: (g) Slc2a1 (encoding GLUT1) and (h) Hk2 (encoding hexokinase 2); data are expressed as fold change from untreated naive controls (n=9, 3 experiments). (i) Extracellular acidification rate (ECAR; mpH/min) from polarized macrophages incubated for 2 hours in the presence or absence of 1 μg/ml insulin. (n=12 or 15; 3 experiments). (j) ECAR measurements from M1 polarized macrophages acutely treated with or without 1 μg/ml insulin and 10 μM LY294002 or 10 μM U0126 (vertical line indicates treatment start, n=9; 3 experiments) (k) Glucose uptake in naive macrophages (control; n=21; 3 experiments) or incubated for 3 hours with 1 μg/ml insulin alone (n=28; 3 experiments) or in combination with 1 ng/ml IL-1β (n=15; 3 experiments). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Statistical significance (P) was determined by ANOVA and in (b, g) by Student’s t test. All error bars denote s.e.m.

Figure 6

Insulin stimulates IL-1β secretion of macrophages.

(a) Two-hour IL-1β secretion from 2-hour polarized macrophages isolated from WT or Nlrp3−/− mice incubated with or without 1 μg/ml insulin. (b-d) Two-hour IL-1β protein release from 2-hour polarized M1 macrophages with or without 1 μg/ml insulin and in combination with or without (b) 10 μM LY294002 or 20 μM rapamycin (n=9-12; 3 experiments), (c) 50 μM fasentin (n=14, 3 experiments), and (d) 2-deoxyglucose (2DG) or mitoTEMPO (n=6, 3 experiments): data are presented as fold stimulation from non-treated cells. (e) IL-1Ra and (f) IL-1β protein release from 16-hour polarized macrophages incubated for 12 hours with or without 1 μg/ml insulin (n=9, 3 experiments). (g) Circulating IL-1β levels in mice treated with or without 1 unit/kg insulin (15 minutes post injection; n=10). *P < 0.05, **P < 0.01, ***P < 0.001. Statistical significance (P) was determined by ANOVA. All error bars denote s.e.m.
IL-1β shifts glucose uptake to immune cells.

In vitro glucose uptake in (a) macrophages incubated with or without 1 ng/ml IL-1β for 2 hours (n=15 and 11, respectively; 3 experiments) and, in (b) macrophages incubated with or without 1 µg/ml IL-1Ra for 3 hours (n=10 and 14, respectively; 3 experiments). (c-g) WT mice were injected i.p. with either saline or 1 µg/kg IL-1β or 1 µg/kg insulin (n=4 per group) 18 minutes prior to an injection of 10 µCi 3H labeled 2DG. Mice were sacrificed 48 minutes after the first injection followed by assessment of glucose uptake in spleen (c), circulating leukocytes (d), visceral adipose tissue (epididymal fat pads; e), adipocytes isolated from epididymal fat pads (f) and muscle (g). (h) Number of peritoneal cells and (i) ex vivo glucose uptake in macrophages from mice injected once a day for 3 days with 35 µg/kg IL-1β (n=5 per group). (j) Ex vivo glucose uptake in peritoneal macrophages from mice acutely injected with saline or 10 mg/kg IL-1Ra. (k) Circulating blood glucose and insulin levels during an intra-peritoneal glucose tolerance test after treatment with 1 µg/kg IL-1β in macrophage-depleted Rag2-/- mice using an injection of 10 ml/kg clodronate or PBS liposomes (PBS; n=13, clodronate; n=8). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Statistical significance (P) was determined by Student’s t test and in (c-g by ANOVA). All error bars denote s.e.m.
Online methods

Human pancreatic islets

Human islets were isolated in the islet transplantation centres of Lille and Geneva from pancreata of cadaver organ donors in accordance with the local Institutional Ethical Committee. They were obtained via the “islet for research distribution program” through the European Consortium for Islet Transplantation, under the supervision of the Juvenile Diabetes Research Foundation (31-2008-416). Islets were cultured in CMRL-1066 medium containing 5 mmol/l glucose, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM Glutamax and 10 % FCS (Invitrogen) on extracellular matrix-coated 24-well plates (Novamed Ltd.) in humid environment containing 5 % CO₂.

Mouse pancreatic islets

To isolate mouse islets, pancreata were perfused through the sphincter of oddi with a collagenase solution (1.4 gr/l; collagenase type 4 Worthington) and digested in the same solution at 37°C, followed by sequential filtration through 500 µm and 70 µm cell strainers (BD). Islets were handpicked and cultured on extracellular matrix-coated 24-well plates (Novamed Ltd.) in RPMI-1640 (GIBCO) containing 11.1 mM glucose, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM Glutamax, 50 µg/ml gentamycin, 10 µg/ml Fungison and 10 % FCS. Islets were either collected directly for RNA isolation extraction or cultured for 36 hours on extracellular matrix-coated 24-well plates for subsequent glucose-stimulated insulin secretion experiments.

Glucose-stimulated insulin secretion assay in islets and ENDOC cells

For glucose-stimulated insulin secretion experiments, islets or the human β-cell line ENDOC cells (kindly provided by R. Sharfmann⁵¹) were cultured for 2 days and pre-incubated for 30
minutes in modified Krebs-Ringer bicarbonate buffer (KRB; 115 mM NaCl, 4.7 mM KCl, 2.6 mM CaCl\(_2\) \(2\)H\(_2\)O, 1.2 mM MgSO\(_4\) \(2\)H\(_2\)O, 10 mM HEPES, 0.5 % bovine serum albumin, pH 7.4) containing 2.8 mM glucose. KRB was then replaced by KRB with 2.8 mM glucose and collected after 1 hour to determine the basal insulin release. IL-1\(\beta\) was added at the indicated concentrations for the last 30 minutes of the 1-hour period of the basal insulin release (priming). This was followed by 1 hour in KRB with 16.7 mM glucose to determine the stimulated insulin release. The stimulatory index was defined as the ratio of insulin secretion at 16.7 mM to 2.8 mM glucose/hour and expressed as percentage of untreated control.

Animal experiments

All animal experiments were performed in mice on a C57BL/6N background unless otherwise specified. Normal mice were obtained from Charles River. For the diet induced obesity (DIO) experiments, 4 week old mice were fed a high fat diet (D12331, Research Diets; containing 58, 26 and 16 % calories from fat, carbohydrate and protein, respectively) for 20-25 weeks. Leptin receptor deficient (db/db) mice were obtained from Jackson laboratories at the age of 4 weeks and housed until the age of 16 weeks. Irak4\(^{-/-}\) mice on a Balb/c background were kindly provided by Amgen. IL1b\(^{-/-}\) mouse strains on a C57BL/6N background produced either by gene targeting\(^{52}\) or with Eucomm embryonal stem cells were used. To generate the macrophage specific IL-1\(\beta\) knock-out mouse (Il1b\(^{fl/fl}\)Lyz2-Cre\(^{+/}\)) chimeric mice were produced from ES cells containing a modified Il1b allele, which contains loxP sites flanking exons 4 and 5 and a frt-LacZ-loxP-neo-Frt cassette introduced between exon 3 and 4 (Eucomm clone HEPD0840_C04). Chimeric mice were then crossed with C57BL6/N mice and the offspring with germ line transmission of the recombined allele were crossed with Flp deleter mice\(^{53}\) on Bl6/N background to excise the FRT flanked lacZ neo cassette and to obtain the Il1b\(^{fl/fl}\) mouse. These mice were next crossed with Lyz2-Cre mice\(^{54}\), that were previously

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backcrossed to a C57BL6/N background. As littermate control mice we used the cre recombinase negative $\text{Il}1\beta^{\text{fl/fl}}$ mice, and as myeloid cells specific $\text{Il}1\beta^{-/-}$ knock-out mice the $\text{Il}1\beta^{\text{fl/fl}}$Ly2-Cre$^{+/−}$ mice (Supplementary Fig. 1c). $\text{Nlrp3}^{-/-}$ mice were generated as described previously$^{55}$. $\text{Rag2}^{-/-}$ mice were bred in house. $\text{Il}1\text{r1}^{-/-}$ mice and immunodeficient female swiss nude were obtained from Charles River laboratories. $\text{Glpr}^{+/−}/\text{Grp}^{+/−}$ mice were generated as described$^{56}$.

All animal experiments were conducted according to the Swiss Veterinary Law and Institutional Guidelines and were approved by the Swiss Authorities. All animals were housed in a temperature-controlled room with a 12 h light – 12 h dark cycle and had free access to food and water.

All metabolic experiments using transgenic mice were performed with wild-type littermates as controls. The mice were between 12 and 29 weeks of age. Mice that did not gain weight in diet-induced obesity experiments were excluded. All experiments were performed at least twice with weight-matched mice and with at least a total of 4 animals per group. For drug applications (apart from antibiotics treated mice that can receive commensal bacteria from non treated controls) each cage included mice receiving all treatments in order to avoid cage dependent differences.

**In vivo treatment administration**

Recombinant mouse IL-1$\beta$ (R&D) was injected i.p. at the indicated time and dose.

IL-1Ra (Anakinra; 10 mg/kg body weight) was injected i.p. 3 hours prior to intervention. 2-deoxyglucose (1 g per kg body weight) was injected i.p 1 hour prior to refeeding.

Canagliflozin (100 mg per kg body weight) or placebo control were homogenised and orally
administered 1 hour prior to refeeding. LPS (InvivoGen; 1 mg/kg body weight) was injected i.p. at the indicated time.

**Glucose tolerance tests (GTTs)**

For glucose tolerance testing, mice were fasted for 6 hours starting in the morning and 2 g glucose per kg body weight was injected i.p. Blood glucose was measured using a glucometer (Freestyle; Abbott Diabetes Care Inc.).

**Urine glucose levels**

Glycosuria was assessed according to manufacturer’s instructions (Accu Check Diabur test strips, Roche).

**Fasting and refeeding experiments**

Before blood collection, fasted mice were provided free access to water but not to food for 12 hours. Refed mice were treated in the same manner as the fasted mice; however, prior to blood collection, refed mice had access to food for 2 hours. In experiments done with antibiotics or with IL-1Ra injection or with IL1β−/− mice and littermate wild-type mice, blood was collected immediately before refeeding (time 0) and 2 hours after feeding. To avoid potential confounding effects due to circadian-mediated fluctuation in circulating IL-1β levels all experiments were performed at the same time of the day (between 8 to 10 am).

**Glucose clamp studies**

Glucose clamp studies were performed in freely moving mice as previously described57. Steady state glucose infusion rate was calculated once glucose infusion reached a constant rate with blood glucose levels at 5 mmol/l (70-80 minutes after the start of insulin infusion). Thereafter, blood glucose concentration was kept constant at 5 mmol/l for 15-20 minutes.
and glucose infusion rate was calculated. Glucose disposal rate, and hepatic glucose production were calculated as previously described\textsuperscript{57}.

**Antibiotics treatment**

One week before fasting-refeeding experiment, 1 g/l antibiotic concoction consisting of vancomycin 10 mg/ml, neomycin 20 mg/ml, metronidazol 20 mg/ml (all purchased from Sigma), was administered by gavage every 12 hours. Gavage volume of 5 ml/kg body weight was delivered with a stainless steel tube. Fresh antibiotics concoction was mixed every day.

**Streptozotocin induced β-cell death and Islet transplantation.**

Streptozotocin (Sigma) was dissolved in citrate buffer (pH 4.5) and was i.p. injected to immunodeficient nude mice at 240 mg/kg. Only hyperglycemic mice (i.e. blood glucose > 14mM) were subjected to transplantation with 500 wild-type or \textit{Il1r1}\textsuperscript{-/-} mouse islets, under the kidney capsule as described in\textsuperscript{58}.

**Macrophage ablation**

Clodronate or PBS liposomes (ordered from ClodronateLiposomes.org) were injected i.p. (100 μl per 10 grams bodyweight) 3 days before the start of the assays. Mice were sacrificed at the end of the procedure. To verify macrophage depletion, peritoneal cells were isolated as described above and analyzed by FACS as described below. Macrophages were defined as CD11b\textsuperscript{+} F4/80\textsuperscript{-} double positive cells.

**Primary cell isolation and culture**

Cells were isolated from male C57BL/6 mice following euthanasia in a CO\textsubscript{2} chamber. To obtain circulating leukocytes, the heart was punctured and the collected blood was incubated briefly with red blood cells lysis buffer (154mM NH\textsubscript{4}Cl, 10mM KHCO\textsubscript{3}, 0.1mM
EDTA). To isolate macrophages, the peritoneum was infused with PBS containing 1% FBS and the lavage was filtered through 70 µm cell strainer (BD). Kupffer cells were isolated from the liver perfused with 3mL collagenase through the ductal vein followed by a 30 minute incubation step and two centrifugation steps: 50 x g for 3 minutes at 4 °C, collection of the upper phase and 350 x g for 5 minutes at 4°C. Intestinal macrophages were isolated after removal of the intestinal peyer patches, cut in pieces and washed twice (20 min shaking in PBS, 5mM EDTA), followed by 30min incubation in collagenase type 4 (1.4 g/l). Cells from omental and epididymal fat pads were isolated after shaking with collagenase type 4 (1.4 g/l) for 30 min at 37°C. Spleens were pushed through a 70 µm cell strainer and red cells were lysed using lysis buffer (154mM NH₄Cl, 10mM KHCO₃, 0.1mM EDTA). For islet resident macrophages, handpicked pancreatic islets were dissociated by accutase (Biolegend, 50%, 2 minutes, 37 degrees) and washed. All cells were filtered through a 70 µm cell strainer washed and resuspended in FACS buffer (PBS 0.5% BSA and 5mM EDTA). For macrophage culture, cells were allowed to adhere for at least 4 hours in 48 or 96 well plates (TPP) and non-adherent cells were washed away, naive macrophages were used for glucose uptake assays or were polarized to M1 or M2 phenotypes as follows: 2 hours (serum free) or 16 hours treatment with LPS (100 ng/ml) + IFNγ (10 ng/ml) for M1 and IL-13 (10 ng/ml) + IL-4 (10 ng/ml) for M2 polarization, followed by 2 or 12 hours, with or without 1 µg/ml insulin in the presence or absence of fasentin (50 µM; Sigma), 2-deoxyglucose (2DG; 10mM; Sigma), mito-TEMPO (100 µM; Sigma), LY294002 (10 µM; Sigma), U0126 (10 µM; Sigma), rapamycin (20 µM; Sigma) or followed by a 30 minute incubation with ATP (5mM; Sigma). Supernatants were collected, centrifuged (at 4°C, 2000 x g for 5 minutes) and stored at -80°C and cells were either harvested for RNA extraction (see RNA extraction and qPCR) or assayed by flow cytometer for cell survival using Annexin V apoptosis detection kit (eBioscience).

RNA extraction and qPCR
Total RNA was extracted using the Nucleo Spin RNA II Kit (Macherey Nagel) or using RNeasy Lipid Tissue (QIAGEN). RNA concentrations were normalized and cDNA was prepared with random hexamers (Microsynth) and Superscript II (Invitrogen) according to the instructions of the supplier. RNA expression was determined with TaqMan assays and the real time PCR system 7500 (Applied Biosystems). The following TaqMan assays were used:

Mouse:  
- *Gadph*: Mm99999915_g1,  
- *Actb*: Mm00607939_s1,  
- *Slc2a1* (encoding GLUT1): Mm00441480_m1,  
- *Il1b*: Mm0043228_m1,  
- *InsR*: Mm01211875_m1,  
- *Cxcl1*: Mm04207460_m1,  
- *Hk2*: Mm00443395_m1,  
- *Il1a*: Mm00439621_m1,  
- *Il1r1*: Mm00434237_m1,  
- *Ccl2*: Mm00441242_m1,  
- *Tnf*: Mm00443258_m1,  
- *Il6*: Mm004461920_m1.

Data were normalized with the geometrical mean of *Gadph* and *Actb* for macrophage mRNA and quantified using the comparative $2^{-\Delta\Delta Ct}$ method.

**Protein and protein-phosphorylation measurement assays**

Insulin concentrations were determined using human insulin ultrasensitive ELISAs (Mercodia) or mouse/rat insulin kits (Mesoscale Discovery). Mouse active GLP-1 was assayed using a Mesoscale discovery kit. Protein phosphorylation was assayed using whole cell lysates (10 μg total protein) and Mesoscale Discovery kinase phosphorylation assay kits (AKT signalling panel; K15177D and MAP kinase panel; K15101D) according to manufacturer’s instructions. Mouse cytokine concentrations were assayed using the V-plex mouse IL-1β TNFα, IL-6, and CXCL1 kit from Mesoscale Discovery with the following modifications: for circulating cytokine assay, after withdrawal, blood was incubated for 30 minutes at room temperature and sera were collected after 20 minutes of centrifugation (4°C, 2000 x g). Samples were incubated in the assay plate overnight at 4°C with gentle shaking. IL-1Ra was determined using ELISA assays (R&D).

**Glucose bio-distribution assay**
Male C57BL/6 mice were fasted for 3 hours in the morning, i.p. injected with IL-1β (1 μg/kg body weight) or saline and 18 minutes later with $^3$H labeled 2-deoxy glucose (10 μCi per mouse, Perkin Elmer). After 30 minutes mice were sacrificed, quadriceps muscle, epididymal adipose tissue and spleen were weighed, washed immediately in ice cold PBS and incubated with lysis buffer (10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, 62.5 mM Tris pH 6.8, 6M urea) followed by sonication. Leukocytes were isolated as described above, washed twice with ice cold PBS, counted and lysed with 0.1% SDS. Triplicate samples were then measured in a beta counter. Data are presented for organs as percentage beta counts per minute per mg tissue and as beta counts per cell for leukocytes. In experiments with refeed mice, fluorescent 2-deoxyglucose (2NBDG 500nmole per mouse; Invitrogen) was i.p. injected immediately after a refeeding window. Mice were sacrificed 1 hour later, peritoneal cells were harvested and analyzed by flow cytometer (BD Acurri).

**In vitro glucose uptake assay**

For *in vitro* treatment, macrophages were pre-incubated for 2 hours with KRB containing 1 mM glucose (as described in GSIS section) with or without the indicated treatment. To determine glucose uptake, macrophages were then incubated for 30 minutes with 0.4 nCi $^3$H labeled 2-deoxy glucose (Perkin Elmer), washed twice with ice cold PBS, lysed with 0.1% SDS and transferred into scintillation fluid. $^3$H labeled 2-deoxyglucose uptake was measured in a beta counter.

**Immunoblotting**

We separated proteins (8-12 μg) in 4-12% NuPAGE gels (Invitrogen), blotted them onto nitrocellulose membranes (Bio-Rad) and incubated them with antibodies against total AKT (pan AKT; #4691), pAKT (s473; #9271) and Insulin receptor β (488; #3025.) All antibodies
were purchased from Cell Signaling. Blots were analyzed using image lab 4.1 software (Bio-Rad).

**Flow cytometry**

To obtain single cells, islets were dispersed with trypsin (Invitrogen) for 6 minutes at 37°C, washed with PBS, centrifuged at 300 x g, 5 minutes, 4°C and resuspended in FACS buffer (PBS with 0.5% BSA and 2 mM EDTA). After 15 minutes incubation with an Fc blocker (Anti-mouse CD16/CD32; eBioscience 14-0161) peritoneal cells or single islet cells were stained with the appropriate antibodies for 30 minutes at 4°C in the dark. To verify the effect of the clodronate depletion, cells from Rag⁻/⁻ mice were stained with anti F4/80 (clone BM8) and anti CD11b (clone M1/70). To determine the peritoneal cell composition, cells were stained with anti CD3e (clone 145-2C11), anti GR-1 (clone RB6-8C5), anti CD11b (clone M1/70), anti F4/80- (clone BM8), anti CD19 (clone RA3-6B2) and anti Siglec-F-APC (clone E50-2440).

Single cells from adipose tissue, islets, Liver, small intestine, and colon were stained with CD45 (clone 30-F11) for immune cells (antibodies were purchased from eBioscience, Siglec-F was purchased from BD Pharmingen). For additional intra-cellular Insulin receptor intensity in tissue resident macrophages, cells were also incubated with intracellular fixation buffer (eBioscience) following incubation with permeabilization buffer (eBioscience) according to manufacturer’s instructions. Anti InsR β (Cell Signaling; 3025S) was added following secondary conjugated donkey anti rabbit antibody (Invitrogen). Stained cells were washed twice with FACS buffer prior to FACS acquisition. Cells were analyzed with an Accuri C6 flow cytometer or LSR-Fortessa (BD Bioscience). Dispersed islet cells were analyzed and sorted with a FACS ARIA III cell sorter (BD Biosciences) using FACS Diva software (BD Biosciences).

All samples were stained with appropriate isotype control antibodies; viability staining was done using 7-AAD (Sigma) or DAPI (Biolegend). Macrophages were defined as CD11b⁺ F4/80⁺ double positive cells. Data were analyzed using Flow Jo 9.4 software (Tree Star).
Extracellular acidification measurements

An XF24 or an XF96e Extracellular Flux analyzer (Seahorse Biosciences) was used to determine the bioenergetic profile of FACS sorted macrophages (F4/80 and CD11b double positive peritoneal cells). Cells were plated at a density of 500'000 or 300'000 cells per well in XF24 or XF96 plate accordingly, incubated for 4 hours and washed before being stimulated with LPS (100 ng/ml) + IFN\(\gamma\) (10 ng/ml) or IL-4\(\delta\)IL-13 (10 ng/ml) for the indicated times. Insulin was injected or added to the media (1 \(\mu\)g/ml end concentration) for the indicated time. Prior to the assay, cells were incubated in unbuffered RPMI (Seahorse Biosciences) containing 11.1 mM glucose for 1 hour. Then extracellular acidification rate were assessed during 2 minutes. Basal measurements were followed by measurements upon injection of the following agents: Glucose (26.8 mM), oligomycin (1 \(\mu\)M), carbonilcyanide p-trifluoromethoxyphenylhydrazone (FCCP) (2 \(\mu\)M), rotenone (1 \(\mu\)M), and 2-deoxyglucose (2DG; 10mM). To activate cells with insulin during Seahorse measurements cells were incubated in unbuffered RPMI containing 5mM glucose for 1 hour, then extracellular acidification rate were assessed during 2 minutes for 6 basal measurements followed by injections of inhibitors (LY294002, 10 \(\mu\)M; U0126, 10 \(\mu\)M) or DMSO as control and insulin (1 \(\mu\)g/ml). Oligomycin, FCCP, and rotenone were purchased from Sigma.

Immunofluorescence staining

Pancreata were fixed overnight in 4% paraformaldehyde at 4°C, followed by paraffin embedding. Sections were deparaffinized, re-hydrated and incubated 1 hour at room temperature with guinea pig anti-insulin antibody (Dako; A0564), followed by detection with a fluorescein-conjugated donkey anti-guinea pig antibody (Dako). Subsequently, the sections were labeled for IL-1R1 with goat anti IL-1-R1 antibody (R&D; AF771), followed by detection with a fluorescein-conjugated donkey anti-goat antibody (Invitrogen).
Statistics

Appropriate statistical tests were performed where required. Comparisons between groups were performed using unpaired, two-sided $t$-test for normally distributed data. For grouped comparisons, one-way ANOVA or two-way ANOVA followed by Sidak's multiple comparisons analysis were used where appropriate. Statistically significant outliers were assessed using ROUT's test ($O = 1\%$) and were excluded from analysis. Data analysis was performed using GraphPad Prism v7.0a Software

Excluding diet induced obesity experiments, all animal studies were performed on weight matched mice. There was no other prior randomization or blinding. Data are expressed as means ± s.e.m. and statistical significance is denoted as $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and, $^{****}P < 0.0001$. $n$ numbers indicate biological replicates for in vitro experiments or number of mice for in vivo experiments.

Method-only references


52. Horai, R., et al. Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. The Journal of experimental medicine 187, 1463-1475 (1998).


Figure a: Glucose levels in WT and Il1b−/− mice during fasting and refeeding.*** indicates statistical significance.

Figure b: Flow cytometry analysis of macrophages stained with F4/80 and CD11b.

Figure c: Glucose levels in mice treated with saline or IL-1Ra. * indicates statistical significance.