

Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells

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Exercise, obesity and type 2 diabetes are associated with elevated plasma concentrations of interleukin-6 (IL-6). Glucagon-like peptide-1 (GLP-1) is a hormone that induces insulin secretion. Here we show that administration of IL-6 or elevated IL-6 concentrations in response to exercise stimulate GLP-1 secretion from intestinal L cells and pancreatic alpha cells, improving insulin secretion and glycemia. IL-6 increased GLP-1 production from alpha cells through increased proglucagon (which is encoded by *GCG*) and prohormone convertase 1/3 expression. In models of type 2 diabetes, the beneficial effects of IL-6 were maintained, and IL-6 neutralization resulted in further elevation of glycemia and reduced pancreatic GLP-1. Hence, IL-6 mediates crosstalk between insulin-sensitive tissues, intestinal L cells and pancreatic islets to adapt to changes in insulin demand. This previously unidentified endocrine loop implicates IL-6 in the regulation of insulin secretion and suggests that drugs modulating this loop may be useful in type 2 diabetes.

Increased systemic IL-6 concentrations are associated with the pathophysiology of type 2 diabetes, with adipose tissue being the major source of this cytokine (refs. 1–3). Under these conditions, IL-6 is thought to contribute to the induction of insulin resistance and the deterioration of glucose homeostasis⁴. In contrast to the deleterious actions of systemically elevated IL-6, contracting skeletal muscle during exercise also increases circulating IL-6 concentrations^{5,6}. During exercise, it is proposed that IL-6 promotes nutrient availability and improves whole-body insulin sensitivity^{7,8}. These conflicting observations have led to a debate regarding the role of IL-6 in metabolism^{9–12}.

We recently found that the pancreatic alpha cell is a primary target of IL-6 action¹³. IL-6 promotes alpha cell proliferation and inhibits apoptosis. In response to a high-fat diet, alpha cell mass expands in an IL-6-dependent manner, corroborating these findings¹³. Furthermore, whole-body IL-6 knockout mice with no alpha cell expansion show increased glycemia after feeding caused by impaired insulin secretion. Thus, we proposed that alpha cell expansion in response to a high-fat diet may be required for functional beta cell compensation and that systemically increased IL-6 abundance induced by a high-fat diet is an adaptive response necessary to maintain proper insulin secretion and glucose homeostasis¹³. However, the mechanism linking alpha cell expansion to beta cell adaptation remains enigmatic when considering

the accepted role of alpha cells: increased alpha cell mass is normally expected to lead to an increase in glucagon production that would in turn lead to increased hepatic glucose output and a deterioration rather than the observed improvement in metabolic control.

GLP-1 is an incretin hormone secreted from intestinal L cells in response to nutrient intake and acts on beta cells to induce insulin secretion in a glucose-dependent manner^{14,15}. GLP-1 is liberated from its precursor proglucagon in intestinal L cells through processing by the enzyme PC1/3 (refs. 16–19).

In the pancreatic alpha cell, proglucagon is processed by PC2 to yield glucagon^{20,21}. Adult alpha cells are thought to produce little GLP-1. However, induction of diabetes in rodents leads to increased GLP-1 production in alpha cells together with increased alpha cell expression of PC1/3 (refs. 22–26).

In an attempt to explain how elevated IL-6 concentrations during obesity or exercise may improve beta cell insulin secretion, we hypothesized that IL-6 promotes GLP-1 production and secretion from intestinal L cells and pancreatic alpha cells, and here we show that is indeed the case, leading to improved beta cell insulin secretion and glucose tolerance. Thus, IL-6 is a hormone that mediates crosstalk between insulin-sensitive tissues and pancreatic islets through GLP-1.

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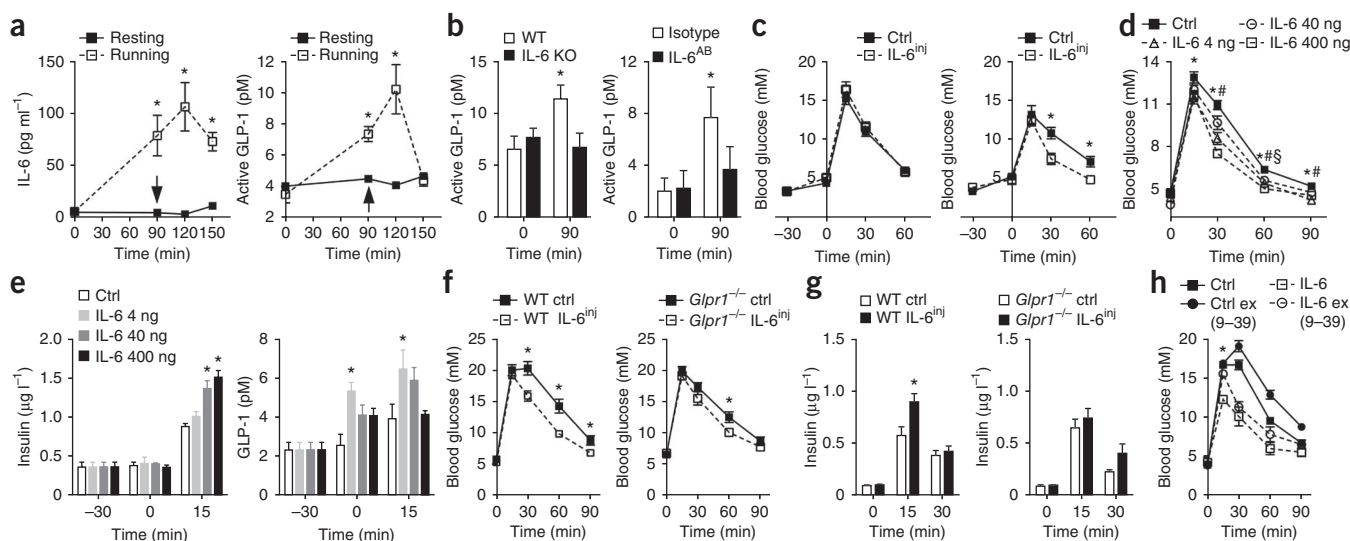


Figure 1 Effect of acute IL-6 on GLP-1 and insulin secretion *in vivo*. (a) Plasma IL-6 (left; resting, $n = 3$ and running, $n = 12$) and GLP-1 (right; resting, $n = 3$ and running, $n = 4$) in resting and running male mice. Arrows at 90 min indicate exhaustion and blood sampling. (b) Plasma GLP-1 in male mice before and after 90 min of running ($n = 6$). KO, knockout; IL-6^{AB}, antibodies to IL-6. (c) Intraperitoneal glucose tolerance test (ipGTT) (left) and oral glucose tolerance test (OGTT) (right) in female mice after a single injection of NaCl (ctrl) or 400 ng of IL-6 ($n = 4$). IL-6^{inj}, mice injected with IL-6. (d) OGTT after a single injection of NaCl or IL-6 in female mice ($n = 12$). (e) Plasma insulin (left) and GLP-1 (right) concentrations in female mice in response to oral glucose after a single injection of NaCl or IL-6 ($n = 4$); -30 min indicates the baseline measurement before NaCl or IL-6 injection, and 0 min indicates time point of glucose administration. (f) OGTT in male wild-type (WT) littermates (left) and GLP-1-receptor knockout (*Glp1r*^{-/-}) (right) mice after a single injection of NaCl or 400 ng of IL-6 ($n = 6-10$). (g) Oral-glucose-stimulated insulin secretion in male WT littermate (left) and *Glp1r*^{-/-} (right) mice after a single injection of NaCl or 400 ng of IL-6 ($n = 6-10$). (h) OGTT in male mice after a single injection of NaCl or 400 ng of IL-6 in the absence and presence of exendin (ex) (9-39) ($n = 4$). Data represent means \pm s.e.m. * $P < 0.05$, determined by Student's *t* test comparing control to IL-6 injection, resting to running or IL-6 to IL-6 plus exendin (9-39) (a-c, f-h). * $P < 0.05$ (400 ng IL-6), $^{\$}P < 0.05$ (40 ng IL-6), $^{\#}P < 0.05$ (4 ng IL-6), determined by analysis of variance (ANOVA) comparing control to IL-6 injections (d). * $P < 0.05$ comparing 400 ng IL-6, 40 ng IL-6 and 4 ng IL-6 to controls (e).

RESULTS

Exercise induces GLP-1 in an IL-6-dependent manner

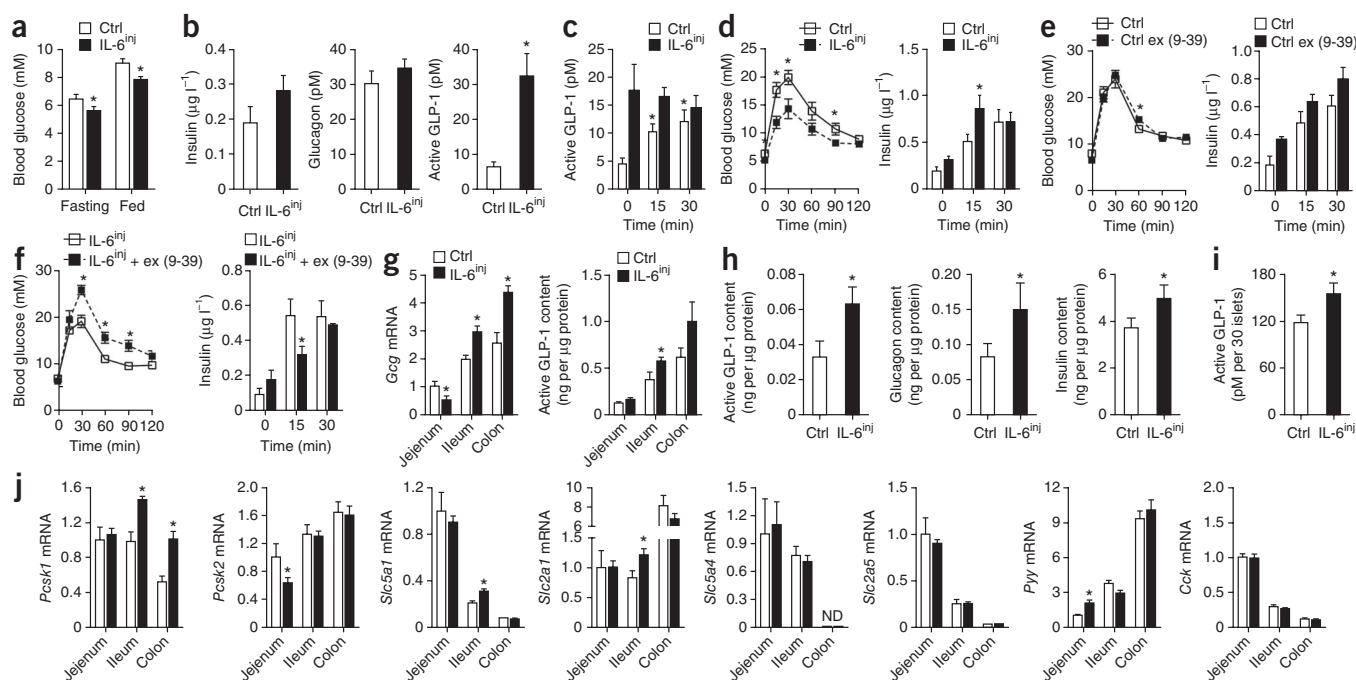
To address whether elevated systemic IL-6 levels have a physiological role in regulating plasma GLP-1 levels, we used exercise (treadmill running) as a model. Systemic IL-6 concentrations increased to 100 ± 20 pg ml⁻¹ (mean \pm s.e.m.) in response to exercise (Fig. 1a). Together with elevated IL-6 concentrations, we found a 2.5-fold increase in circulating active GLP-1 in response to 90 min of exercise (Fig. 1a). To address whether IL-6 is required for this exercise-induced increase in active GLP-1, we first subjected IL-6 knockout mice to a bout of exercise and found they were unable to increase plasma GLP-1 concentrations in response to this manipulation (Fig. 1b). In another set of experiments, we administered an antibody to IL-6 to block systemic IL-6 actions. We confirmed the specificity of the antibody by showing that it blocked IL-6-stimulated plasma serum amyloid A, whereas the isotype control did not (Supplementary Fig. 1). Indeed, neutralization of plasma IL-6 also inhibited the exercise-induced increase in plasma GLP-1 (Fig. 1b), confirming that systemic increases in IL-6 caused by exercise increase plasma GLP-1 concentrations.

IL-6 increases insulin secretion through GLP-1

Because systemically elevated IL-6 concentrations during exercise stimulated GLP-1 secretion, we hypothesized that acutely elevated IL-6 may improve oral glucose tolerance through the incretin action of GLP-1. To investigate this hypothesis, we injected a single bolus of 400 ng of IL-6 into mice 30 min before glucose administration (time point -30 min) followed by either intraperitoneal or oral (Fig. 1c) glucose administration (time point 0 min). IL-6 improved oral but

not intraperitoneal glucose tolerance, suggesting enhancement of the incretin axis. Dose-response experiments with 4, 40 and 400 ng of IL-6 led to circulating IL-6 concentrations ranging from 10 to 550 pg ml⁻¹ (Supplementary Fig. 2a), similar to the concentrations observed during exercise or after administration of a high-fat diet¹³ (Fig. 1a). All doses of IL-6 improved glucose tolerance (Fig. 1d), and 40 and 400 ng of IL-6 enhanced insulin secretion in a dose- and glucose-dependent manner (Fig. 1e), along with increasing plasma concentrations of GLP-1 (Fig. 1e) with no impact on insulin sensitivity (Supplementary Fig. 2b). In contrast, in GLP-1-receptor knockout (*Glp1r*^{-/-}) mice, IL-6 no longer enhanced glucose-stimulated insulin secretion or improved glucose tolerance (Fig. 1f,g), and the GLP-1 receptor antagonist exendin (9-39) prevented IL-6 from improving early glucose excursions during oral glucose tolerance testing (Fig. 1h). These data identify GLP-1 as an essential mediator of IL-6 actions on beta cell function and glucose homeostasis.

To study the effects of intermittently increased systemic IL-6 concentrations, we injected 400 ng of recombinant mouse IL-6 into mice twice daily for 7 d. We observed peak plasma IL-6 concentrations in response to a single injection after 30 min (baseline, 9.6 ± 2.5 pg ml⁻¹ compared to 30 min after injection, 552 ± 96 pg ml⁻¹ IL-6 concentrations, mean \pm s.e.m.) (Supplementary Fig. 2c), and the IL-6 concentrations returned to baseline values within 2 h after injection. We obtained all measurements listed below 15-17 h after the last injection of IL-6. After 7 days, both fasting and fed glycemia were lower in IL-6-injected mice compared to saline-injected mice, but we saw little effect on fasting insulin, glucagon (Fig. 2a,b) or GLP-2 (Supplementary Fig. 3). Notably, we detected fivefold higher fasting plasma GLP-1 concentrations in IL-6-injected mice compared



to saline-injected mice (Fig. 2b). However, we did not detect further increases in plasma GLP-1 following oral glucose in IL-6-injected mice (Fig. 2c). We reasoned that increased fasting GLP-1 levels might improve insulin secretion during an intraperitoneal glucose tolerance test. Indeed, compared to saline-injected mice, IL-6-injected mice showed improved glucose tolerance during an intraperitoneal glucose tolerance test, as well as enhanced glucose-stimulated insulin secretion (Fig. 2d). IL-6 injections had no effect on insulin sensitivity (Supplementary Fig. 4). To determine whether basally elevated GLP-1 levels in IL-6-injected mice were responsible for the increased glucose-stimulated insulin secretion and the improved glucose tolerance, we performed intraperitoneal glucose tolerance tests in the presence of the GLP-1 receptor antagonist exendin (9–39). Administration of exendin (9–39) prevented the improvement of glucose tolerance and insulin secretion by IL-6 (Fig. 2e,f). Thus, these data show that intermittently increased systemic IL-6 concentrations improve beta cell function and glucose homeostasis by increasing fasting GLP-1 concentrations and thus enhancing intraperitoneal glucose-stimulated insulin secretion.

IL-6 increases intestinal and pancreatic GLP-1

Next we examined whether IL-6 injections increased tissue *Gcg* mRNA expression and GLP-1 content. Compared to saline-injected mice, mice injected twice daily with IL-6 for 7 d showed higher *Gcg* mRNA expression and active GLP-1 content in the distal gut, where most L cells are localized (Fig. 2g). Furthermore, pancreatic

GLP-1, glucagon and insulin content were higher after injections of IL-6 compared to saline injections (Fig. 2h). In support of an islet origin for pancreatic GLP-1, isolated islets from IL-6-injected mice showed increased GLP-1 release over 24 h compared to saline-injected mice (Fig. 2i).

Analysis of intestinal tissue gene expression revealed higher PC1/3 (encoded by *Pcsk1*) mRNA expression in the ileum and colon of IL-6-injected mice compared to controls, with no difference in PC2 (encoded by *Pcsk2*) mRNA expression (Fig. 2j). IL-6-injected mice also showed higher ileum mRNA expression of sodium glucose transporter 1 (encoded by *Slc5a1*) and glucose transporter 1 (encoded by *Slc2a1*) as well as peptide tyrosine tyrosine (also known as peptide YY, encoded by *Pyy*) in the jejunum compared to saline-injected mice; however, we detected no differences in the levels of mRNA transcripts for sodium glucose transporter 3 (encoded by *Slc5a4*) and glucose transporter 5 (encoded by *Slc2a5*) in the same experiments (Fig. 2j). Finally, intestinal dipeptidylpeptidase 4 (encoded by *Dpp4*) mRNA and plasma *Dpp4* activity were not changed by IL-6, supporting IL-6-induced GLP-1 production rather than reduced clearance of GLP-1 (Supplementary Fig. 5a,b).

IL-6 increases GLP-1 synthesis and secretion in L cells

We next investigated the mechanism by which IL-6 promotes GLP-1 secretion and production in the gut by studying the direct effects of IL-6 on the mouse intestinal L cell line GLUTag. Western blot analysis confirmed expression of the IL-6 receptor in GLUTag cells

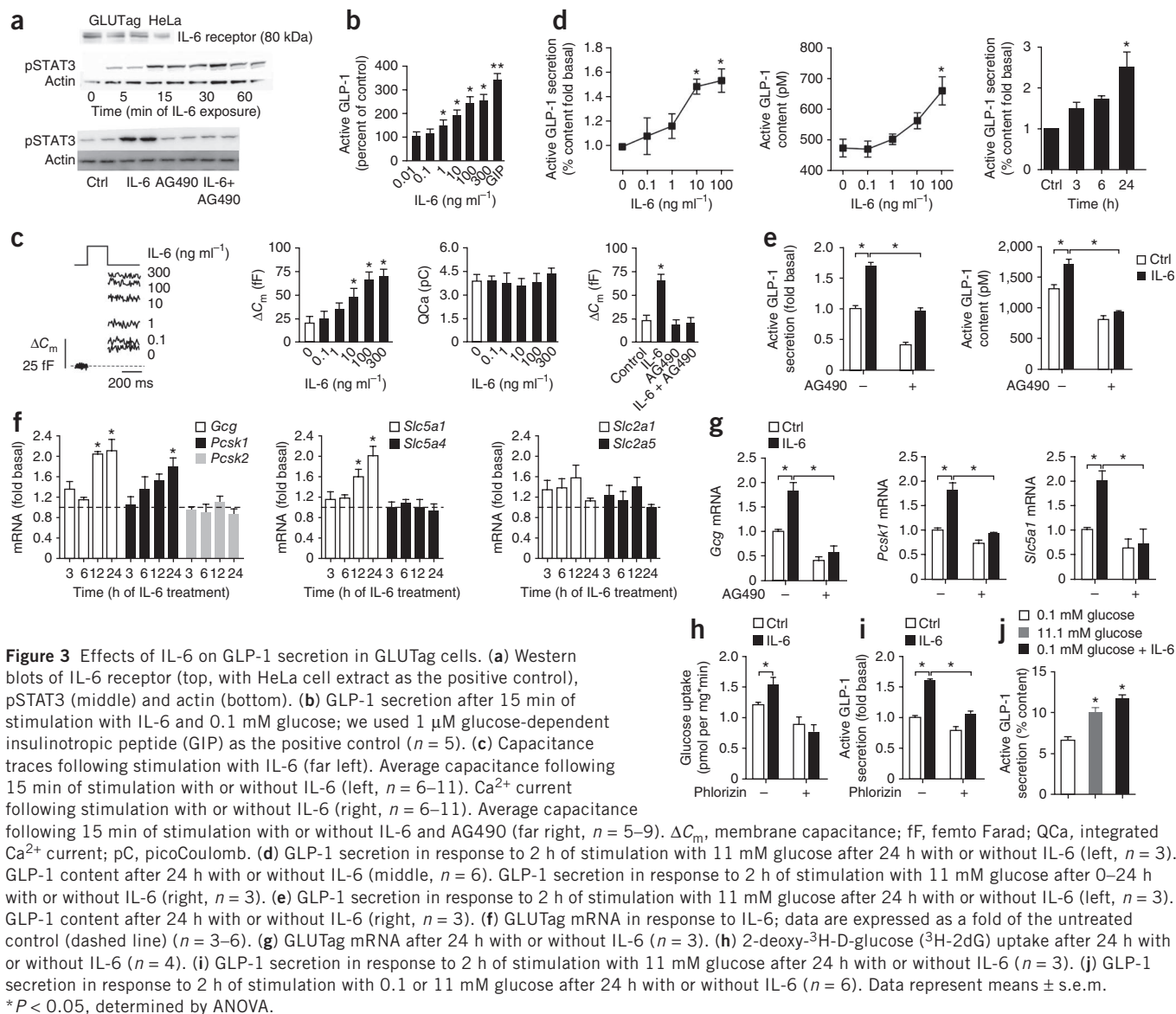


Figure 3 Effects of IL-6 on GLP-1 secretion in GLUTag cells. **(a)** Western blots of IL-6 receptor (top, with HeLa cell extract as the positive control), pSTAT3 (middle) and actin (bottom). **(b)** GLP-1 secretion after 15 min of stimulation with IL-6 and 0.1 mM glucose; we used 1 μ M glucose-dependent insulinotropic peptide (GIP) as the positive control ($n = 5$). **(c)** Capacitance traces following stimulation with IL-6 (far left). Average capacitance following 15 min of stimulation with or without IL-6 (left, $n = 6-11$). Ca^{2+} current following stimulation with or without IL-6 (right, $n = 6-11$). Average capacitance following 15 min of stimulation with or without IL-6 and AG490 (far right, $n = 5-9$). ΔC_m , membrane capacitance; fF, femto Farad; QCa, integrated Ca^{2+} current; pC, picoCoulomb. **(d)** GLP-1 secretion in response to 2 h of stimulation with 11 mM glucose after 24 h with or without IL-6 (left, $n = 3$). GLP-1 content after 24 h with or without IL-6 (middle, $n = 6$). GLP-1 secretion in response to 2 h of stimulation with 11 mM glucose after 0–24 h with or without IL-6 (right, $n = 3$). **(e)** GLP-1 secretion in response to 2 h of stimulation with 11 mM glucose after 24 h with or without IL-6 (left, $n = 3$). GLP-1 content after 24 h with or without IL-6 (right, $n = 3$). **(f)** GLUTag mRNA in response to IL-6; data are expressed as a fold of the untreated control (dashed line) ($n = 3-6$). **(g)** GLUTag mRNA after 24 h with or without IL-6 ($n = 3$). **(h)** 2-deoxy- ^3H -D-glucose (^3H -2dG) uptake after 24 h with or without IL-6 ($n = 4$). **(i)** GLP-1 secretion in response to 2 h of stimulation with 11 mM glucose after 24 h with or without IL-6 ($n = 3$). **(j)** GLP-1 secretion in response to 2 h of stimulation with 0.1 or 11 mM glucose after 24 h with or without IL-6 ($n = 6$). Data represent means \pm s.e.m. * $P < 0.05$, determined by ANOVA.

(Fig. 3a); activation of the IL-6 receptor was coupled to increased phosphorylation of signal transducer and activator of transcription 3 (STAT3) (Fig. 3a), and inhibition of janus kinase 2 (JAK2)-phosphorylated STAT3 (pSTAT3) with AG490 blocked IL-6-induced STAT3 phosphorylation (Fig. 3a).

To determine whether IL-6 directly stimulates GLP-1 secretion, we incubated GLUTag cells with increasing concentrations of IL-6 in the presence of 0.1 mM glucose (Fig. 3b). IL-6 (1–300 ng ml^{-1}) significantly increased GLP-1 secretion in a dose-dependent manner. We monitored increases in cell capacitance as a measure of exocytosis in individual GLUTag cells. IL-6 increased exocytosis in a dose-dependent manner (Fig. 3c). That capacitance increases after IL-6 stimulation independently of an increase in Ca^{2+} current (Fig. 3c) suggests that IL-6 enhances GLP-1 secretion beyond Ca^{2+} entry and thus modulates the exocytotic machinery. Inhibiting STAT3 phosphorylation completely blocked IL-6-induced exocytosis (Fig. 3c).

We next investigated whether IL-6 directly potentiated glucose-stimulated GLP-1 secretion. GLUTag cells treated with 0–100 ng ml^{-1}

of IL-6 for 24 h and then stimulated with 11 mM glucose for 2 h showed significantly increased GLP-1 secretion at 10 and 100 ng ml^{-1} IL-6 (Fig. 3d; unless otherwise indicated, we used 100 ng ml^{-1} of IL-6 in all further experiments). Cellular GLP-1 content was also increased in response to IL-6 (Fig. 3d). Time course experiments showed that the effect of IL-6 to potentiate glucose-stimulated GLP-1 secretion was most pronounced at 24 h after treatment (Fig. 3d). The effect of IL-6 on glucose-induced GLP-1 secretion and GLP-1 content was diminished after JAK2-pSTAT3 inhibition (Fig. 3e). Consistent with the importance of JAK2-STAT3 in the L cell²⁷, IL-6 potentiates glucose-stimulated GLP-1 secretion in a JAK2-STAT3-dependent manner.

To further investigate how IL-6 increases GLP-1 secretion and production in L cells, we analyzed the mRNA expression profiles of the candidate genes. Investigation of mRNA expression of *Gcg*, *Pcsk1*, *Pcsk2*, *Slc5a1*, *Slc5a4*, *Slc2a1* and *Slc2a5* 0 to 24 h after treatment with IL-6 revealed greater amounts of *Gcg*, *Pcsk1* and *Slc5a1* mRNA transcripts at 24 h (Fig. 3f). These mRNA effects were all reversed by JAK2-pSTAT3 inhibition (Fig. 3g), whereas the amount

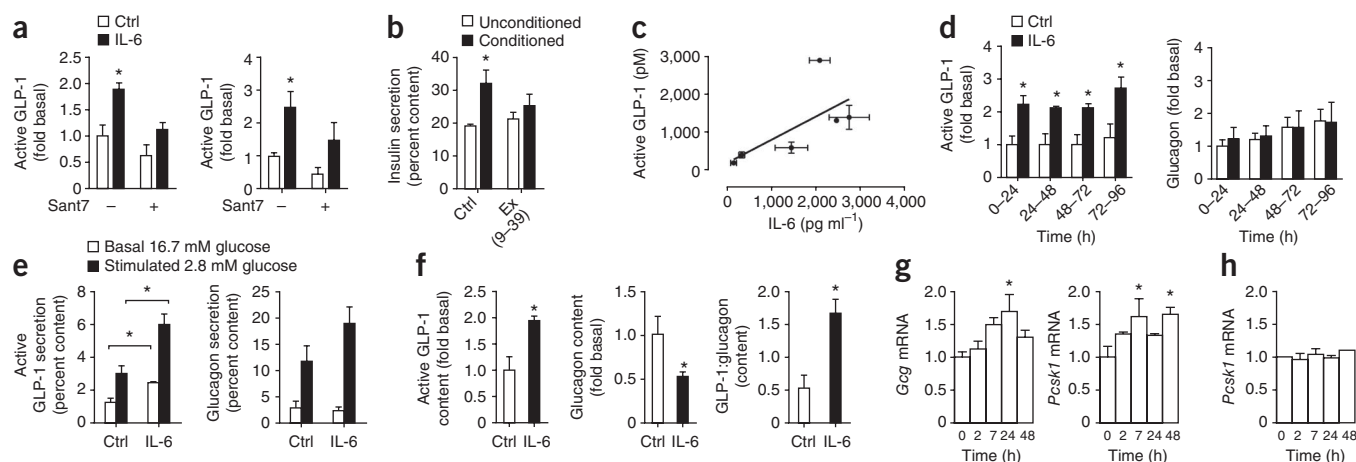


Figure 4 Effects of IL-6 on GLP-1 secretion in human islets and human alpha cells. **(a)** GLP-1 release (left) over 24 h after 4 d with or without IL-6 and with (+) or without (–) the IL-6 receptor antagonist Sant7 ($n = 3$). GLP-1 secretion (right) in response to 1 h of static incubation in 10 mM arginine after 5 days with or without IL-6 and with (+) or without (–) Sant7 ($n = 3$). **(b)** Human islet insulin secretion in response to 1 h of static incubation in unconditioned or conditioned medium from human islets in the absence and presence of exendin (9–39) ($n = 3$). **(c)** Basal GLP-1 and IL-6 released from various human islet preparations ($n = 6$, $r^2 = 0.46$, $P = 0.058$). **(d)** GLP-1 (left) and glucagon (right) released over consecutive 24 h intervals with or without IL-6 ($n = 4$). **(e)** GLP-1 (left) and glucagon (right) secretion in response to 1-h glucose incubations after 4 d with or without IL-6 ($n = 4$). **(f)** GLP-1 content (left), glucagon content (middle) and GLP-1:glucagon content as a molar ratio (right) after 4 days with or without IL-6 ($n = 3–4$). **(g)** FACS-enriched human alpha cell mRNA in response to IL-6 ($n = 3–6$). **(h)** FACS-sorted human beta cell mRNA in response to IL-6 ($n = 4$). Data represent means \pm s.e.m. * $P < 0.05$, determined by Student's t test comparing control to IL-6 or unconditioned to conditioned medium, or determined by ANOVA (**g,h**) comparing control (at time 0) to IL-6.

of *Pcsk2* mRNA transcripts (which is not regulated by IL-6) was not affected by JAK2-STAT3 inhibition (**Supplementary Fig. 6**).

Supporting a functional role for the enhanced expression of sodium glucose transporter 1 (encoded by *Slc5a1*), we observed greater glucose uptake in GLUTag cells after 24 h of IL-6 treatment compared to control cells (**Fig. 3h**), an effect that was abolished by the sodium glucose transporter 1 inhibitor phlorizin (**Fig. 3h**). The ability of IL-6 to potentiate glucose-induced GLP-1 secretion was also inhibited by phlorizin (**Fig. 3i**), suggesting that enhanced glucose uptake is crucial for IL-6 to stimulate GLP-1 secretion. Indeed, 0.1 mM glucose stimulated GLP-1 secretion to a similar degree as 11 mM glucose after IL-6 incubation (**Fig. 3j**).

In summary, these data show that IL-6 acutely increases GLP-1 secretion from GLUTag cells by directly increasing GLP-1 exocytosis, whereas chronic IL-6 exposure increases glucose-stimulated GLP-1 secretion by increasing GLP-1 biosynthesis and glucose uptake, rendering the L cell more responsive to glucose.

IL-6 increases GLP-1 secretion from human islet alpha cells

We used intact and FACS-sorted human islet cells to address whether IL-6 directly acts on alpha cells to increase GLP-1 production. Incubating human islets with IL-6 for 4 d with or without the IL-6 receptor antagonist super antagonist 7 (Sant7) indicated that IL-6 enhances both the constitutive release of GLP-1 as well as acute arginine-stimulated GLP-1 secretion (**Fig. 4a**). These effects were blunted in the presence of Sant7. That Sant7 tended to reduce GLP-1 in the absence of exogenous IL-6 can be explained by the presence of endogenous islet-derived IL-6 (ref. 28).

To assess whether the GLP-1 released from human islets was biologically active, we performed glucose-stimulated insulin secretion experiments using conditioned medium (cell culture medium from untreated human islets containing 11 mM glucose) in the absence and presence of exendin (9–39). These experiments showed improved insulin secretion stimulated by 11 mM glucose in islets incubated

with conditioned medium relative to unconditioned medium, and this improvement was reversed in the presence of the GLP-1 receptor antagonist exendin (9–39) (**Fig. 4b**). Thus, bioactive GLP-1 released from human islets has the ability to improve insulin secretion *in vitro*. Furthermore, we observed a positive correlation between basal IL-6 release and basal GLP-1 release in human islets from six different organ donors (**Fig. 4c**).

FACS-enriched human alpha cells incubated with IL-6 also showed more GLP-1 release compared to control cells (basal GLP-1 release over 0–24 h was 3.1 ± 0.6 nM (mean \pm s.e.m.), whereas glucagon release was not significantly different in cells incubated with IL-6 and control cells (basal glucagon release over 0–24 h was 2.0 ± 0.7 nM, mean \pm s.e.m.) (**Fig. 4d** and **Supplementary Table 1**). After 4 d of exposure to IL-6, human alpha cells showed increased GLP-1 secretion in response to an acute decrease in glucose from 16.7 to 2.8 mM compared to control cells (**Fig. 4e**). Stimulated glucagon secretion trended higher at low glucose concentrations (**Fig. 4e**). In agreement with the increased pancreatic GLP-1 content of IL-6-injected mice (**Fig. 2h**), the cellular GLP-1 content of enriched human alpha cells was 1.9-fold higher after IL-6 treatment, whereas glucagon content was decreased after treatment compared to controls (**Fig. 4f**). These data suggest a shift in the processing of proglucagon from glucagon toward GLP-1, as shown by the expression of the cellular content as the molar ratio of GLP-1:glucagon (**Fig. 4f**). Thus, these data show that IL-6 acts directly on alpha cells to enhance their ability to liberate GLP-1.

Gene expression analysis revealed higher amounts of *Gcg* and *Pcsk1* mRNA in response to IL-6 incubation in FACS-enriched human alpha cells after 24 and 7 h, respectively (**Fig. 4g**). IL-6 had no effect on *Pcsk1* mRNA in purified human beta cells, indicating an alpha cell-specific effect (**Fig. 4h**). These data support the notion that IL-6 increases alpha cell GLP-1 production by increasing both proglucagon gene transcription and its subsequent processing toward GLP-1 through PC1/3. Overall, IL-6 is able to directly increase GLP-1 secretion from the human islet alpha cell.

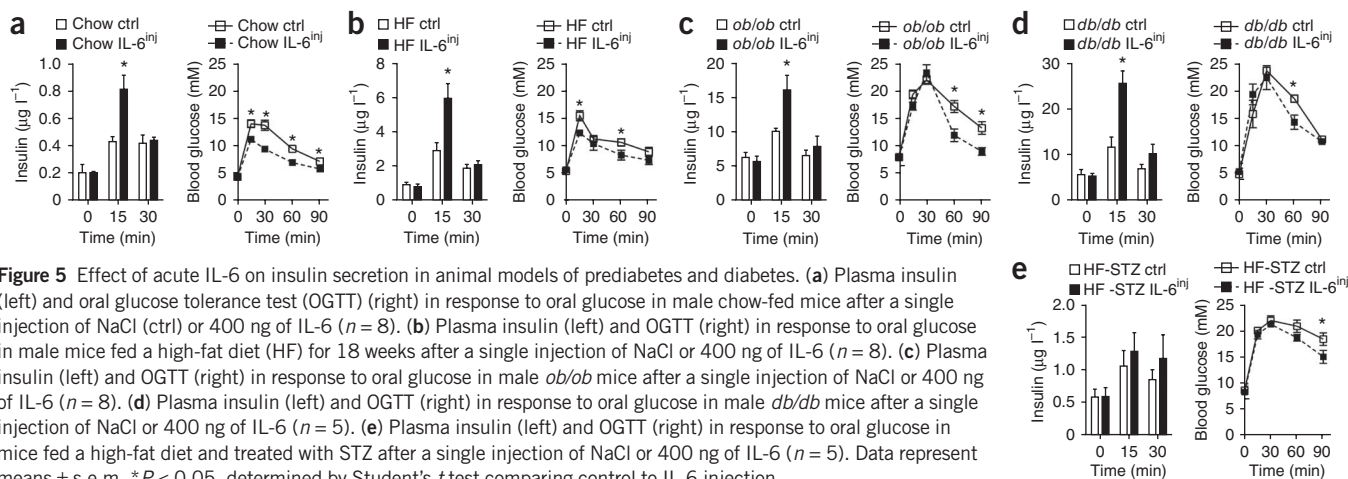


Figure 5 Effect of acute IL-6 on insulin secretion in animal models of prediabetes and diabetes. (a) Plasma insulin (left) and oral glucose tolerance test (OGTT) (right) in response to oral glucose in male chow-fed mice after a single injection of NaCl (ctrl) or 400 ng of IL-6 ($n = 8$). (b) Plasma insulin (left) and OGTT (right) in response to oral glucose in male mice fed a high-fat diet (HF) for 18 weeks after a single injection of NaCl or 400 ng of IL-6 ($n = 8$). (c) Plasma insulin (left) and OGTT (right) in response to oral glucose in male *ob/ob* mice after a single injection of NaCl or 400 ng of IL-6 ($n = 8$). (d) Plasma insulin (left) and OGTT (right) in response to oral glucose in male *db/db* mice after a single injection of NaCl or 400 ng of IL-6 ($n = 5$). (e) Plasma insulin (left) and OGTT (right) in response to oral glucose in mice fed a high-fat diet and treated with STZ after a single injection of NaCl or 400 ng of IL-6 ($n = 5$). Data represent means \pm s.e.m. * $P < 0.05$, determined by Student's *t* test comparing control to IL-6 injection.

Effect of acutely elevated IL-6 in animal models of diabetes

Because plasma concentrations of IL-6 are chronically increased in mouse models of obesity and diabetes^{13,29–31}, we questioned whether these mice still responded to an acute increase in IL-6 by improving beta cell function. Indeed, compared to a saline injection, a single bolus of IL-6 significantly increased glucose-stimulated insulin secretion in mice fed chow (Fig. 5a), mice fed a high-fat diet (Fig. 5b), *ob/ob* mice (Fig. 5c) and *db/db* mice (Fig. 5d). In contrast, in a high-fat diet model with direct beta cell destruction by streptozotocin (STZ), IL-6 failed to enhance insulin secretion (Fig. 5e). Overall, the effect of IL-6 on glucose tolerance (Fig. 5a–e) varied to a greater extent than the effect on insulin secretion, probably because of varying degrees of insulin resistance in the models used.

IL-6 reprograms alpha cells in response to obesity

We previously reported that a high-fat diet leads to an IL-6–dependent expansion of alpha cell mass¹³. However, the expansion of alpha cell mass in wild-type mice was not associated with changes in pancreatic glucagon content (Fig. 6a), whereas the trend for pancreatic GLP-1 content was to increase ($P = 0.08$) (Fig. 6a), leading to a significant increase in the GLP-1:glucagon content in wild-type but not in IL-6 knockout mice (Fig. 6a). This high-fat diet–induced increase in the pancreatic GLP-1:glucagon ratio suggests a shift in proglucagon processing in alpha cells and was associated with increased PC1/3 colocalization to alpha cells in wild-type mice, whereas PC1/3 remained undetectable in alpha cells of IL-6 knockout mice on either a chow or a high-fat diet (Fig. 6b).

Similar to studies in IL-6 knockout mice, high-fat diet feeding alone or in combination with IL-6 injections or IL-6 neutralization did not change the pancreatic glucagon content (Fig. 6c). In contrast, mice fed a high-fat diet had a higher pancreatic GLP-1 content compared to mice fed on chow (Fig. 6c), and, although IL-6 injections had no statistically significant additive effect on pancreatic GLP-1 content, treatment with an antibody to IL-6 blocked this high-fat diet–induced pancreatic GLP-1 (Fig. 6c). Supporting previous data²⁷, high-fat diet feeding was associated with higher GLP-1 content in the colon, an effect that was IL-6 dependent (chow, 1.2 ± 0.1 active GLP-1 ng μg^{-1} protein; high-fat diet, 1.7 ± 0.1 active GLP-1 ng μg^{-1} protein; and high-fat diet plus antibody to IL-6, 0.8 ± 0.2 active GLP-1 ng μg^{-1} protein, mean \pm s.e.m.). Moreover, IL-6 antagonism in mice fed on a high-fat diet worsened fasting glycemia (Supplementary Fig. 7a). Fasting plasma concentrations of GLP-1 were unchanged in response

to a high-fat diet and interventions with IL-6 (chow, 7.4 ± 1.9 pM active GLP-1; high-fat diet, 3.7 ± 0.3 pM active GLP-1; high-fat diet plus injected IL-6 3.9 ± 0.4 pM active GLP-1; and high-fat diet plus antibodies to IL-6, 3.7 ± 0.5 pM active GLP-1, mean \pm s.e.m.). Glucose-stimulated insulin secretion in response to intraperitoneal glucose revealed an enhanced insulin response in mice fed a high-fat diet compared to mice fed chow, and IL-6 injections further increased this response (Supplementary Fig. 7b). As was the case in chow-fed mice, in mice fed high-fat diet IL-6 interventions did not affect insulin tolerance (Supplementary Fig. 7c). Taken together, these data show that short-term antagonism of IL-6 signaling during high-fat diet feeding impairs the ability of alpha cells to increase GLP-1 production and also impairs glycemia.

Increasing high-fat diet–induced IL-6 concentrations by exogenous IL-6 injections revealed lower islet *Tnf* (also known as *Tnf- α*) mRNA expression and higher *Ins1*, *Ins2* and *Pdx1* (also known as *Ipf1*) mRNA expression (Supplementary Fig. 8). Finally, FACS sorting of alpha cells from transgenic mice fed on a high-fat diet expressing a yellow fluorescent protein under the control of the glucagon promoter³² showed that alpha cell PC1/3 mRNA expression was significantly lower in mice treated with antibodies to IL-6 compared to control mice fed a high-fat diet (Fig. 6d). Thus, high-fat diet–induced IL-6 promotes *Pcsk1* mRNA expression, PC1/3 protein expression and GLP-1 production in alpha cells. These data suggest that elevated systemic IL-6 concentrations during obesity increase alpha cell PC1/3 expression, causing a shift from glucagon toward GLP-1 production.

IL-6 antagonism deteriorates glycemia in *db/db* mice

Next we examined whether blocking increased levels of endogenous IL-6 in *db/db* mice^{29,33} precipitates diabetes. We treated *db/db* mice with an antibody to IL-6 for 4 weeks and found impaired fasting glycemia relative to control mice (Fig. 6e) and a severe deterioration of glucose tolerance without any difference in insulin tolerance (Fig. 6f). Beta cell responses to intraperitoneal glucose were absent in these 9- to 10-week-old mice, and antagonizing IL-6 had no effect on insulin secretion (Fig. 6f). Fasting plasma insulin was unchanged, whereas glucagon was increased (Fig. 6g), and GLP-1 concentrations were undetectable in mice treated with antibodies to IL-6 compared to control mice. The insulin and glucagon concentrations in the pancreatic tissue were not different, whereas the GLP-1 content was lower in mice receiving antibody to IL-6 compared to control mice (Fig. 6h).

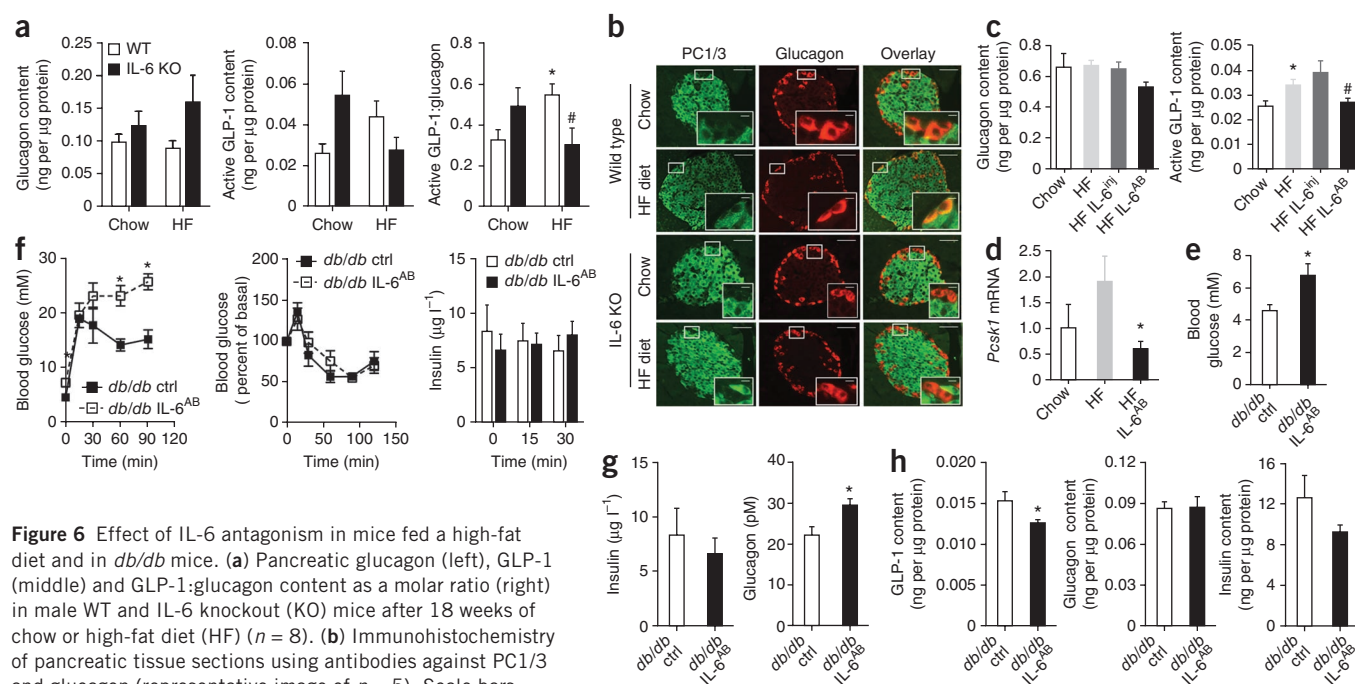


Figure 6 Effect of IL-6 antagonism in mice fed a high-fat diet and in *db/db* mice. **(a)** Pancreatic glucagon (left), GLP-1 (middle) and GLP-1:glucagon content as a molar ratio (right) in male WT and IL-6 knockout (KO) mice after 18 weeks of chow or high-fat diet (HF) ($n = 8$). **(b)** Immunohistochemistry of pancreatic tissue sections using antibodies against PC1/3 and glucagon (representative image of $n = 5$). Scale bars on large image: 50 μm ; on insets, 5 μm . **(c)** Pancreatic glucagon (left) and GLP-1 (right) in male mice fed chow or a high-fat diet for 15 weeks ($n = 7-8$). Mice on a high-fat diet and injected with IL-6 (HF IL-6^{inj}) received IL-6 twice daily for the last 7 days of the study, and mice fed on a high-fat diet and injected with an antibody to IL-6 (HF IL-6^{Ab}) received an antibody that neutralized IL-6 for the last 4 weeks of the study. **(d)** *Pcsk1* mRNA in FACS-sorted alpha cells from male mice expressing a yellow fluorescent protein under the control of the glucagon promoter after 20 weeks on chow or a high-fat diet ($n = 5-8$). **(e)** Blood glucose, **(f)** ipGTT (left), ITT (middle) and plasma insulin (right) in response to intraperitoneal glucose in male *db/db* mice after 4 weeks without (*db/db* ctrl) or with (*db/db* IL-6^{Ab}) treatment with antibodies to IL-6 ($n = 4-5$). **(g)** Fasting plasma hormones in male *db/db* mice with or without treatment with antibodies to IL-6 ($n = 4-5$). **(h)** Pancreatic hormone content in male *db/db* mice with or without treatment with antibodies to IL-6 ($n = 4-5$). Data represent means \pm s.e.m. * $\#P < 0.05$, determined by ANOVA, where the asterisk compares chow to a high-fat diet, and # compares genotypes on a high-fat diet only **(a)** or high-fat diet to a high-fat diet plus antibodies to IL-6 **(c)**. In **d-h**, * $P < 0.05$, determined by Student's *t* test comparing control mice to mice injected with antibodies to IL-6.

DISCUSSION

We identified IL-6 as a key regulator of glucose homeostasis through effects on L cell and alpha cell GLP-1 production and secretion and subsequent improvements in insulin secretion. Acute effects are caused by an IL-6-mediated increase in GLP-1 exocytosis in L cells, whereas chronic effects are caused by an IL-6-mediated increase in glucose responsiveness and GLP-1 production in L and alpha cells.

Exercise is accompanied by an increase in plasma GLP-1 concentrations³⁴⁻³⁷. Here we show that this increase is mediated by skeletal-muscle-derived IL-6. Elevated GLP-1 in response to exercise may be physiologically relevant through its effects on gut motility and satiety, whereas GLP-1 may improve beta cell function after physical training³⁸. Of note, the potentiating actions of GLP-1 on insulin secretion are glucose dependent and require blood glucose concentrations above $\sim 4-5$ mM (ref. 15). Therefore, during exercise, IL-6-induced GLP-1 will not acutely affect insulin secretion in subjects without diabetes. In line with this concept, GLP-1 was found to determine the future insulin secretory response; that is, basal GLP-1 levels help prepare the beta cell for the subsequent meal, resulting in a potentiating effect on glucose-stimulated insulin secretion³⁹⁻⁴¹. Therefore, IL-6-induced GLP-1 released during exercise will promote insulin secretion during a post-exercise meal.

Compared to weight-matched wild-type mice on high-fat diet, IL-6 knockout mice on a high-fat diet show glucose intolerance caused by impaired insulin secretion^{13,42}. In this study, we show that these

mice have reduced pancreatic GLP-1 and that neutralization of IL-6 in wild-type mice fed on a high-fat diet and in *db/db* mice impairs this obesity-induced alpha cell adaptation associated with increased glycemia. This suggests that IL-6 released by adipose tissues has a compensatory role under conditions of obesity by increasing islet GLP-1 production to augment insulin secretion in order to adapt to insulin resistance and prevent diabetes.

GLP-1 produced by L cells is thought to act on beta cells through the circulation. We show that IL-6 increases GLP-1 production in the alpha cell during obesity, probably through increased proglucagon transcription and PC1/3 expression. Previous work has shown that alpha cells can be a source of GLP-1 and increased PC1/3 expression under conditions of beta cell stress²²⁻²⁶. A study describing the architecture in human islets reported direct intercellular contacts between alpha and beta cells, supporting the notion that alpha cell products can act in a paracrine manner to regulate the beta cell⁴³. In support of this, a recent study shows that acetylcholine secreted by alpha cells acts in a paracrine manner to prime the beta cell to respond optimally to subsequent increases in glucose⁴⁴. Along these lines, alpha cell-derived GLP-1 may act locally on the beta cell to potentiate glucose-induced insulin secretion and to promote survival, whereas L cell-derived GLP-1 will act locally to decrease gut motility and enhance satiety. Considering the short half-life of bioactive plasma GLP-1 (< 2 min)⁴⁵, a paracrine rather than an endocrine action of GLP-1 seems more likely. Prolonging the half-life of GLP-1 using

DPP4 inhibitors is a widely used antidiabetic treatment. It remains to be shown whether the main action of DPP4 inhibition occurs at the plasma or tissue level.

Several studies have uncovered IL-6 as a cytokine that regulates glucose tolerance, obesity and insulin action^{46–50}. However, there exists little consensus on whether IL-6 is beneficial or detrimental. We enhanced systemic IL-6 concentrations through exercise, a high-fat diet and acute and intermittent IL-6 injections, and we observed consistent beneficial effects. Thus, IL-6 may adapt the metabolism to exercise and obesity, and during proinflammatory states IL-6 may contribute to adjust the metabolism to the requirement of the immune system.

In this study, we describe an adipose-tissue and skeletal-muscle enteroendocrine-islet axis. This crosstalk between insulin-sensitive tissues and insulin-producing cells is mediated through IL-6 acting on L cells and alpha cells to promote GLP-1 secretion and production, thereby allowing for adaptation to increased insulin demand during obesity and improved beta cell function in response to physical training. Failure to adapt to enhanced insulin demand leads to type 2 diabetes. Thus, understanding this endocrine loop may open the door to new therapeutic approaches or lead to a more judicious use of existing drugs modulating IL-6 and GLP-1.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturemedicine/>.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

H.E. designed and performed experiments, analyzed data and wrote the manuscript. I.H., B.S., A.M.H., L.L.B., D.T.M., E.E., J.G., S.W., A.M.K.H., D.K., M.R., M.G., D.J.D., F.R. and F.M.G. performed experiments. Y.D.M. isolated human islets. K.B. and P.A.H. provided FACS-sorted human islet cells. D.J.D., F.M.G. and F.R. provided mice for this study. J.A.E. and M.Y.D. designed experiments and wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. Male and female C57BL/6J mice (Harlan), male C57BL/6J wild-type and IL-6 knockout B6;129S2-*Il6^{tm1Kopf}*/J mice, male *db/db* and *ob/ob* mice (Jackson Laboratories), male wild-type (*Glp1r^{+/+}*) and GLP-1-receptor knockout (*Glp1r^{-/-}*) littermates and male transgenic mice expressing a yellow fluorescent protein under the control of the glucagon promoter were used. We followed the guidelines for laboratory animals from the University of Zurich and Mt. Sinai Hospital, Toronto and the Zurich, Mt. Sinai Hospital Cantonal Animal Experimentation Committee granted ethical approval. The UK Home Office and Local Ethical Committee approved the work done in Cambridge. We fed mice standard chow or a high-fat diet for 15 to 22 weeks¹³. After 3 weeks, we injected some of the mice with 130 mg per kg of body weight of STZ and continued the high-fat diet.

Injections of IL-6 and antibodies to IL-6. We injected mice either twice daily for 7 d with 400 ng of carrier-free recombinant IL-6 (R&D Systems) or saline (NaCl) or with a single bolus of 4, 40 or 400 ng of IL-6 30 min before administration of glucose or insulin. We injected mice fed on a high-fat diet and *db/db* mice with 500 µg of an antibody that neutralizes IL-6 (R&D Systems) or PBS twice weekly for the final 2–4 weeks of the experiment. One hour before treadmill running, we injected mice with 500 µg of antibody that neutralizes IL-6 or an isotype control. We used a Luminex kit (Millipore) to measure plasma IL-6 concentrations.

Glucose and insulin tolerance tests. We performed glucose tolerance tests by administering 2 g of glucose per kg of body weight orally (gavage) or by injecting 2 g of glucose per kg of body weight intraperitoneally. In some experiments, we injected 25 nM per kg body weight exendin (9–39) (a GLP-1 receptor antagonist; Bachem) either 1 min (for ipGTTs) or 15 min (for OGTTs) before glucose administration. To analyze the plasma, we used insulin ELISA (Millipore and ALPCO), GLP-1 Luminex and GLP-1 ELISA (Millipore), the in-house Novo Nordisk assay²⁶ and GLP-2 ELISA (Labodia). We performed insulin tolerance tests injecting 0.75–4 U of insulin per kg of body weight (Novo Nordisk).

Treadmill running. Mice ran 10 m per min at a 0° inclination for 30 min followed by a gradual increase by 3 m per min and a 2.5° inclination every 10 min for 90 min until exhaustion. Exhaustion has been defined previously⁵¹.

Tissue content. We determined the hormone content in homogenized pancreatic and intestinal tissues after overnight HCl-EtOH (0.18 M HCl in 70% ethanol) extraction at 4 °C. We diluted the supernatant in H₂O and measured active GLP-1 and glucagon using the in-house Novo Nordisk kit²⁶ and radioimmunoassay (Millipore). Insulin was measured using ELISA (Mercodia), and protein concentration was measured using a bicinchoninic acid assay (Pierce).

Immunohistochemistry. We used pancreatic paraffin-embedded tissue sections for double immunofluorescence^{52,53} with primary antibodies to PC1/3 (Millipore) and glucagon (Linco).

Human islets. We isolated human islets from pancreata of organ donors at the University of Geneva Medical Center, Geneva, Switzerland, and cultured them as previously described²⁸. We used 1 mM diprotin A (Dpp4 inhibitor; Sigma), 200 ng ml⁻¹ recombinant human IL-6 (R&D Systems), 200 ng ml⁻¹ Sant7 (an IL-6 receptor antagonist provided by Sigma-Tau⁵⁴) and 100 nM exendin (9–39). We used an IL-6 ELISA (R&D Systems) to measure IL-6 concentrations.

Fluorescence-activated cell sorting. We sorted human beta cells by FACS after labeling with Newport Green⁵⁵ giving rise to a cell population consisting of, on average, 96% beta cells. We also used the FACS-sorted non-beta-cell fraction enriched in alpha cells (on average, 50% alpha cells). We cultured cells as human islets and assessed active GLP-1 and glucagon secretion by 1 h static incubations in 16.7 and 2.8 mM glucose. We extracted the cellular content with HCl-EtOH. We separated fluorescent alpha cells from transgenic mice by FACS as previously described³².

Mouse islets. We isolated and cultured mouse pancreatic islets as described²⁸ in the presence of 1 mM diprotin A.

GLUTag cells. We cultured GLUTag cells as described⁵⁶ and treated them with 100 ng ml⁻¹ mouse IL-6 (unless otherwise indicated) and 1 mM diprotin A. We assessed active GLP-1 secretion by 2 h static incubations in 0.1 or 11.1 mM glucose and used 50 µM of the JAK2-pSTAT3 inhibitor AG490 and 100 µM phlorizin (Fluka). GLP-1 and glucagon radioimmunoassays (Millipore) were used for all *in vitro* experiments. We used the perforated-patch whole-cell configuration of the patch-clamp technique and an EPC9 patch-clamp amplifier (Heka Elektronik) to monitor changes in cell capacitance as a measure of exocytosis.

Western blotting. We separated proteins (20–50 µg) on 4–12% NuPAGE gels (Invitrogen), blotted them onto nitrocellulose membranes (Bio-Rad) and incubated them with antibodies against pSTAT3 (Tyr705) (Cell Signaling), actin and IL-6 receptor (Santa Cruz).

RNA extraction and real-time PCR. We extracted tissue and cellular RNA according to the manufacturer's instructions (QIAGEN and MACHEREY-NAGEL respectively, or Ambion for FACS fluorescent alpha cells) and performed real-time PCR using primers and the ABI 7500 and 7900 systems (Applied Biosystems). We calculated changes in mRNA expression using differences of C_T values compared to housekeeping genes (18S or β actin).

2-deoxy-³H-D-glucose uptake. We incubated GLUTag cells with or without 100 ng ml⁻¹ of IL-6 and determined uptake of 2-deoxy-³H-D-glucose (³H-2dG) (10 µM) as reported⁵⁷ with or without 100 µM phlorizin (Fluka).

Statistics. Data are expressed as means ± s.e.m. We considered a value of *P* < 0.05 to be statistically significant.

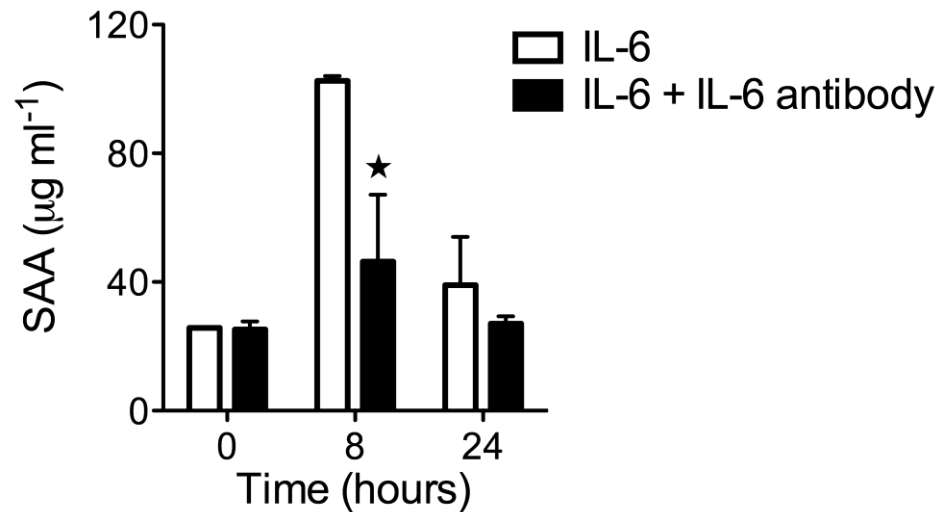
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Supplementary online material to

Interleukin-6 enhances insulin secretion by increasing L cell and α cell glucagon-like peptide-1 secretion

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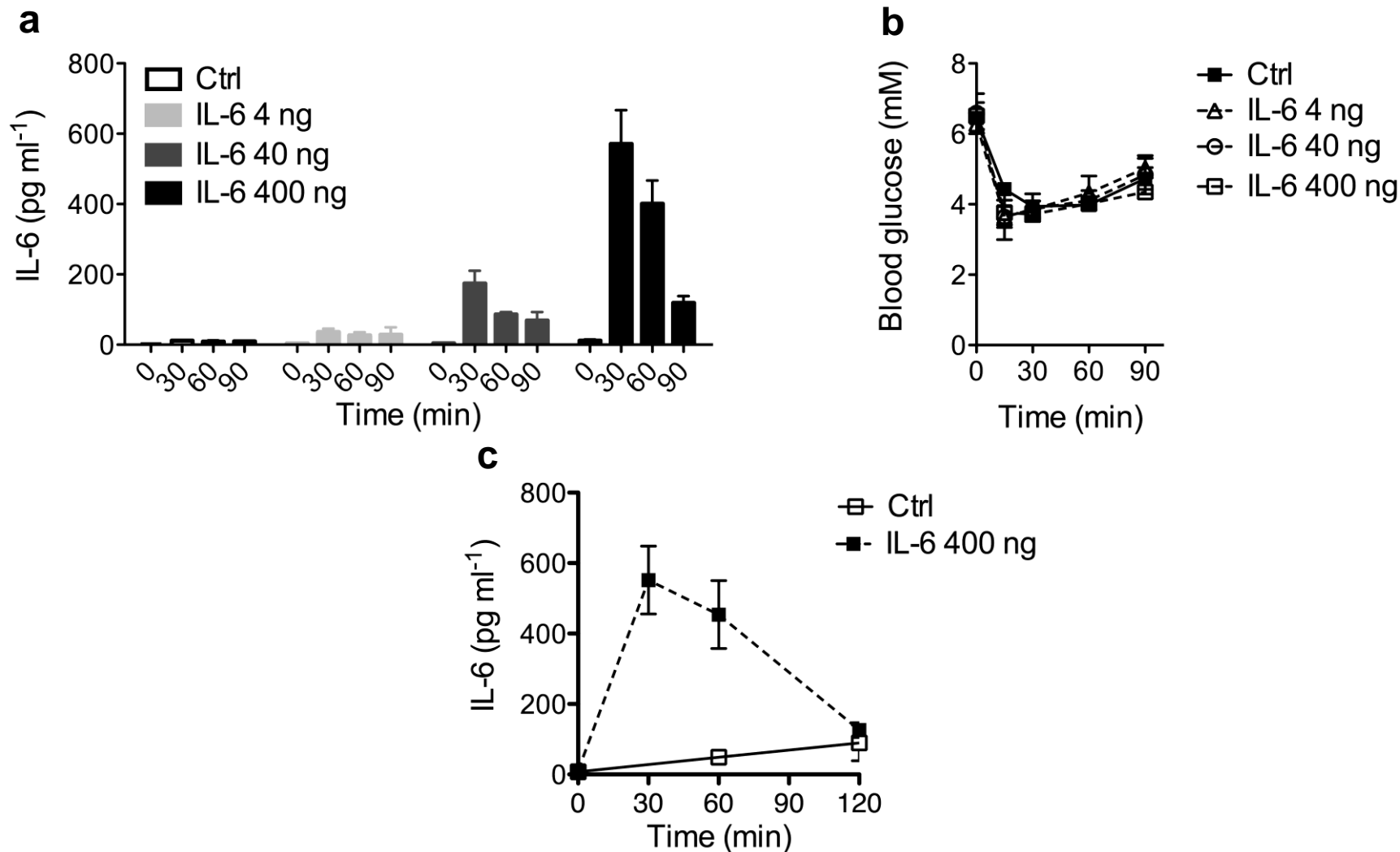
Supplementary Figure 1. Validation of IL-6 antibody.



Supplementary Figure 1. Validation of IL-6 antibody.

Plasma Serum Amyloid A (SAA) in response to a single injection of 100 ng IL-6 in the presence of an isotype control or an IL-6 neutralizing antibody ($n = 3$). Data represents mean \pm s.e.m. * $P < 0.05$ by Student's t test.

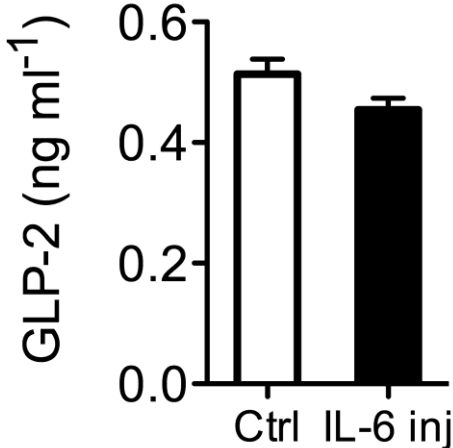
Supplementary Figure 2. Plasma IL-6 and insulin tolerance after injection with IL-6.



Supplementary Figure 2. Plasma IL-6 and insulin tolerance after injection with IL-6.

(a) Plasma IL-6 concentrations in male mice in response to a single intraperitoneal injection of IL-6 ($n = 6$). (b) Insulin tolerance test in male mice after a single injection of IL-6 ($n = 6$). (c) Plasma IL-6 in male mice in response to a single IL-6 injection ($n = 4-6$). Data represents mean \pm s.e.m.

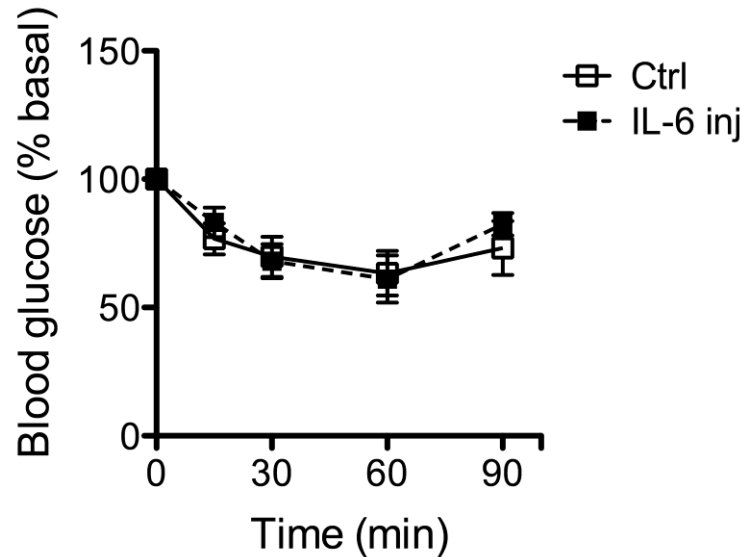
Supplementary Figure 3. IL-6 effects on GLP-2.



Supplementary Figure 3. IL-6 effects on GLP-2.

Fasting plasma GLP-2 in male mice injected with or without 400 ng IL-6 twice daily for 7 days ($n = 10$). Data represents mean \pm s.e.m.

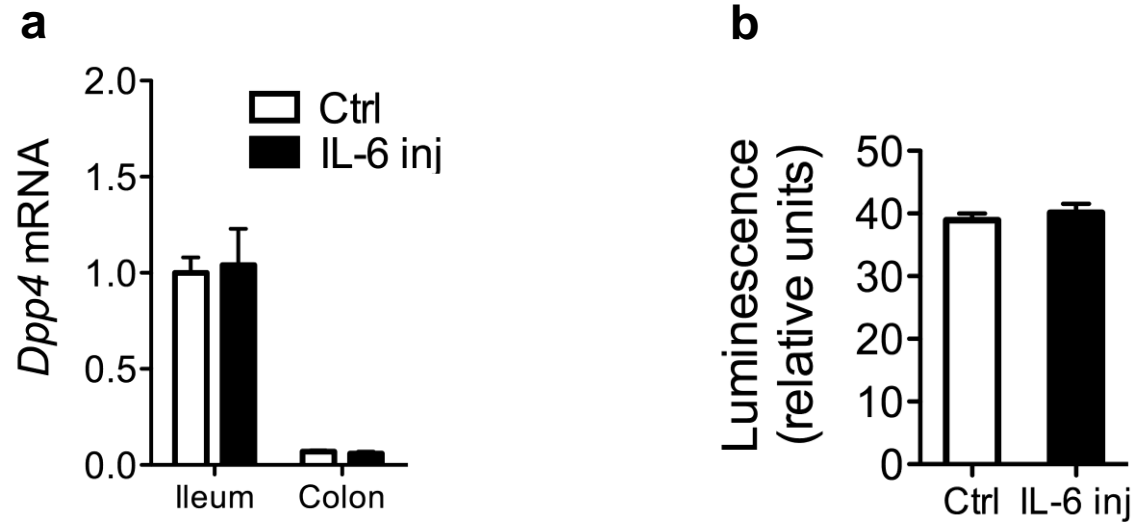
Supplementary Figure 4. Insulin tolerance test.



Supplementary Figure 4. Insulin tolerance test.

Insulin tolerance test in in male mice injected with or without 400 ng IL-6 twice daily for 7 days ($n = 8$). Data represents mean \pm s.e.m.

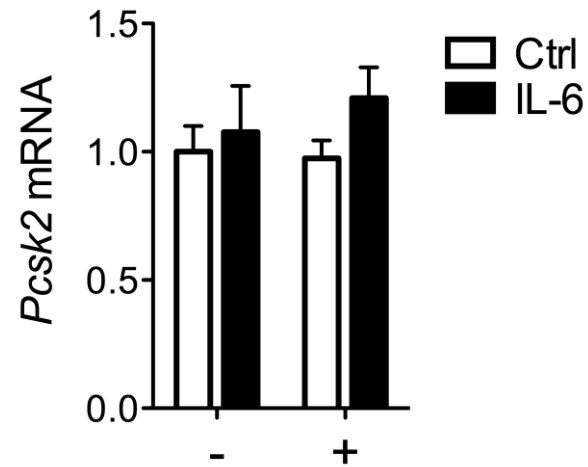
Supplementary Figure 5. IL-6 effects on DPP-4.



Supplementary Figure 5. IL-6 effects on DPP-4.

(a) Intestinal mRNA expression in male mice injected with or without 400 ng IL-6 twice daily for 7 days ($n = 6-8$). Data expressed relative to ileum control. (b) DPP-4 activity in plasma from male mice 30 min after a single injection of 400 ng IL-6 ($n = 5$). Data represents mean \pm s.e.m.

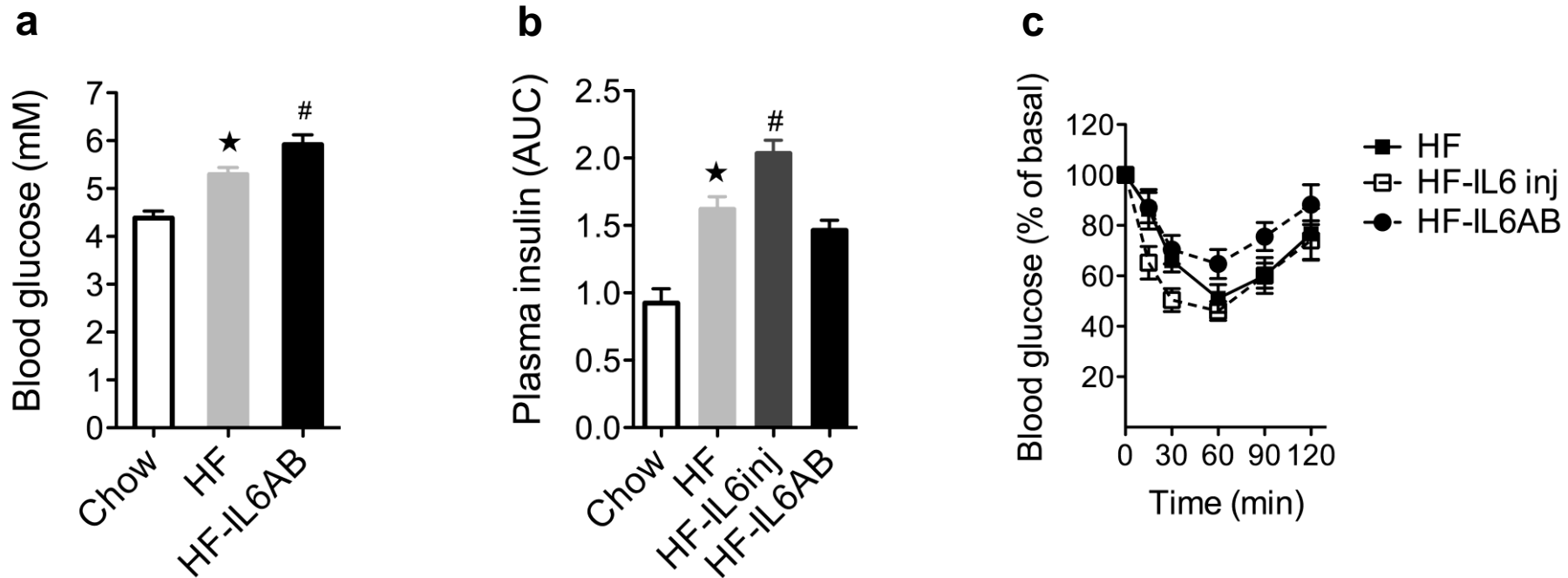
Supplementary Figure 6. Prohormone convertase 2 in GLUTag cells.



Supplementary Figure 6. Prohormone convertase 2 in GLUTag cells.

GLUTag prohormone convertase 2 (*pcsk2*) mRNA after 24 h \pm 100 ng ml⁻¹ IL-6 in the absence (-) or presence (+) of 50 μ M AG490. Data expressed relative to control ($n = 3$). Data represents mean \pm s.e.m.

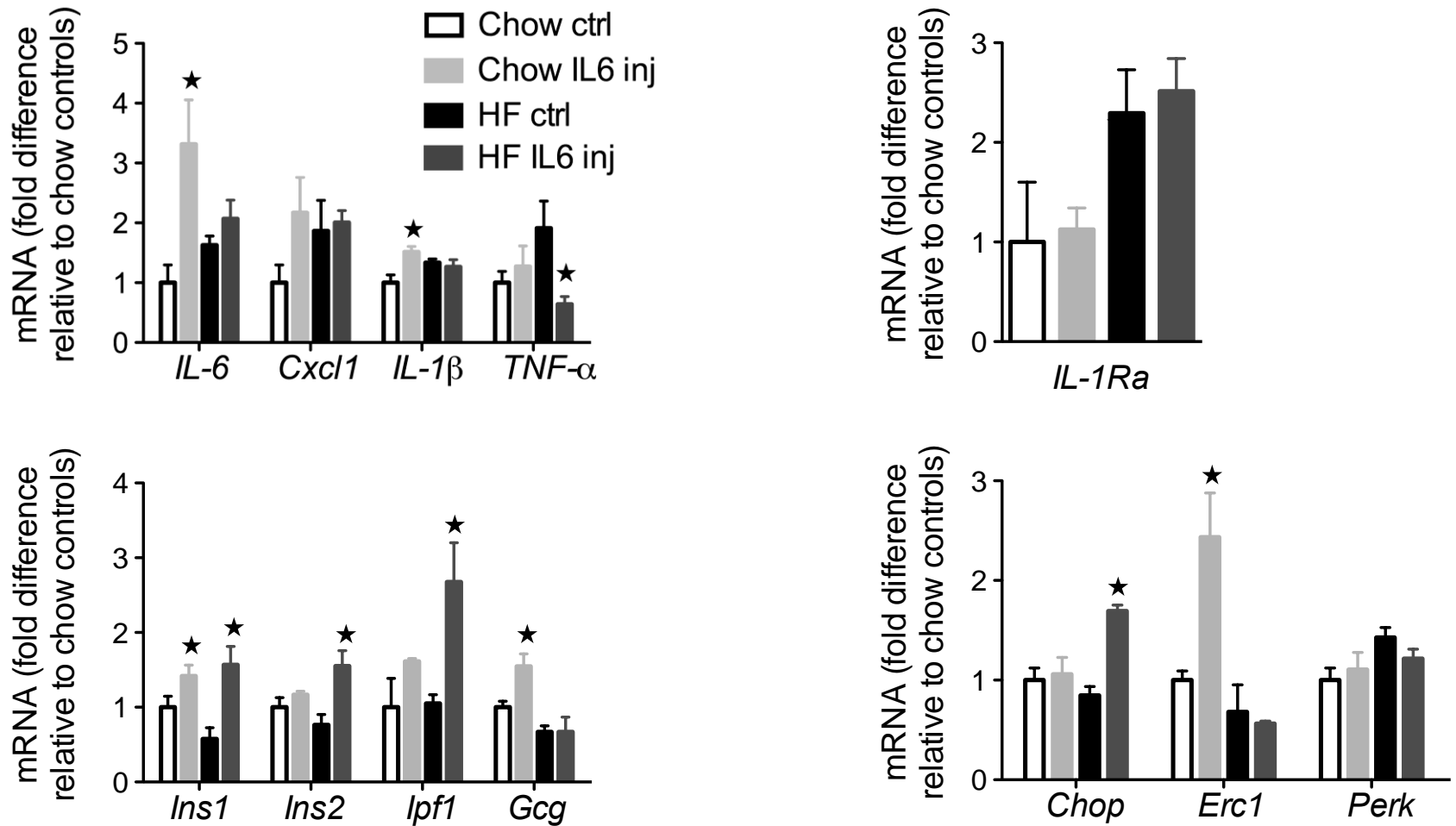
Supplementary Figure 7. IL-6 interventions in high fat (HF) diet fed mice.



Supplementary Figure 7. IL-6 interventions in high fat (HF) diet fed mice.

(a) Fasting blood glucose. (b) Insulin secretion in response to intraperitoneal glucose (c) Insulin tolerance test. Male mice were fed chow or HF diet for 15 weeks, HF-IL6inj mice were injected twice daily with 400 ng IL-6 for the last 7 days of the study, whereas HF-IL6AB mice were injected twice weekly with a neutralizing IL-6 antibody for the last four weeks of the study ($n = 7-8$). Data represents mean \pm s.e.m. * $P < 0.05$ by Anova comparing chow vs. HF and # comparing HF vs. HF-IL6AB (panel a) and HF vs. HF-IL6inj (panel b).

Supplementary Figure 8. Pancreatic islet gene expression.



Supplementary Figure 8. Pancreatic islet gene expression.

mRNA expression in islets from male mice fed chow or high fat (HF) diet for 18 weeks and Injected with 400 ng IL-6 or NaCl (Ctrl) twice daily for the last 7 days of the experiment ($n = 4$). Data represents mean \pm s.e.m. * $P < 0.05$ by Student's t test comparing changes from baseline (chow controls and HF controls respectively).

Supplementary Table 1. GLP-1 and glucagon in FACS sorted human α cells.

Time (hours)	Active GLP-1 (nM)	Glucagon (nM)
0 - 24	3.1 \pm 0.6	2.0 \pm 0.7
24 - 48	2.7 \pm 0.5	2.4 \pm 0.8
48 - 72	3.2 \pm 0.6	3.2 \pm 1.2
72 - 96	3.7 \pm 1.0	3.6 \pm 1.4
Content	17.6 \pm 3.4	35.0 \pm 4.6

Supplementary Table 1. GLP-1 and glucagon in FACS sorted human α cells. Active GLP-1 and glucagon accumulation into culture media is provided for four consecutive 24 h intervals. Cellular content was extracted after 96 h ($n = 4$). Data represents mean \pm s.e.m