

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

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### Abstract

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.

### Reference

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## Interleukin-6 enhances insulin secretion by increasing L cell and $\alpha$ cell glucagon-like peptide-1 secretion

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### ABSTRACT

Exercise, obesity, and type 2 diabetes are all associated with elevated plasma levels of interleukin-6 (IL-6). Glucagon-like peptide-1 (GLP-1) is an incretin hormone acting on pancreatic  $\beta$  cells to induce insulin secretion in a glucose-dependent manner. Here we show that elevated IL-6 levels in response to exercise, and acute and chronic IL-6 administration can stimulate GLP-1 secretion and production from intestinal L cells and pancreatic  $\alpha$  cells leading to improved insulin secretion and glucose homeostasis. *In vitro*, IL-6 increased GLP-1 synthesis and secretion from enteroendocrine L cells both acutely and chronically via direct effects on exocytosis and glucose uptake respectively. IL-6 also increased GLP-1 synthesis and secretion from human pancreatic  $\alpha$  cells in association with increased proglucagon and prohormone convertase 1/3 transcription. In models of obesity and type 2 diabetes, the beneficial effects of acutely elevating IL-6 were maintained. Indeed, IL-6 neutralization in these models deteriorated glycemia and was associated with reductions in pancreatic GLP-1 content. Hence, IL-6 mediates cross talk between insulin sensitive tissues, L cells and pancreatic islets to adapt to changes in insulin demand by increasing L cell GLP-1 secretion and reprogramming  $\alpha$  cells to process proglucagon to GLP-1. This novel endocrine loop implicates IL-6 in the regulation of  $\beta$  cell insulin secretion in both health and disease, and suggests how drugs modulating this loop may be useful in obesity and type 2 diabetes.

Increased systemic IL-6 levels are associated with the pathophysiology of obesity and type 2 diabetes with adipose tissue being the major source<sup>1,2,3</sup>. Under these conditions, IL-6 is thought to contribute to the induction of insulin resistance and the deterioration of glucose homeostasis<sup>4</sup>. In contrast to these deleterious actions of elevated IL-6 levels, contracting skeletal muscle during exercise also increases systemic IL-6 levels<sup>5,6</sup>. During exercise, it is proposed that IL-6 promotes nutrient availability and improves whole body insulin sensitivity<sup>7,8</sup>. These conflicting observations have led to a debate regarding the physiological role of elevated systemic IL-6 levels<sup>9,10,11,12</sup>.

We recently found that the pancreatic  $\alpha$  cell is a primary target of IL-6 actions<sup>13</sup>. IL-6 promotes  $\alpha$  cell proliferation and inhibits  $\alpha$  cell apoptosis in the presence of toxic levels of nutrients. In response to high fat (HF) diet,  $\alpha$  cell mass expanded in an IL-6-dependent manner, corroborating these findings<sup>13</sup>. Most strikingly however, whole body IL-6 knockout (IL-6 KO) mice with no  $\alpha$  cell expansion displayed increased fed glycemia due to impaired insulin secretion. Thus, we proposed that  $\alpha$  cell expansion in response to HF diet may be required for functional  $\beta$  cell compensation and that systemically increased IL-6 induced by HF diet was an adaptive response necessary to maintain proper insulin secretion and glucose homeostasis<sup>13</sup>. Yet, the mechanism linking  $\alpha$  cell expansion to  $\beta$  cell adaptation remained enigmatic considering the accepted role of  $\alpha$  cells, with increased  $\alpha$  cell mass 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 integral to the maintenance of glucose homeostasis<sup>14,15,16</sup>. It is secreted from intestinal L cells in response to nutrient intake and acts on the  $\beta$  cell to induce insulin secretion in a glucose-dependent manner<sup>17</sup>. GLP-1 is liberated from its precursor proglucagon in intestinal L cells via processing by the enzyme prohormone convertase (PC) 1/3<sup>18</sup>. In the pancreatic  $\alpha$  cell, proglucagon is processed by PC2 to yield glucagon<sup>19</sup>. Posttranslational processing of proglucagon is thus tissue-specific and determined by the expression pattern of these two enzymes. Normal

adult pancreatic  $\alpha$  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<sup>20,21,22,23,24</sup>. Thus, increasing PC1/3 and consequently GLP-1 production in  $\alpha$  cells may be an adaptive mechanism used by islets to promote  $\beta$  cell regeneration and insulin secretion under certain conditions.

In an attempt to explain how elevated IL-6 levels during obesity or exercise may improve  $\beta$  cell insulin secretion, we hypothesized that IL-6 promotes GLP-1 secretion from intestinal L cells and pancreatic  $\alpha$  cells. Here we show that increased systemic IL-6 levels enhance GLP-1 secretion and production in L cells and  $\alpha$  cells, leading to improved  $\beta$  cell insulin secretion and glucose tolerance. Thus, IL-6 presents itself as a hormone mediating cross-talk between insulin sensitive tissues and pancreatic islets via GLP-1.

## RESULTS

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

To address whether elevated systemic IL-6 levels play a physiological role in regulating plasma GLP-1 levels we used exercise (treadmill running) as a model. Systemic IL-6 levels increased to 100 +/- 20 pg/ml in response to exercise (**Fig. 1a**). Together with elevated IL-6 levels, we found a 2.5-fold increase in circulating levels of active GLP-1 in response to 90 min exercise (**Fig. 1b**). To address whether IL-6 is required for this exercise-induced increase in active GLP-1 we first subjected IL-6 KO mice to an exercise bout. IL-6 KO mice were unable to increase plasma GLP-1 levels in response to exercise (**Fig. 1c**). In another set of experiments we administered an IL-6 antibody 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, while 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. 1d**), confirming that systemic increases in IL-6 due to exercise increase plasma GLP-1 levels.

### Acutely elevated IL-6 improves oral glucose tolerance via GLP-1-stimulated insulin secretion

Since systemically elevated IL-6 levels during exercise stimulated GLP-1 secretion, we hypothesized that acutely elevated IL-6 may improve oral glucose tolerance via GLP-1. To address this, we injected a single bolus of 400 ng IL-6 into mice at time point -30 min followed by either intraperitoneal (**Fig. 1e**) or oral (**Fig. 1f**) glucose administration at 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 levels ranging from 10 to 550 pg/ml (**Supplementary Fig. 2a**), similar to those obtained during exercise or HF diet (**Fig. 1a** and <sup>13</sup>). All doses of IL-6 improved glucose tolerance (**Fig. 1g**) and 40 and 400 ng IL-6 enhanced insulin secretion in a dose and glucose-dependent manner (**Fig. 1h**) along with increased plasma levels of GLP-1 (**Fig. 1h**) with no impact on insulin sensitivity (**Supplementary Fig. 2b**). In contrast, in GLP-1

receptor KO (Glp1r<sup>-/-</sup>) mice IL-6 no longer enhanced glucose-stimulated insulin secretion or improved glucose tolerance (**Fig. 1i,j**) and the GLP-1 receptor antagonist exendin (9-39) prevented IL-6 from improving early glucose excursions during oral glucose tolerance testing (**Fig. 1k**). These data identify GLP-1 as an essential mediator of IL-6 actions on  $\beta$  cell function and glucose homeostasis.

### **Intermittently elevated IL-6 improves glucose-stimulated insulin secretion via increased GLP-1 levels**

To study the effects of intermittently increased systemic IL-6 levels we injected 400 ng recombinant mouse IL-6 into mice twice daily for seven days. Peak plasma IL-6 levels in response to a single injection were observed after 30 min (baseline:  $9.6 \pm 2.5$  vs. 30 min:  $552 \pm 96$  pg/ml IL-6) (**Supplementary Fig. 2c**) and reached baseline values within 2 h after injection. All measurements below were performed 15 to 17 h after the last injection of IL-6. After 7 days, both fasting and fed glycemia were reduced in IL-6-injected (IL-6<sup>inj</sup>) animals, with little effect on fasting insulin, glucagon (**Fig. 2a,b**) or GLP-2 (**Supplementary Fig. 3**). Strikingly, we detected 5-fold increased fasting plasma GLP-1 levels in IL-6<sup>inj</sup> animals (**Fig. 2b**). However, further increases in plasma GLP-1 following oral glucose were not detected in IL-6<sup>inj</sup> animals (**Fig. 2c**). We reasoned that increased fasting GLP-1 levels might improve insulin secretion during an intraperitoneal glucose tolerance test. Indeed, IL-6<sup>inj</sup> animals showed improved glucose tolerance during an intraperitoneal glucose tolerance test, together with enhanced glucose-stimulated insulin secretion (**Fig. 2d,e**). There was no effect of IL-6 injections on insulin sensitivity (**Supplementary Fig. 4**). To determine whether basally elevated GLP-1 levels in IL-6<sup>inj</sup> mice were responsible for the increased glucose-stimulated insulin secretion and improved glucose tolerance, we performed intraperitoneal glucose tolerance tests in the presence of the GLP-1 receptor antagonist exendin (9-39). Administration of 25 nmol/l exendin (9-39) 1 min prior to glucose injection prevented the improvement by IL-6 of glucose tolerance and insulin secretion (**Fig. 2f-i**). Thus, these data show that intermittently increased systemic IL-6 levels improve  $\beta$  cell function and glucose homeostasis by

increasing fasting GLP-1 levels and thus enhancing intraperitoneal glucose-stimulated insulin secretion.

### **Elevated systemic IL-6 levels increase intestinal and pancreatic GLP-1 levels**

Next we examined whether IL-6 injections increased tissue proglucagon (*gcg*) mRNA and GLP-1 content. Twice daily injections of IL-6 for seven days increased *gcg* mRNA and active GLP-1 content in the distal gut, where most L cells are localized (**Fig. 2j,k**). Furthermore, pancreatic GLP-1, glucagon and insulin content were increased following injections of IL-6 (**Fig. 2l-n**). In support of an islet origin for pancreatic GLP-1, isolated islets from IL-6-injected mice displayed increased GLP-1 release over 24 h compared to saline-injected mice (**Fig. 2o**).

Analysis of intestinal tissue gene expression revealed increased PC1/3 (*pcsk1*) mRNA expression in the ileum and colon of IL-6-injected mice compared to controls, with no increase in PC2 (*pcsk2*) mRNA expression (**Fig. 2p**). IL-6 also enhanced ileum expression of sodium glucose transporter 1 (SGLT1) (*slc5a1*) and glucose transporter 1 (GLUT1) (*slc2a1*) as well as PYY in the jejunum, however no changes in levels of mRNA transcripts for sodium glucose transporter 3 (SGLT3) (*slc5a4*) and glucose transporter 5 (GLUT5) (*slc2a5*) were detected in the same experiments (**Fig. 2p**). Finally, intestinal *DPP4* mRNA expression 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 GLUTag 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 (**Fig. 3a**); activation of the IL-6 receptor was coupled to increased STAT3 phosphorylation (**Fig. 3b**), and inhibition of JAK2/STAT3 with AG490, blocked IL-6-induced STAT3 phosphorylation (**Fig. 3c**).

To determine whether IL-6 directly stimulates GLP-1 secretion, GLUTag cells were incubated with increasing concentrations of IL-6 in the presence of 0.1 mmol/l glucose. (**Fig. 3d**). IL-6 (1 to 300 ng/ml) significantly increased GLP-1 secretion in a dose-dependent manner. To understand how IL-6 induced GLP-1 secretion we used the patch-clamp technique to monitor increases in cell capacitance as a measure of exocytosis in individual GLUTag cells. IL-6 increased exocytosis in a dose-dependent manner (**Fig. 3e,f**). Figure 3e shows representative capacitance traces elicited by 200 ms depolarizations from  $-70$  to zero mV following stimulation with increasing concentrations of IL-6. That capacitance increases following IL-6 stimulation independently of an increase in  $Ca^{2+}$  current (**Fig. 3g**) suggests that IL-6 enhances GLP-1 secretion beyond  $Ca^{2+}$  entry and thus modulates the exocytotic machinery. To examine whether these IL-6 effects involved activation of STAT3, we repeated the experiments in the presence of AG490. Inhibiting STAT3 phosphorylation completely blocked IL-6-induced exocytosis (**Fig. 3h**).

We next investigated whether IL-6 directly potentiated glucose-stimulated GLP-1 secretion. GLUTag cells treated with 0 to 100 ng/ml IL-6 for 24 h and then stimulated with 11 mmol/l glucose for 2 h showed significantly increased GLP-1 secretion at 10 and 100 ng/ml IL-6 (**Fig. 3i**; unless otherwise indicated 100 ng/ml IL-6 was used in all further experiments). Cellular GLP-1 content was also increased in response to IL-6 (**Fig. 3j**). Time course experiments showed that the effect of IL-6 to potentiate glucose-stimulated GLP-1 secretion was most pronounced at 24 h (**Fig. 3k**). The effect of IL-6 on glucose-induced GLP-1 secretion and GLP-1 content was diminished after JAK2/pSTAT3 inhibition (**Fig. 3l,m**). Consistent with the demonstrated importance of JAK2/STAT3 in the L cell<sup>25</sup>, our data demonstrates that 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 mRNA expression profiles of candidate genes. Investigation of *gcg*, *pcsk1*, *psck2*, *slc5a1*, *slc5a4*, *slc2a1*, and *slc2a5* mRNA expression from 0 to 24 h after treatment with IL-6 revealed an increase in *gcg*, *pcsk1* and *slc5a1* mRNA transcripts by 24 h (**Fig. 3n**). These



mRNA effects were all reversed by JAK2/pSTAT3 inhibition (**Fig. 3o**), whereas *pcsk2*, which was not IL-6-regulated, was not affected by JAK2/STAT3 inhibition (**Supplementary Fig. 6**).

Supporting a functional role for the enhanced expression of sodium glucose transporter 1 (*slc5a1*), we observed increased glucose uptake in GLUTag cells after 24 h of IL-6 treatment (**Fig. 3p**), an effect that was abolished by the sodium glucose transporter 1 inhibitor, phlorizin (**Fig. 3p**). Finally, the ability of IL-6 to potentiate glucose-induced GLP-1 secretion was also inhibited by phlorizin (**Fig. 3q**), suggesting that enhanced glucose uptake is critical for the effect of IL-6 to stimulate GLP-1 secretion. Indeed, 0.1 mmol/l glucose was able to stimulate GLP-1 secretion to a similar degree as 11 mmol/l glucose after IL-6 incubation (**Fig. 3r**).

In summary, these data demonstrate 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 glucose responsive.

### **IL-6 increases GLP-1 secretion from human islets and human $\alpha$ cells**

To address whether IL-6 directly acts on  $\alpha$  cells to increase GLP-1 production we used intact human islets, a FACS sorted islet cell fraction enriched in human  $\alpha$  cells and FACS purified human  $\beta$  cells. Incubating human islets with IL-6 for 4 days in the presence or absence of the IL-6 receptor antagonist Super Antagonist 7 (Sant7) indicated that IL-6 enhances both the constitutive release of GLP-1 (**Fig. 4a**), as well as acute arginine-stimulated GLP-1 secretion (**Fig. 4b**). These effects were blunted in the presence of the IL-6 receptor antagonist. The fact 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<sup>26</sup>. To assess whether the GLP-1 released from human islets was biologically active, we performed glucose-stimulated insulin secretion experiments using conditioned media (cell culture media from untreated human islets containing 11 mmol/l glucose) in the absence and presence of exendin (9-39). These experiments revealed improved 11 mmol/l glucose-

stimulated insulin secretion in islets incubated with conditioned media relative to unconditioned media, and this improvement was reversed in the presence of the GLP-1 receptor antagonist exendin (9-39) (**Fig. 4c**). 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. 4d**).

FACS-enriched human  $\alpha$  cells incubated with IL-6 also displayed increased GLP-1 release (**Fig. 4e**, basal GLP-1 release over 0 – 24 h was  $3.1 \pm 0.6$  nmol/l, **Supplementary Table 1**), whereas glucagon release was not significantly different (**Fig. 4f**, basal glucagon release over 0 – 24 h was  $2.0 \pm 0.7$  nmol/l, **Supplementary Table 1**). After 4 days 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 mmol/l (**Fig. 4g**). Stimulated glucagon secretion at low glucose trended higher but was not significantly different (**Fig. 4h**). In agreement with the increased pancreatic GLP-1 content of IL-6<sup>inj</sup> mice (**Fig. 2l**), the cellular GLP-1 content of enriched human  $\alpha$  cells was increased 1.9-fold after IL-6 treatment (**Fig. 4i**) whereas glucagon content was decreased (**Fig. 4j**). These data suggest a shift in the processing of proglucagon from glucagon towards GLP-1, as demonstrated by expressing the cellular content as the molar ratio of GLP-1 to glucagon (**Fig. 4k**). Thus, these data show that IL-6 acts directly on  $\alpha$  cells to enhance their ability to liberate GLP-1.

Gene expression analysis revealed increased proglucagon (*gcg*) mRNA and PC1/3 (*pcsk1*) mRNA in response to IL-6 incubation in FACS-enriched human  $\alpha$  cells after 24 and 7 h respectively (**Fig. 4l**). There was no effect of IL-6 on PC1/3 (*pcsk1*) mRNA in FACS purified human  $\beta$  cells, indicating an  $\alpha$  cell specific effect (**Fig. 4m**). These data support the notion that IL-6 increases  $\alpha$  cell GLP-1 production by increasing both proglucagon gene transcription and its subsequent processing towards GLP-1 via PC1/3. Overall, IL-6 is able to directly increase GLP-1 secretion from the human islet  $\alpha$  cell.

### **The effect of acutely elevated IL-6 is maintained in animal models of prediabetes and diabetes**

Because plasma levels of IL-6 are chronically increased in animal models of obesity and diabetes<sup>13,27,28,29</sup> we questioned whether these mice still responded to an acute increase in IL-6 by improving  $\beta$  cell function. Indeed, IL-6 significantly increased glucose-stimulated insulin secretion in chow fed (**Fig. 5a**), HF diet fed (**Fig. 5c**), *ob/ob* (**Fig. 5e**) and *db/db* (**Fig. 5g**) mice. In contrast, in a HF diet model with direct  $\beta$  cell destruction by streptozotocin (HF-STZ) IL-6 failed to enhance insulin secretion (**Fig. 5i**). Overall, the effect of IL-6 on glucose tolerance (**Fig. 5 b, d, f, h & j**) varied to a greater extent than the effect on insulin secretion, likely due to varying degrees of insulin resistance in the models used. Furthermore, the small difference in glucose tolerance in chow vs. HF diet fed mice is likely due to the adaptive increase in insulin production in the face of increased insulin resistance in HF diet fed mice.

### **IL-6 is required for obesity induced $\alpha$ cell expression of PC1/3 and GLP-1**

We previously reported that HF diet chronically increased circulating IL-6 levels of wild type mice<sup>13</sup>. Weight-matched IL-6 KO mice on HF diet displayed no  $\alpha$  cell mass expansion and further impairments in glucose tolerance and insulin secretion<sup>13</sup>.  $\alpha$  cell mass expansion in wild type mice was not associated with changes in pancreatic glucagon content (**Fig. 6a**), while pancreatic GLP-1 content tended to increase ( $p=0.08$ ) (**Fig. 6b**) leading to a significant increase in GLP-1:glucagon content in wild type but not in IL-6 KO mice (**Fig. 6c**). This HF diet-induced increase in pancreatic GLP-1 to glucagon ratio suggests a shift in proglucagon processing in  $\alpha$  cells and was associated with increased PC1/3 co-localization to  $\alpha$  cells in wild type mice while PC1/3 remained undetectable in  $\alpha$  cells of IL-6 KO animals on chow or HF diet (**Fig. 6d**).

Similar to studies in IL-6 KO mice, HF diet feeding alone, or in combination with IL-6 injections or IL-6 neutralization did not change pancreatic glucagon content (**Fig. 6e**). In contrast, HF diet increased pancreatic GLP-1 content (**Fig. 6f**) and while IL-6 injections had no significant additive effect on pancreatic GLP-1 content, treatment with an IL-6 antibody

blocked this HF diet-induced adaptation (**Fig. 6f**). Also GLP-1 content in the colon was increased in response to HF diet in an IL-6 dependent manner (chow:  $1.2 \pm 0.1$ ; HF:  $1.7 \pm 0.1$ ; HF<sup>IL6AB</sup>  $0.8 \pm 0.2$  active GLP-1 ng/ $\mu$ g protein). Moreover, IL-6 antagonism worsened fasting glycemia (**Supplementary Fig. 7a**). Fasting plasma levels of GLP-1 were unchanged in response to HF diet and IL-6 interventions (chow:  $7.4 \pm 1.9$ ; HF:  $3.7 \pm 0.3$ ; HF<sup>IL6inj</sup>  $3.9 \pm 0.4$ ; HF<sup>IL6AB</sup>  $3.7 \pm 0.5$  pmol/l active GLP-1). Glucose-stimulated insulin secretion in response to intraperitoneal glucose revealed an enhanced insulin response in HF diet fed mice and IL-6 injections further increased this response (**Supplementary Fig. 7b**). Similar to chow fed mice, insulin tolerance was not affected by IL-6 interventions in HF fed mice (**Supplementary Fig. 7c**). Taken together, these data show that short-term antagonism of IL-6 signaling during HF diet feeding impairs the ability of the  $\alpha$  cells to increase GLP-1 production and moreover impairs glycemia.

Increasing HF diet-induced IL-6 levels by exogenous IL-6 injections reduced islet *TNF- $\alpha$*  mRNA expression and had otherwise no profound effect on the pro- or anti-inflammatory gene expression profile in isolated islets, however, *Ins1*, *Ins2* and *Ipf1* mRNA were all significantly increased in HF diet fed mice injected with IL-6 (**Supplementary Fig. 8**).

Finally, FACS sorting of pure  $\alpha$  cells from HF diet fed transgenic mice expressing a yellow fluorescent protein under the control of the glucagon promoter<sup>30</sup> with and without neutralization of IL-6, demonstrated that  $\alpha$  cell PC1/3 mRNA was significantly reduced in IL-6 antibody treated mice compared to HF diet control mice (**Fig. 6g**). Thus, HF diet-induced IL-6 promotes PC1/3 mRNA, PC1/3 protein expression and GLP-1 production in  $\alpha$  cells.

While also previous work has demonstrated that HF diet feeding leads to enhanced GLP-1 content in the intestine together with reduced circulating GLP-1<sup>25</sup>, these data suggest that increased systemic IL-6 levels during obesity increase pancreatic GLP-1, perhaps as an adaptive mechanism required for functional  $\beta$  cell compensation in response to obesity. These data also implicate IL-6 in the regulation of  $\alpha$  cell PC1/3 expression, suggesting a shift from glucagon towards GLP-1 production in the pancreas.

### **IL-6 antagonism deteriorates glucose homeostasis in *db/db* mice**

Next we examined whether blocking increased levels of endogenous IL-6 in *db/db* mice<sup>27,31</sup> precipitates diabetes.

*Db/db* mice treated with an IL-6 antibody for four weeks demonstrated impaired fasting glycemia (**Fig. 6h**) and a severe deterioration of glucose tolerance (**Fig. 6i**) without any difference in insulin tolerance (**Fig. 6j**).  $\beta$  cell responses to intraperitoneal glucose were absent in these 9-10 week old mice and antagonizing IL-6 had no effect on insulin secretion (**Fig. 6k**). Fasting plasma insulin was unchanged (**Fig. 6l**), while glucagon was increased (**Fig. 6l**) and GLP-1 levels were undetectable. Pancreatic tissue content of insulin and glucagon revealed no differences, while GLP-1 content was reduced in mice receiving IL-6 antibody (**Fig. 6m**).

## DISCUSSION

The present study has uncovered IL-6 as a key regulator of glucose homeostasis via effects on L cell and  $\alpha$  cell GLP-1 and subsequent improvements in insulin secretion. IL-6 can have both acute and chronic effects on these parameters via direct actions on L cells and  $\alpha$  cells. Acute effects are due to IL-6 increasing GLP-1 exocytosis, while chronic effects are due to IL-6 increasing glucose-responsiveness and GLP-1 production.

In addition to its role as an adipokine, IL-6 may also act as a myokine in response to exercise. Indeed, research investigating the interplay between exercise and satiety has drawn attention to the effect of exercise on gut hormones, demonstrating that exercise is accompanied by an acute increase in plasma GLP-1 levels<sup>32,33,34</sup>. Moreover, a physical training intervention has been shown to enhance the secretory capacity of L cells resulting in increased GLP-1 secretion in response to physiological stimuli<sup>35</sup>. In the present study, we show, that exercise-induced increases in GLP-1 levels are IL-6-dependent and thus reveal a physiological role for skeletal muscle-derived IL-6 during exercise. Elevated GLP-1 in response to exercise may be physiologically relevant through its inhibitory effects on gastric emptying, whereas long-term it may explain how exercise improves  $\beta$  cell function<sup>36</sup>. Of note, the potentiating actions of GLP-1 on insulin secretion are known to be glucose-dependent, and require systemic glucose levels to be above  $\sim 4\text{--}5$  mmol/l<sup>17</sup>. Therefore, during exercise, when glucose levels are not elevated, IL-6 induced GLP-1 will not acutely impact on insulin secretion in non-diabetic subjects. In line with this concept, GLP-1 has been 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 secretion<sup>37,38,39</sup>.

That acutely increased plasma IL-6 levels improve insulin secretion in animal models of obesity and prediabetes indicates that a beneficial effect of exercise on  $\beta$  cell function may still be achieved under these conditions.

IL-6 KO mice on HF diet display glucose intolerance due to impaired insulin secretion<sup>13,40</sup>. In the present study we show that these mice display

reduced pancreatic GLP-1, and that neutralization of IL-6 in wild type HF diet fed mice and *db/db* mice impairs this  $\alpha$  cell adaptation to obesity and is associated with increased glycemia. In support hereof, IL-6 appears as a positive regulator of GLP-1 production and secretion in the pancreatic  $\alpha$  cell, resulting in elevated islet GLP-1 levels that are coupled to improved insulin secretion and glycemia. This suggests that IL-6 plays a compensatory role under conditions of obesity by increasing islet GLP-1 production to augment insulin secretion in order to adapt to insulin resistance.

GLP-1 produced by L cells is thought to act on  $\beta$  cells via the circulation. Using IL-6 KO mice and pharmacological immunoneutralization of IL-6 we show that IL-6 was required to increase GLP-1 production in the pancreatic  $\alpha$  cell during obesity. The potentiation of GLP-1 secretion from  $\alpha$  cells by IL-6 is likely due to both increased proglucagon transcription and PC1/3 expression. Previous work has also demonstrated that the  $\alpha$  cell can be a source of GLP-1 and increased PC1/3 expression under conditions of  $\beta$  cell stress, such as during partial pancreatectomy, streptozotocin-induced diabetes and obesity<sup>20,21,22-24</sup>. A recent study describing the islet architecture in human islets reveals previously unexpected direct intercellular contacts between  $\alpha$  and  $\beta$  cells, supporting the notion that  $\alpha$  cell products could act in a paracrine manner to regulate the  $\beta$  cell<sup>41</sup>. Thus, these data suggest that in addition to IL-6-mediated L cell effects, the pancreatic  $\alpha$  cell may also produce GLP-1 to enhance  $\beta$  cell insulin secretion under conditions of  $\beta$  cell adaptation. However, the relative contribution of IL-6-induced L cell versus  $\alpha$  cell-derived GLP-1 remains to be elucidated.

IL-6 is a pleiotropic signaling molecule having multiple effects in immunity, acting as a pro-inflammatory<sup>42,43</sup> and sometimes as an anti-inflammatory cytokine. More recently, several studies uncovered its role as a cytokine regulating glucose tolerance, central control of obesity and insulin action<sup>44,45,46,47,48</sup>. This led to the hypothesis that acutely elevated IL-6 is beneficial while chronically elevated IL-6 is detrimental. In the present study we enhanced systemic IL-6 levels via exercise, HF diet, and acute and intermittent IL-6 injections, observing consistent effects. Possibly, the role of

IL-6 is more dependent on the context or cellular source rather than on whether it is activated in an acute or chronic fashion<sup>6</sup>.

In the present study we describe an adipose tissue and skeletal muscle enteroendocrine-islet axis. This cross talk between insulin-sensitive tissues and insulin-producing cells is mediated via 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 novel therapeutic approaches or lead to a more judicious use of existing drugs modulating IL-6 and GLP-1.



## FIGURE LEGENDS

**Figure 1** Effect of acute IL-6 on GLP-1 and insulin secretion *in vivo*. **(a)** Plasma IL-6 in resting and running mice ( $n=3$  for resting,  $n=12$  for running). **(b)** Plasma GLP-1 in resting and running mice ( $n=3$  for resting,  $n=4$  for running). **(c)** Plasma GLP-1 in WT and IL-6 KO mice before and after 90 min running to exhaustion ( $n=6$  per group). **(d)** Plasma GLP-1 before and after 90 min running to exhaustion in mice injected with isotype control or IL-6 neutralizing antibody ( $n=6$  per group). The arrow at time point 90 min indicates the time point when mice were exhausted and blood was sampled. **(e)** IPGTT **(f)** OGTT after single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=4$  per group). **(g)** OGTT after single injection of NaCl (ctrl) or IL-6 ( $n=12$  per group). **(h)** Plasma insulin and GLP-1 in response to oral glucose after single injection of NaCl (ctrl) or IL-6 ( $n=4$  per group), -30 min indicates baseline before NaCl or IL-6 injection, 0 min indicates time point for glucose administration. **(i)** OGTT in WT ( $Glp1r^{+/+}$ ) and  $Glp1$  receptor KO ( $Glp1r^{-/-}$ ) mice after single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=6-10$  per group). **(j)** Oral glucose-stimulated insulin secretion in WT ( $Glp1r^{+/+}$ ) and  $Glp1$  receptor KO ( $Glp1r^{-/-}$ ) mice after single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=6-10$  per group). **(k)** OGTT after single injection of NaCl (ctrl) or 400 ng IL-6 in the absence and presence of exendin 9-39 ( $n=4$  per group). Data represent means  $\pm$  s.e.m. \*  $p < 0.05$  by Student's  $t$  test comparing control vs. IL-6 injection, resting vs. running (panel a – d) or IL-6 vs. IL-6<sup>ex 9-39</sup> (panel k). \* \$ #  $p < 0.05$  by Anova comparing control vs. IL-6 injections (panel g,h).

**Figure 2** Effect of 1-week IL-6 injections on glucose homeostasis and GLP-1 production. **(a)** Fasting and fed blood glucose in control and IL-6<sup>inj</sup> mice ( $n=8$  per group). **(b)** Fasting plasma hormones in control and IL-6<sup>inj</sup> mice ( $n=6-8$  per group). **(c)** Plasma GLP-1 in response to oral glucose in control and IL-6<sup>inj</sup> mice ( $n=8$  per group). **(d)** IPGTT in control and IL-6<sup>inj</sup> mice ( $n=8$  per group). **(e)** Plasma insulin in response to intraperitoneal glucose in control and IL-6<sup>inj</sup> mice ( $n=8$  per group). **(f)** IPGTT in control mice in the absence or presence of exendin (9-39) ( $n=4$  per group). **(g)** Plasma insulin in control mice in response

to intraperitoneal glucose in the absence or presence of exendin (9–39) ( $n=4$  per group). (h) IPGTT in IL-6<sup>inj</sup> mice in the absence or presence of exendin (9–39) ( $n=4$  per group). (i) Plasma insulin in response to intraperitoneal glucose in IL-6<sup>inj</sup> mice in the absence or presence of exendin (9–39) ( $n=4$  per group). (j) Intestinal *gcg* mRNA expression in control and IL-6<sup>inj</sup> mice ( $n=8$  per group). (k) Intestinal GLP-1 content in control and IL-6<sup>inj</sup> mice ( $n=8$  per group). (l–n) Pancreatic hormone content in control and IL-6<sup>inj</sup> mice ( $n=8$  per group). (o) GLP-1 release over 24 h from isolated mouse islets from control and IL-6<sup>inj</sup> mice ( $n=5$  per group). (p) Intestinal mRNA expression in control and IL-6<sup>inj</sup> mice. Data expressed as fold of jejunum control ( $n=8$  per group). nd: not detectable. Data represent means  $\pm$  s.e.m. \*  $p < 0.05$  by Student's *t* test comparing control vs. IL-6<sup>inj</sup> mice.

**Figure 3** Effects of IL-6 on GLP-1 secretion in GLUTag cells. (a) IL-6 receptor protein expression in GLUTag cells ( $n=3$ ) and HeLa cell extract (positive control). (b,c) Western blot of pSTAT3 and actin. (d) GLP-1 secretion in response to 15 min stimulation with IL-6 in the presence of 0.1 mmol/l glucose, 1  $\mu$ mol/l GIP was used as positive control ( $n=5$ ). (e) Representative capacitance traces following stimulation with IL-6 (0.1–300 ng/ml). (f) Average capacitance following 15 min stimulation in the absence or presence of IL-6 ( $n=6–11$ ). (g)  $Ca^{2+}$  current following stimulation with IL-6 ( $n=6–11$ ). (h) Average capacitance following 15 min stimulation with IL-6 in the absence and presence of AG490 ( $n=5–9$ ). (i) GLP-1 secretion in response to 2 h 11 mmol/l glucose stimulation after 24 h treatment +/- IL-6 ( $n=3$ ). (j) GLP-1 content after 24 h treatment +/- IL-6 ( $n=6$ ). (k) GLP-1 secretion in response to 2 h 11 mmol/l glucose stimulation after 0 to 24 h treatment +/- IL-6 ( $n=3$ ). (l) GLP-1 secretion in response to 2 h 11 mmol/l glucose stimulation after 24 h treatment +/- IL-6 in the absence (-) or presence (+) of AG490 ( $n=3$ ). (m) GLP-1 content after 24 h treatment +/- IL-6 in the absence (-) or presence (+) of AG490 ( $n=3$ ). (n) GLUTag mRNA expression in response to IL-6, data expressed as fold of untreated control (indicated by broken line). (o) GLUTag mRNA expression after 24 h treatment +/- IL-6 in the absence (-) or presence (+) of AG490 ( $n=3$ ). (p)  $^3H$ -2dG glucose uptake after 24 h treatment +/- IL-6 in the absence (-) or

presence (+) of phlorizin ( $n=4$ ). (q) GLP-1 secretion in response to 2 h 11 mmol/l glucose stimulation in the absence (-) or presence (+) of phlorizin. Cells were pretreated for 24 h +/- IL-6 ( $n=3$ ). (r) GLP-1 secretion in response to 2 h 0.1 or 11 mmol/l glucose stimulation after 24 h treatment +/- IL-6 ( $n=6$ ). Data represent means  $\pm$  s.e.m. \*  $p < 0.05$  by ANOVA.

**Figure 4** Effects of IL-6 on GLP-1 secretion in human islets and human  $\alpha$  cells. (a) GLP-1 release over 24 h after 4 days treatment +/- IL-6 in the absence (-) or presence (+) of Sant7 ( $n=3$ ). (b) GLP-1 secretion in response to 1 h static incubation in 10 mmol/l arginine after 5 days treatment +/- IL-6 in the absence (-) or presence (+) of Sant7 ( $n=3$ ). (c) Human islet insulin secretion in response to 1 h static incubation in un/conditioned media from human islets in the absence and presence of exendin (9–39) ( $n=3$ ). (d) Basal GLP-1 and IL-6 release from various human islet preparations ( $n=6$ ). (e) GLP-1 release over consecutive 24 h intervals +/- IL-6 ( $n=4$ ). (f) Glucagon release over consecutive 24 h intervals +/- IL-6 ( $n=4$ ). (g) GLP-1 secretion in response to 1 h glucose incubation after 4 days treatment +/- IL-6 ( $n=4$ ). (h) Glucagon secretion in response to 1 h glucose incubation after 4 days treatment +/- IL-6 ( $n=4$ ). (i) GLP-1 content after 4 days treatment +/- IL-6 ( $n=4$ ). (j) Glucagon content after 4 days treatment +/- IL-6 ( $n=3$ ). (k) GLP-1 and glucagon content expressed as molar ratio ( $n=4$ ). (l) FACS enriched human  $\alpha$  cell mRNA expression in response to IL-6 ( $n=3-6$ ). (m) FACS sorted  $\beta$  cell mRNA expression in response to IL-6 ( $n=4$ ). Data represent means  $\pm$  s.e.m. \*  $p < 0.05$  by Student's  $t$  test comparing control vs. IL-6, or unconditioned vs. conditioned media, or by ANOVA (panel l, and m) comparing control (0) vs. IL-6.

**Figure 5** Effect of acute IL-6 on insulin secretion in animal models of prediabetes and diabetes. (a) Plasma insulin (b) OGTT in response to oral glucose in chow-fed mice after a single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=8$  per group). (c) Plasma insulin (d) OGTT in response to oral glucose in mice fed HF diet for 18 weeks after single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=8$  per group). (e) Plasma insulin (f) OGTT in response to oral glucose in

*ob/ob* mice after a single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=8$  per group). (g) Plasma insulin (h) OGTT in response to oral glucose in *db/db* mice after a single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=5$  per group). (i) Plasma insulin (j) OGTT in response to oral glucose in HF-STZ mice after a single injection of NaCl (ctrl) or 400 ng IL-6 ( $n=5$  per group). Data represent means  $\pm$  s.e.m. \*  $p < 0.05$  by Student's *t* test comparing control vs. IL-6 injection.

**Figure 6** Effect of IL-6 antagonism in HF diet fed mice and *db/db* mice. (a) Pancreatic glucagon and (b) GLP-1 content in WT and IL-6 KO mice after 18 weeks on chow or HF diet ( $n=8$  per group). (c) Pancreatic GLP-1:glucagon content as molar ratio. (d) Immunohistochemistry of pancreatic tissue sections from chow or HF diet fed wild type and IL-6 KO mice using antibodies against PC1/3 and glucagon (representative image of  $n=5$ ). (e) Pancreatic glucagon and (f) GLP-1 content in mice fed chow or HF diet for 15 weeks, HF<sup>IL6inj</sup> received IL-6 injections twice daily for the last 7 days of the study, HF<sup>IL6AB</sup> received an IL-6 neutralizing antibody for the last 4 weeks of the study ( $n=7-8$ ). (g) PC1/3 (*Pcsk1*) mRNA expression in FACS sorted  $\alpha$  cells from transgenic mice expressing a yellow fluorescent protein under the control of the glucagon promoter after 20 weeks on chow or HF diet ( $n=5-8$ ). (h) Blood glucose, (i) intraperitoneal glucose tolerance (j) and insulin tolerance test in *db/db* mice after 4 weeks without (*db/db*<sup>Ctrl</sup>) or with (*db/db*<sup>IL6AB</sup>) IL-6 antibody treatment ( $n=4-5$ ). (k) Insulin in response to intraperitoneal glucose in *db/db* mice with or without IL-6 antibody treatment ( $n=4-5$ ). (l) Fasting plasma hormones in *db/db* mice with or without IL-6 antibody treatment ( $n=4-5$ ). (m) Pancreatic hormone content in *db/db* mice with or without IL-6 antibody treatment ( $n=4-5$ ). Data represent means  $\pm$  s.e.m. \* #  $p < 0.05$  by ANOVA where \* compares chow vs. HF diet and # compares genotypes on HF diet (panel c) or HF vs. HF<sup>IL6AB</sup> (panel e and f). In panels h – m \*  $p < 0.05$  by Student's *t* test comparing control vs. IL-6<sup>AB</sup> injected mice.

## METHODS

**Animals.** Male and female C57BL/6J mice (Harlan), male C57BL/6J wild type and B6;129S2-*Il6*<sup>tm1Kopf</sup>/J (IL-6 KO) mice backcrossed for 11 generations and maintained on a C57BL/6J background (Jackson), male *db/db* and *ob/ob* mice (Jackson), male wild type (*Glp1r*<sup>+/+</sup>) and *Glp1r* receptor knockout (*Glp1r*<sup>-/-</sup>) littermates on a C57BL/6 background (Daniel J. Drucker) and male transgenic mice expressing a yellow fluorescent protein under the control of the glucagon promoter (Fiona M. Gribble/ Frank Reimann) were used. Guidelines for the use and care of laboratory animals at the University of Zurich and Mt. Sinai Hospital were followed, and the Zurich, Mt. Sinai Hospital Cantonal Animal Experimentation Committee granted ethical approval. Work in Cambridge was approved by the Home Office and Local Ethical Committee. Mice received a standard chow, or a HF diet for 15 - 22 weeks as previously described<sup>13</sup>. HF-STZ (streptozotolin) mice were fed a HF diet for 3 weeks, injected with a single bolus of 130 mg/kg STZ and continued on HF diet.

**IL-6 and IL-6 antibody injections.** In all injection studies lasting 7 days, mice were injected intraperitoneally twice daily (08:00 and 17:00) with 400 ng carrier free recombinant mouse IL-6 (R&D) or saline (NaCl). No injections were performed on days of glucose and insulin tolerance testing. In acute experiments 4, 40 or 400 ng IL-6 or saline was injected intraperitoneally 30 minutes prior to the glucose tolerance tests. In HF diet fed mice and *db/db* mice IL-6 neutralizing antibody 500 µg (R&D) or PBS was injected intraperitoneally twice weekly for the final 2 - 4 weeks of the experiment. In exercise experiments mice were injected intraperitoneally 1 h prior to running with 500 µg IL-6 antibody (R&D) or isotype control. Plasma IL-6 was determined using a Luminex kit (Millipore).

**Glucose and insulin tolerance testing.** Glucose tolerance tests were performed on overnight fasted mice by administering 2 g/kg glucose orally (gavage), or by injecting 2 g/kg intraperitoneally. In some experiments 25 nmol/l exendin (9-39) (GLP-1 receptor antagonist; Bachem) was injected intraperitoneally 1 min (for ipGTT's) and 15 min (for OGTT's) prior to glucose injection. Glucose-induced insulin and active GLP-1 secretion were

determined in plasma using insulin ELISA (Millipore and AlpcO) and GLP-1 Luminex™ kits and ELISA (both Millipore) and the in-house Novo Nordisk assay<sup>24</sup>. GLP-2 was determined using an ELISA (Labodia). We performed insulin tolerance tests on 3 h fasted mice by injecting 0.75 – 4 U/kg insulin (Novo Nordisk).

**Treadmill running.** Mice were adapted to the treadmill (Columbus Instruments) as previously described<sup>49</sup>. Exercise started at 10 meters per minute at 0° inclination for 30 min, followed by a gradual increase in intensity by 3 meters per minute and 2.5° inclination every 10 min until exhaustion. The protocol was adapted so mice were exhausted after 90 min running. Exhaustion was defined as described previously<sup>49</sup>.

**Tissue content.** We determined hormone content in homogenized pancreatic and intestinal tissues after overnight HCL/EtOH (0.18 M HCl in 70% ethanol) extraction at 4°C. Samples were centrifuged and supernatant diluted 1 to 200 in H<sub>2</sub>O. Active GLP-1 and glucagon were determined using the in-house Novo Nordisk kit<sup>24</sup> and RIA (Millipore). Insulin using ELISA (Mercodia), and protein concentration using a BCA assay (Pierce).

**Histochemical analysis.** Pancreatic paraffin-embedded tissue sections were used for double immunofluorescence<sup>50,51</sup>. Antigen retrieval solution was used according to the manufacturers protocol (Dako) and antibodies to PC1/3 (Millipore), and glucagon (Linco) were used.

**Human islets.** Human islets were isolated from pancreata of organ donors at the University of Geneva Medical Center and cultured as previously described<sup>26</sup> in the presence of 1 mmol/l diprotin A (DPP4 inhibitor, Sigma), 200 ng/ml recombinant human IL-6 (R&D), the Super Antagonist 7 (200 ng/ml Sant7, an IL-6 receptor antagonist kindly provided by Sigma Tau<sup>52</sup>, 100 nmol/l exendin (9-39) (GLP-1 receptor antagonist, Bachem). IL-6 was determined by ELISA (R&D).

**Fluorescence-activated cell sorting (FACS).** Human β cells were sorted by FACS following labeling with Newport Green<sup>53</sup>. We mostly used the FACS sorted non β cell fraction enriched in α cells (on average 50% α cells), referred to as FACS enriched human α cells. The β cell fraction consisted of on average 96% β cells. FACS enriched human α cells were cultured as for

human islets. Active GLP-1 and glucagon secretion were assessed by 1 h incubation in 16.7 mmol/l glucose (basal secretion) followed by a 1 h incubation in 2.8 mmol/l glucose (stimulated secretion). Cellular active GLP-1 and glucagon content were extracted with HCL/EtOH.  $\alpha$ -cells from transgenic mice expressing fluorescent protein under control of the proglucagon promoter were separated by FACS as described previously<sup>28</sup>, and collected directly into lysis buffer for mRNA extraction.

**Mouse islet.** Mouse pancreatic islets were isolated and cultured as previously described<sup>26</sup> in the presence of 1 mmol/l diprotin A (Sigma).

**GLUTag cell culture and GLP-1 secretion.** GLUTag cells were cultured as previously described<sup>54</sup> and treated in the presence of 100 ng/ml recombinant mouse IL-6 (unless otherwise indicated) and 1 mmol/l diprotin A (Sigma). Active GLP-1 secretion was assessed by 2 h incubation in glucose (0.1 or 11 mmol/l). In some experiments the JAK2/pSTAT3 inhibitor AG490 (50  $\mu$ mol/l) was present throughout the 24 h treatment, and in some experiments 100  $\mu$ mol/l Phlorizin (Fluka) was added to the stimulation solution. For all *in vitro* experiments, active GLP-1 and glucagon were measured by RIA's (Millipore). Exocytosis was monitored as changes in cell capacitance using the perforated patch whole-cell configuration of the patch-clamp technique and an EPC9 patch-clamp amplifier (Heka Elektronik, Lambrecht/Pfalz, Germany). The pipette solution for the perforated patch configuration consisted of (in mM) 76 Cs<sub>2</sub>SO<sub>4</sub>, 10 NaCl, 10 KCl, 1 MgCl<sub>2</sub>, 5 HEPES (pH 7.35 with CsOH) and 0.24 mg/ml amphotericin B. The extracellular medium was composed of (in mM) 118 NaCl, 20 tetraethylammonium-Cl, 5.6 KCl, 1.2 MgCl<sub>2</sub>, 2.6 CaCl<sub>2</sub>, 5 HEPES (pH 7.40 using NaOH) and 0.1 glucose. The capacitance measurements were performed at 33 °C .

**Western blotting.** Proteins (20–50  $\mu$ g) were separated on 4–12% NuPAGE gels (Invitrogen) and blotted onto nitrocellulose membranes (Bio-Rad). Membranes were incubated with primary antibodies against phospho-STAT3 (Tyr705) (Cell Signalling), actin (C2) (Santa Cruz) and IL-6 receptor (Santa Cruz).

**RNA extraction and real-time PCR.** Total tissue RNA and cellular RNA was extracted according to manufacturer's instructions (Qiagen, Macherey Nagel



respectively, or Ambion for FACS purified fluorescent  $\alpha$ -cells). Mouse and human primers (Applied Biosystems) were used and real-time PCR performed using the ABI 7500 or 7900 system (Applied Biosystems). Changes in mRNA expression were calculated using difference of  $C_T$  values as compared to a housekeeping gene (18S or  $\beta$ -actin) and expressed relative to controls.

**Determination of 2-deoxy- $^3$ H-D-glucose uptake.** GLUTag cells were incubated in the presence or absence of 100 ng/ml IL-6 (R&D). Uptake of 2-deoxy- $^3$ H-D-glucose ( $^3$ H-2dG) (10  $\mu$ mol/l) was determined as previously reported<sup>55</sup>, in the presence or absence of 100  $\mu$ mol/l Phlorizin (Fluka).

**Statistics.** Data are expressed as means  $\pm$  s.e.m. with the number of individual experiments presented in the figure legends. All data were analyzed using the nonlinear regression analysis program PRISM (GraphPad), and significance was tested using Student's *t*-test and analysis of variance (ANOVA) with Dunnett's or Bonferroni's post hoc test for multiple comparison analysis. Significance was set at  $p < 0.05$ .



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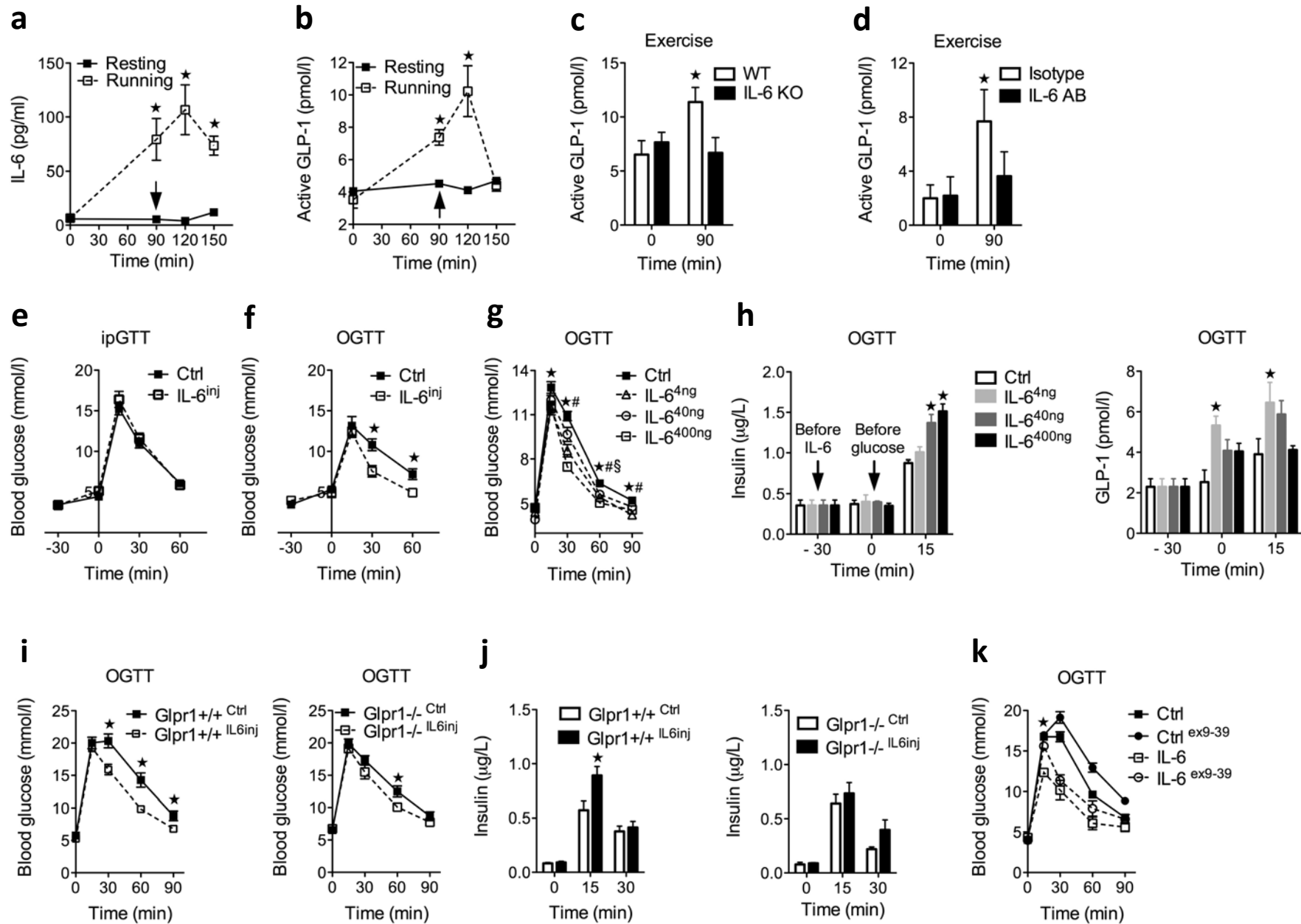
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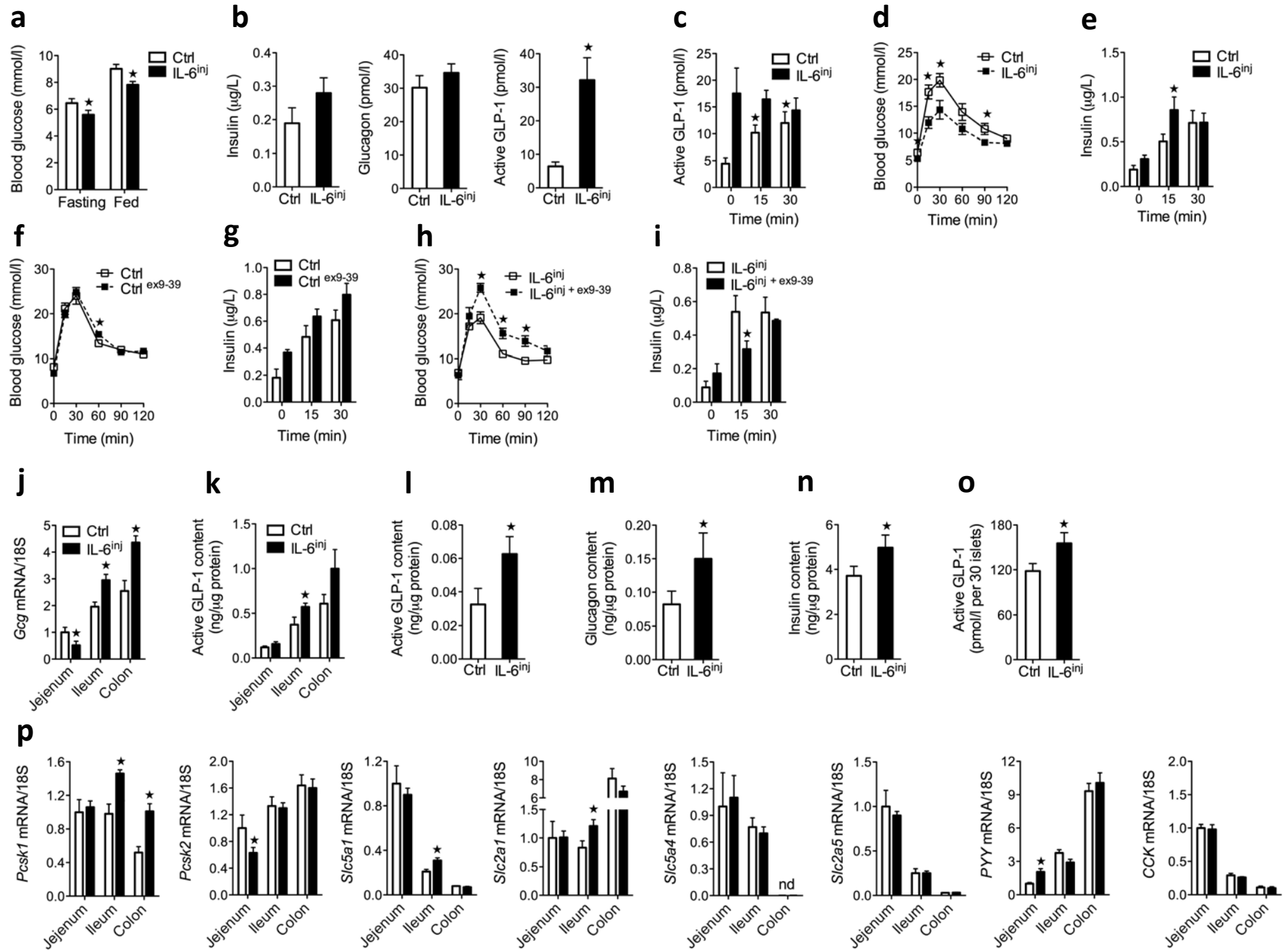
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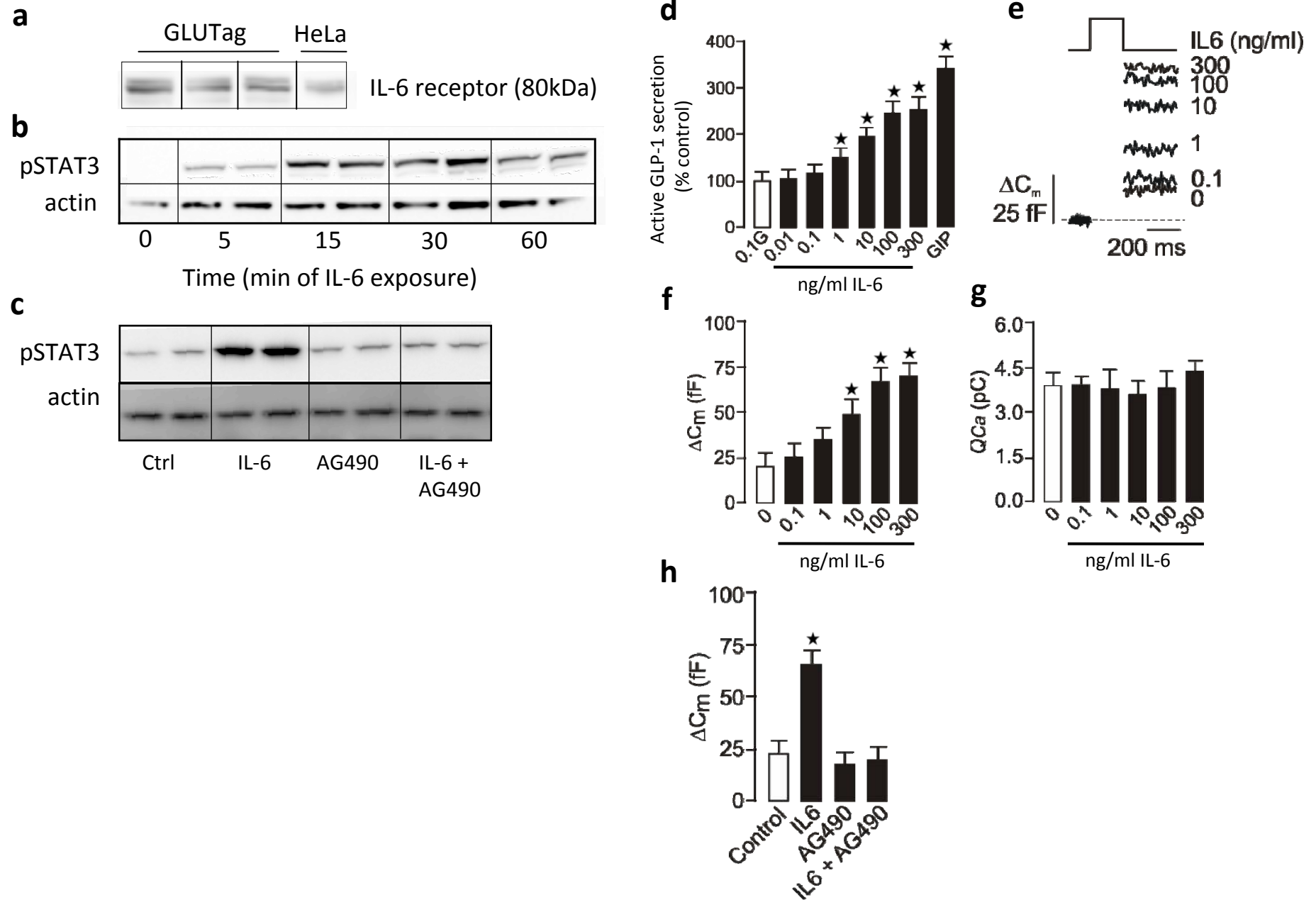
**Figure 1** Effect of acute IL-6 on GLP-1 and insulin secretion *in vivo*



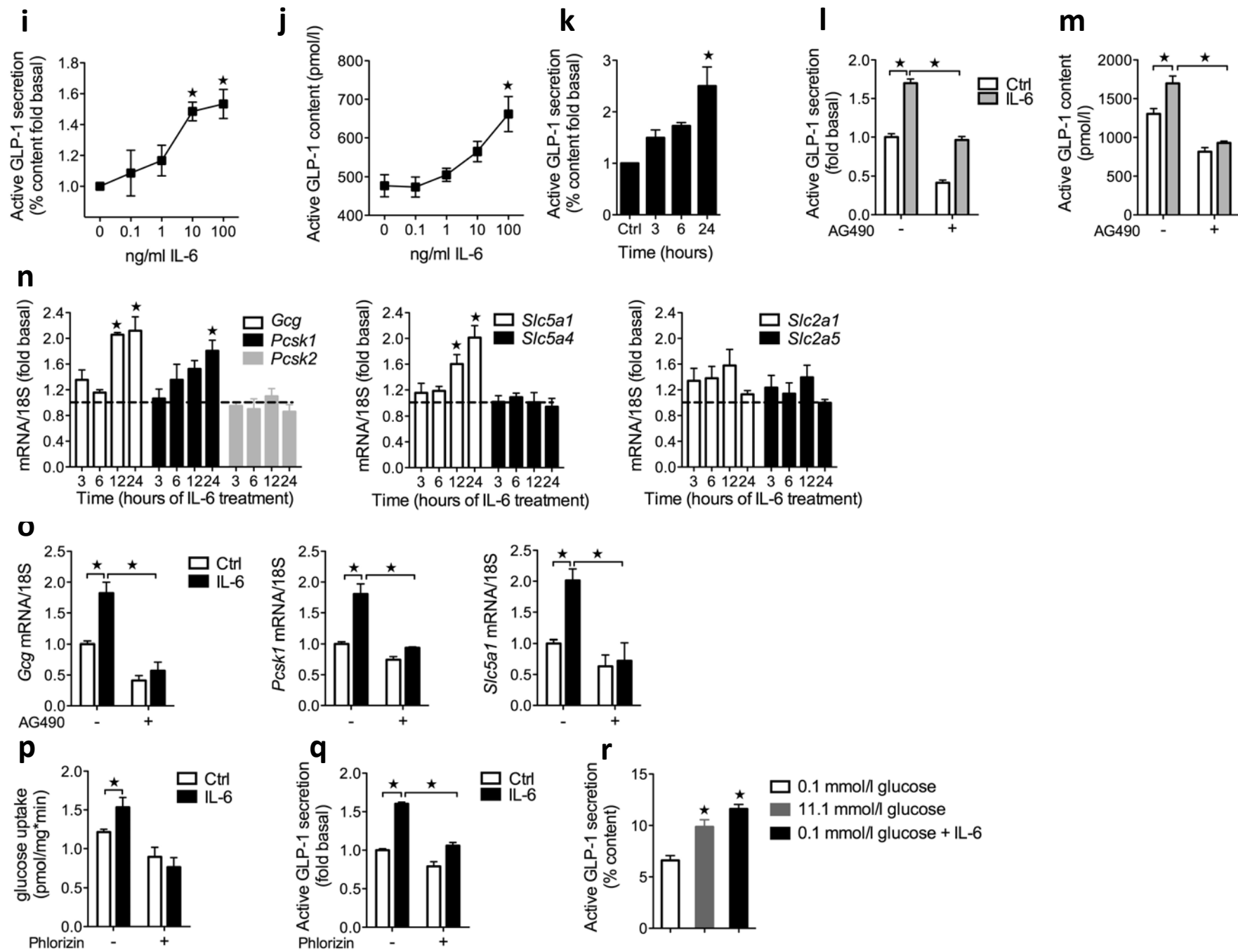
**Figure 2** Effect of 1-week IL-6 injections on glucose homeostasis and GLP-1 production



**Figure 3** Effects of IL-6 on GLP-1 secretion in GLUTag cells

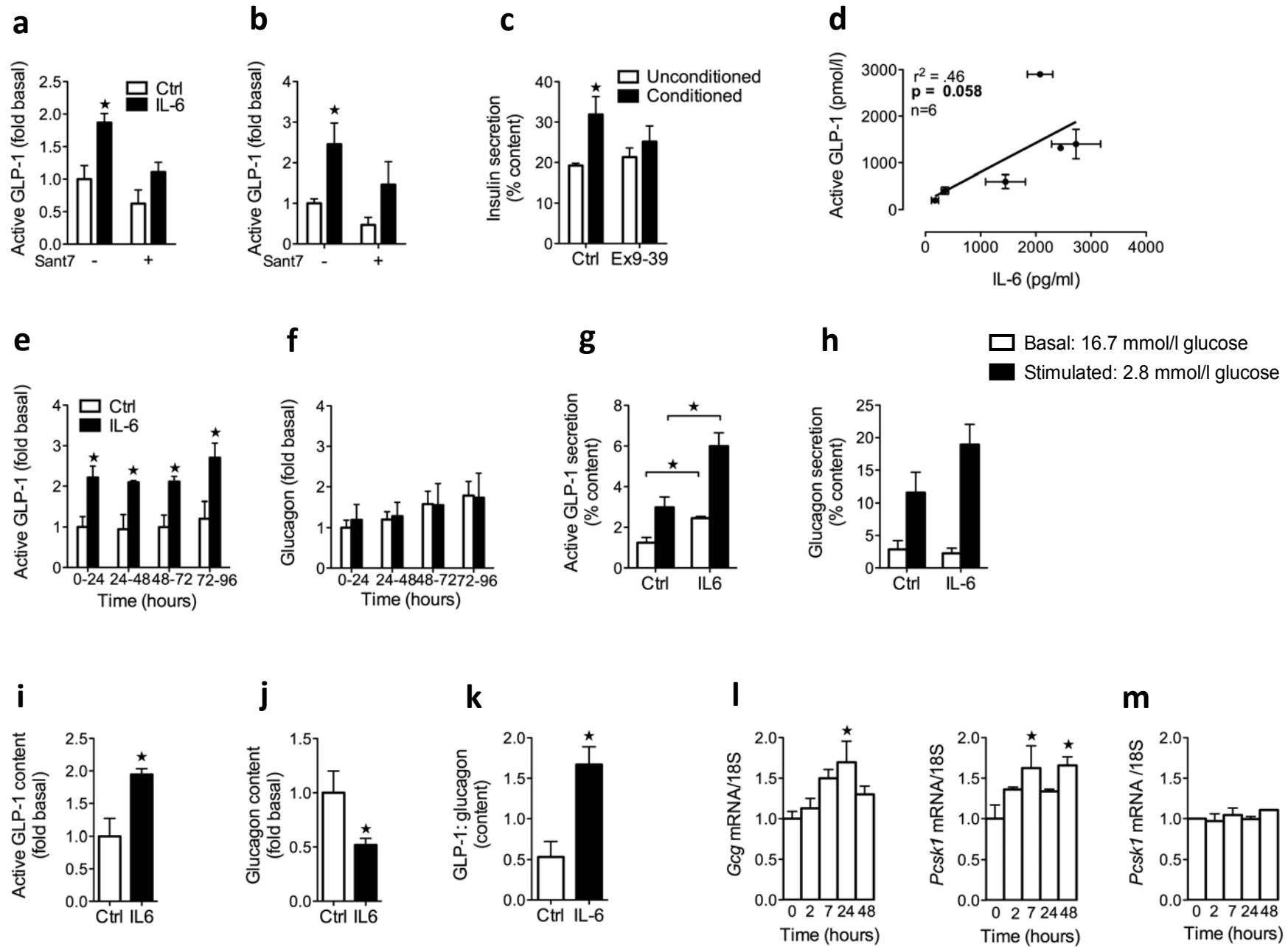


**Figure 3** Effects of IL-6 on GLP-1 secretion in GLUTag cells

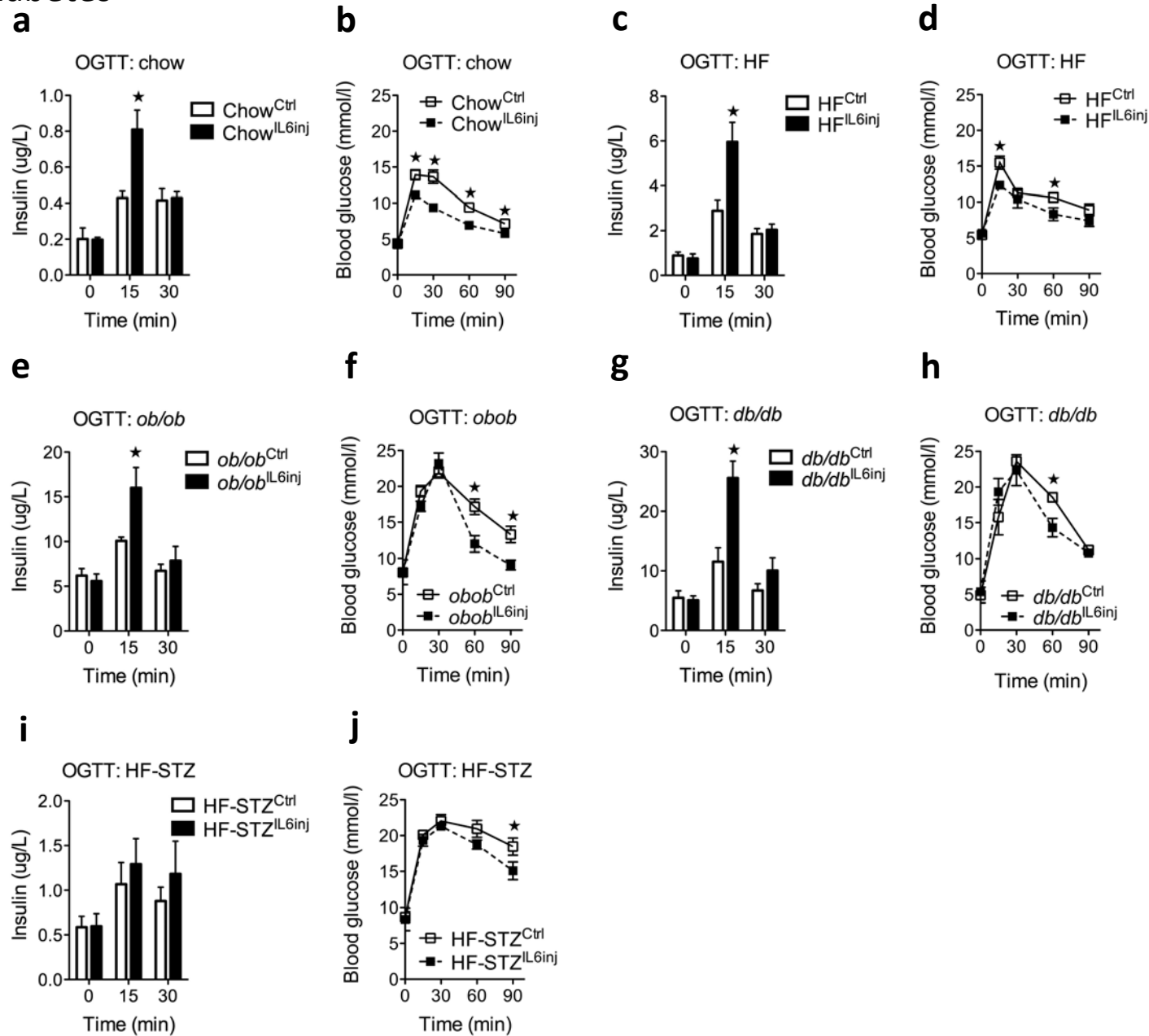




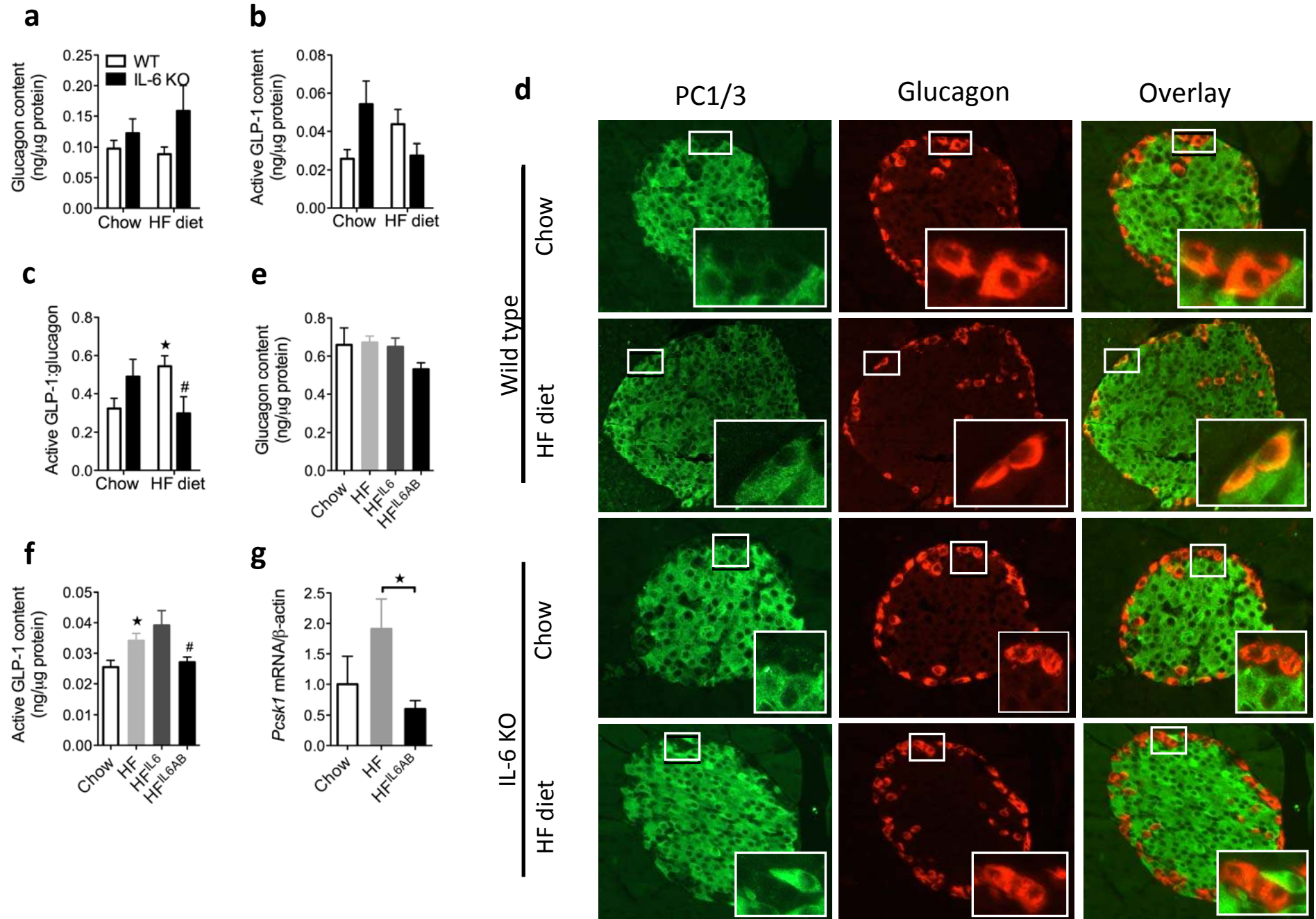
**Figure 4** Effects of IL-6 on GLP-1 secretion in human islets and human  $\alpha$  cells



**Figure 5** Effect of acute IL-6 on insulin secretion in animal models of prediabetes and diabetes



**Figure 6** Effect of IL-6 antagonism in HF diet fed mice and *db/db* mice



**Figure 6** Effect of IL-6 antagonism in HF diet fed mice and *db/db* mice

