IL-6-type Cytokine Signaling in Adipocytes Induces Intestinal GLP-1 Secretion

Stephan Wueest[1,2], Céline I. Laesser[1,2], Marianne Böni-Schnetzler[3,4], Flurin Item[1,2], Fabrizio C. Lucchini[1,2,5], Marcela Borsigova[1,2], Werner Müller[6], Marc Y. Donath[3,4] and Daniel Konrad[1,2,5]

[1]Division of Pediatric Endocrinology and Diabetology and [2]Children's Research Center, University Children's Hospital, Zurich, Switzerland

[3]Clinic of Endocrinology, Diabetes and Metabolism University Hospital Basel and

[4]Department Biomedicine, University of Basel, Basel, Switzerland

[5]Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland

[6]Faculty of Biology, Medicine and Health, University of Manchester, Manchester, U.K.

All correspondence and requests for reprint to:

Daniel Konrad, MD PhD

University Children's Hospital

Department of Endocrinology and Diabetology

Steinwiesstrasse 75

CH-8032 Zurich

Tel: ++41-44-266 7966; Fax: ++41-44-266 7983

Email: daniel.konrad@kispi.uzh.ch

or:

Stephan Wueest, PhD

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Email: stephan.wueest@usz.ch

Abstract

We recently showed that IL-6-type cytokine signaling in adipocytes induces FFA release from visceral adipocytes thereby promoting obesity-induced hepatic insulin resistance and steatosis. In addition, IL-6-type cytokines may increase the release of leptin from adipocytes and by that means induce GLP-1 secretion. We thus hypothesized that IL-6-type cytokine signaling in adipocytes may regulate insulin secretion. To this end, mice with adipocyte-specific knockout of gp130, the signal transducer protein of IL-6, were fed a high fat diet for 12 weeks. Compared to control littermates, knockout mice showed impaired glucose tolerance and circulating leptin, GLP-1 and insulin levels were reduced. In line, leptin release from isolated adipocytes was reduced and intestinal *Pcsk1* expression, the gene encoding PC1/3, which controls GLP-1 production, was decreased in knockout mice. Importantly, treatment with the GLP-1 receptor antagonist exendin 9-39 abolished the observed difference in glucose tolerance between control and knockout mice. Ex vivo, supernatant collected from isolated adipocytes of gp130 knockout mice blunted *Pcsk1* expression and GLP-1 release from GLUTag cells. In contrast, glucose and GLP-1 stimulated insulin secretion was not affected in islets of knockout mice. In conclusion, adipocyte-specific IL-6 signaling induces intestinal GLP-1 release to enhance insulin secretion thereby counteracting insulin resistance in obesity.

Introduction

High production and release of interleukin-6 (IL-6) from adipose tissue may contribute to dys-regulated metabolism in obesity and, thus, contribute to the development of insulin resistance (1-3). Consistently, we recently reported a role of IL-6 signaling in adipocytes in the development of obesity-associated liver insulin resistance and steatosis by making use of adipocyte-specific glycoprotein 130 (gp130) knockout ($gp130^{\Delta adipo}$) mice (4). gp130 is a common signal transducer protein of all IL-6-type cytokines which comprises eight different cytokines such as IL-6, interleukin 11 (IL-11) and leukemia inhibitor factor (LIF) (5). It was previously suggested that these IL-6-type cytokines may impact on leptin production in adipose tissue (6-8), thereby potentially mediating a protective effect on glucose metabolism. Interestingly, leptin was found to stimulate the release of glucagon-like peptide-1 (GLP-1) from enteroendocrine cells (9). GLP-1 is an important inducer of glucose-stimulated insulin release *in vivo* and it also inhibits gastric emptying and glucagon secretion (10). Hence, identification of factors promoting endogenous GLP-1 release is of interest since enhancing endogenous GLP-1 secretion may be a useful strategy to prevent pancreatic β-cell failure in insulin-resistant obese patients and subsequent development of type 2 diabetes (11). Active GLP-1 is mainly secreted into circulation from intestinal L cells after being cleaved from its precursor proglucagon by the prohormone convertase 1/3 (PC1/3) (10; 12; 13). The latter is encoded by *proprotein convertase subtilisin/kexin type 1 (Pcsk1*) that is expressed in intestinal L cells as well as alpha cells of pacreatic islets (13). Accordingly, GLP-1 can also be produced in pancreatic islets where it shows paracrine actions (12). In fact, it has recently been proposed that GLP-1 originating from pancreatic islets may

be more important for glucose homeostasis than GLP-1 produced in intestinal L cells (14; 15).

In the present study, we hypothesized that adipocyte-specific IL-6-type cytokine signaling induces leptin-mediated GLP-1 release in obese mice thereby improving glucose-stimulated insulin release and glucose tolerance.

Research Design and Methods

Animals

Adipocyte-specific gp130 knockout mice ($gp130^{\Delta adipo}$) on a C57BL/6J background were generated by crossing gp130 floxed ($gp130^{F/F}$) mice (16) with animals expressing the Cre recombinase controlled by the Adipoq promoter (AdipoqCre mice; purchased from The Jackson Laboratory, Bar Harbor, ME, USA). Six weeks-old male mice were fed *ad libitum* with standard rodent diet (chow) or high fat diet (HFD; D12331, Research Diets, New Brunswick, USA) for 12 weeks. HFD consisted of 58% of calories derived from fat, 28% from carbohydrate and 16% from protein. All protocols conformed to the Swiss animal protection laws and were approved by the Cantonal Veterinary Office in Zurich, Switzerland.

Intraperitoneal glucose and insulin tolerance test

Intraperitoneal glucose and insulin tolerance tests were performed as described (17). To block GLP-1 action, exendin 9-39 (25 nM/kg body weight; Bachem, Bubendorf, Switzerland), was injected intraperitoneally 1 minute prior to glucose injection. Blood

glucose concentration was measured in blood from tail-tip bleedings using a glucometer (AccuCheck Aviva, Roche Diagnostics, Rotkreuz, Switzerland).

Oral glucose tolerance test

Glucose (2g/kg body weight) was orally administered in overnight fasted mice. To block GLP-1 action, exendin 9-39 (25 nM/kg body weight), was injected intraperitoneally 15 minutes prior to oral glucose administration. Blood glucose concentration was measured in blood from tail-tip bleedings using a glucometer (AccuCheck Aviva).

Food intake

Food intake was determined using a metabolic and behavioral monitoring system (PhenoMaster, TSE Systems, Bad Homburg, Germany) as described (18).

Leptin release from isolated adipocytes

Adipocytes were isolated as described (19). Isolated adipocytes were incubated in the absence or presence of 1 nM/l CNTF (BioLegend, San Diego, CA, USA), 1 nM/l CT-1 (BioLegend), 5 nM/l recombinant IL-6 (AdipoGen), 10 nM/l IL-11 (Lucerna Chem, Luzern, Switzerland), 2.5 nM/l LIF (Miltenyi Biotec, Bergisch Gladbach, Germany) or 1 nM/l OSM (BioLegend) for four hours. Leptin levels were measured using MSD technology (Meso Scale Discovery, Gaithersburg, MD, USA).

Blood sampling

To prevent GLP-1 degradation, sitagliptine (25µg/g body weight; Sigma, Buchs, Switzerland) was injected intraperitoneally 30 minutes before oral or intraperitoneal

glucose administration. Of note, sitagliptine was demonstrated to increase circulating GLP-1 levels in mice (20). EDTA (5mM) and Diprotin A (0.1 mM; Sigma) were added to the collected blood followed by immediate centrifugation at 4°C and plasma was stored at -80°C until further processing.

Determination of plasma parameters

Plasma active GLP-1 and leptin were determined using MSD technology. Plasma insulin levels were measured using an ELISA kit as described previously (21). ELISA kits were used to measure plasma adiponectin (AdipoGen, Liestal, Switzerland), DPP IV (Abnova, Taipei City, Taiwan) and IL-6 (BioLegend, San Diego, CA, USA).

RNA extraction and quantitative reverse transcription-PCR (RT-PCR)

Total RNA was extracted (NucleoSpin, Macherey-Nagel, Düren, Germany) and concentration was determined spectrophotometrically (Nanodrop 1000; Nanodrop Technologies, Boston, MA). RNA was reverse transcribed with GoScript Reverse Transcription System (Promega, Madison, WI, USA). Taqman assays (Applied Biosystems, Rotkreuz, Switzerland) were used for real-time PCR amplification. The following PCR primers (Applied Biosystems) were used: leptin: Mm00434759 m1; pcsk1: Mm00479023_m1. Relative gene expression was obtained after normalization to 18sRNA (Applied Biosystems), using the formula $2^{-\Delta\Delta cp}$ (22).

Experiments in isolated islets

For mouse islet isolation, pancreata were perfused via the pancreatic duct with a collagenase solution (Worthington, Lakewood, NJ), excised and digested in the same

solution at 37°C for 27 minutes. The digest was sequentially filtered through 500 µm and 70 µm cell strainers (BD Falcon). Islets were handpicked under a microscope and cultured in 24-well ECM coated plates (Novamed Ltd., Jerusalem, Israel) for 40 hours in RPMI-1640 containing 11.1 mM glucose, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamax, 50 µg/ml gentamycin, 10 µg/ml Fungison and 10 % FCS at a density of 25 islets/well. To determine glucose stimulated insulin secretion islets were preincubated for 30 minutes in modified Krebs-Ringer bicarbonate buffer (KRB) containing 115 mM NaCl, 4.7 mM KCl, 2.6 mM $CaCl_2$ $2H_2O$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ $7H_2O$, 10 mM HEPES, 0.5 % bovine serum albumin, pH 7.4 and 2.8 mM glucose. KRB was then replaced by KRB 2.8 mM glucose and collected after 1 hour (basal insulin release), followed by collection of 1 hour release in KRB 16.7 mM glucose (stimulated insulin release) or KRB 16.7 mM glucose with 100 nM GLP-1(7-36) amide (Bachem, Switzerland). For determination of the insulin content islet cells were extracted with 0.18 N HCl in 70 % EtOH. Insulin concentrations were determined using mouse insulin ultrasensitive mouse/rat insulin kit (Mesoscale Discovery, Rockville, MD).

Ileum explants

Terminal ileum was dissected, flushed in PBS and cut into smaller pieces of 0.5 cm length. Explants were maintained in culture medium (Krebs buffer containing 0.1% BSA, 5 mM D-Glucose and 100 µM of Diprotin A (Sigma)). After 1 hour, medium was changed and supplemented with 15 mM D-Glucose or 0.1 µM recombinant leptin (Bio-Techne Ltd, Abingdon, U.K.) for 2 hours. Thereafter, supernatants were collected and explants were snap frozen until further processing.

GLUTag cell culture

GLUTag cells were grown in DMEM (1g/L glucose with L-glutamine and pyruvate) supplemented with 10% FBS and 1% penicillin-streptomycin. For experiments, cells were seeded in 12 well (200'000 cells/well) or 24 well (100'000 cells/well) plates. After one day, cells were fasted overnight in serum-free medium and subsequently stimulated with or without 0.1 µM recombinant leptin (Bio-Techne Ltd) for 6 hours. Alternatively, fasted GLUTag cells were treated with supernatants of isolated adipocytes for 6 hours in the presence or absence of neutralizing leptin antibody (0.2 µg/ml; Bio-Techne).

Data analysis

Data are presented as means ± SEM. Data were analyzed by unpaired two-tailed Student's *t* test, one sample *t* test, one-way ANOVA with Newman-Keuls correction for multiple group comparisons or two-way ANOVA with Bonferroni multiple comparisons. All statistical tests were calculated using the GraphPad Prism 5.04 (GraphPad Software, San Diego, CA, USA). P values < 0.05 were considered to be statistically significant.

Results

Impaired glucose tolerance in HFD-fed gp130$^{\Delta adipo}$ mice

To investigate a possible role of adipocyte-specific IL-6-type cytokine signaling in glucose metabolism, intraperitoneal glucose tolerance tests were performed in adipocyte-specific gp130 knockout mice (gp130$^{\Delta adipo}$) and control littermate mice (gp130$^{F/F}$) fed a chow or high fat diet (HFD) for twelve weeks. As previously shown, gp130 protein levels are reduced in isolated adipocytes but not in skeletal muscle and

liver of gp130$^{\Delta adipo}$ mice compared to control littermates, and body weight (4) as well as food intake was similar in both genotypes (Supplemental Fig. 1). As depicted in Fig. 1A and B, depletion of gp130 in adipocytes led to a stronger deterioration of intraperitoneal glucose tolerance in HFD-fed mice. In contrast, intraperitoneal insulin tolerance was improved in HFD-fed gp130$^{\Delta adipo}$ mice (Fig. 1C and D), confirming previous findings obtained from hyperinsulinemic-euglycemic clamp studies (4). Such data indicate that impaired glucose tolerance in knockout mice may be the result of blunted glucose-stimulated insulin secretion. Indeed, glucose-stimulated circulating insulin levels were significantly lower after intraperitoneal glucose injection in HFD-fed gp130$^{\Delta adipo}$ compared to gp130$^{F/F}$ mice (Fig. 1E). To investigate whether impaired β-cell function may drive the observed phenotype, glucose-stimulated insulin secretion (GSIS) was assessed in islets *ex vivo*. Of note, GSIS was not impaired in islets isolated from HFD-fed gp130$^{\Delta adipo}$ mice when compared to control littermates (Fig. 1F). Moreover, insulin content was higher in HFD-fed knockout compared to control mice (Supplemental Fig. 2). Hence, deteriorated glucose-stimulated insulin levels *in vivo* do not result from defective β-cell function. Rather, impaired incretin secretion may constitute the observed metabolic phenotype.

Reduced circulating GLP-1 levels in obese gp130$^{\Delta adipo}$ mice

To assess a potential involvement of impaired incretin secretion, oral glucose tolerance tests were performed. As depicted in Fig. 2A and B, oral glucose tolerance was significantly impaired in HFD-fed knockout mice, paralleled by a ~40% reduction in circulating insulin levels (Fig. 2C). Of note, the difference between knockout and control mice was even more pronounced after oral compared to intraperitoneal glucose

administration [~20% difference in area under the curve (AUC) between HFD-fed gp130$^{\Delta adipo}$ and control littermates after oral administration (Fig. 2B) vs. ~10% difference in AUC after intraperitoneal glucose injection (Fig. 1B)]. Since glucose clearance during oral glucose tolerance test is augmented by incretins (23), such data further support a role for decreased incretin secretion as a cause for impaired glucose tolerance in gp130 knockout mice. Indeed, basal as well as glucose-stimulated levels of glucagon-like peptide-1 (GLP-1) were reduced by ~50% in knockout mice (Fig. 2D). Importantly, the GLP-1 receptor antagonist exendin 9-39 (24) blunted the difference in glucose tolerance between HFD-fed control and knockout mice (Figs. 2E and F). In fact, exendin 9-39 significantly blunted AUC in gp130$^{F/F}$ mice (1272±57 mmol/l*min in control vs. 1500±51 mmol/l*min in exendin 9-39 treated mice; $p<0.05$) but not in gp130$^{\Delta adipo}$ mice (1507±34 mmol/l*min in control vs. 1567±34 mmol/l*min in exendin 9-39 treated mice; $p=0.23$). Besides affecting oral glucose tolerance, exendin 9-39 treatment abrogated impaired intraperitoneal glucose tolerance in gp130$^{\Delta adipo}$ mice (Fig. 2G), suggesting that reduced basal GLP-1 levels (Fig. 2D) contributed to impaired intraperitoneal glucose tolerance in untreated mice (Fig. 1A and B). Of note, *ex vivo* stimulation of pancreatic islets with high glucose concentration in combination with GLP-1 revealed similar degrees of insulin secretion in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice (Fig. 2H) indicating similar GLP-1 sensitivity of *β*-cells. Taken together, adipocyte-specific depletion of IL-6-type cytokine signaling reduces GLP-1 release in in HFD-fed mice thereby impairing glucose tolerance.

IL-6 signaling in adipocytes contributes to obesity-induced circulating leptin levels

Reduced circulating GLP-1 levels in HFD-fed gp130$^{\Delta adipo}$ mice may result from enhanced degradation by dipeptidyl peptidase IV (DPP IV) that is increasingly released from adipose tissue in obesity (10; 25). However, plasma DPP IV levels were similar in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice (Supplemental Fig. 3), indicating that IL-6-type cytokine signaling did not affect circulating DPP IV levels and, hence, was not responsible for blunted GLP-1 levels in knockout mice. Circulating GLP-1 derives almost completely from enteroendocrine L cells (12). Thus, reduced circulating GLP-1 levels in gp130 knockout mice may result from blunted intestinal secretion. Indeed, glucose-stimulated GLP-1 secretion from intestinal explants harvested from HFD-fed gp130$^{\Delta adipo}$ mice was reduced (Fig. 3A), whereas its release from islets isolated from HFD-fed knockout mice remained unaffected (Supplemental Fig. 4). Hence, reduced circulating GLP-1 levels in HFD-fed gp130$^{\Delta adipo}$ mice resulted from its blunted release from enteroendocrine cells.

Recently, different adipose-derived circulating factors were shown to affect GLP-1 release from intestinal cells. In fact, circulating IL-6 was shown to stimulate GLP-1 secretion thereby enhancing glucose-stimulated insulin secretion (26). Moreover, the two adipokines adiponectin and leptin were reported to induce GLP-1 secretion from intestinal L cells (9; 27). As depicted in Supplemental Fig. 5, circulating IL-6 and adiponectin levels were similar between HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. In contrast, plasma leptin levels were reduced by ~40% in gp130$^{\Delta adipo}$ mice (Fig. 3B) despite similar body weight (4), potentially contributing to their reduced GLP-1 levels. To analyze the impact of IL-6-cytokine signaling on leptin production in adipocytes, its expression and release were analyzed in isolated adipocytes of chow- and HFD-fed

knockout and control mice. As expected, HFD induced leptin mRNA expression in and release from adipocytes of control mice (Fig. 3C and D). Importantly, leptin release from adipocytes was significantly blunted in HFD-fed knockout mice (Fig. 3D), whereas its expression was unchanged (Fig. 3C). In addition, leptin content in isolated adipocytes of HFD-fed mice was similar between the genotypes (Fig. 3E). Hence, IL-6-type cytokine signaling in adipocytes affects leptin release but not its transcription and protein synthesis. We next wanted to investigate which member of the IL-6-type cytokine family mediated the effect on leptin release. To this end, isolated adipocytes of chow-fed $gp130^{F/F}$ and $gp130^{\Delta adipo}$ mice were incubated with the IL-6-type cytokine family members ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), IL-6, IL-11, LIF or oncostatin M (OSM), which were previously suggested to affect leptin production or were suggested as potential targets to treat obesity (5-8; 28). While CNTF, CT-1, IL-11, LIF and OSM had no significant effect on leptin release, IL-6 stimulated leptin secretion in isolated adipocytes gp130-dependently (Fig. 3F). Taken together, IL-6 induced leptin release from adipocytes thereby contributing to elevated circulating leptin levels in HFD-fed mice.

Leptin induces Pcsk1 expression and GLP-1 release in enteroendocrine cells

Leptin induces GLP-1 release from the enteroendocrine cell line GLUTag cells (9; 29). Such release of GLP-1 is regulated by PC1/3 in intestinal cells (10; 13). Indeed, expression of *proprotein convertase subtilisin/kexin type 1 (Pcsk1),* the gene encoding PC1/3 (13), was significantly increased by leptin treatment (Fig. 4A). Importantly, leptin also stimulated *Pcsk1* expression in L cell-rich ileum (14) explants harvested from HFD-fed $gp130^{F/F}$ mice (Fig. 4B). Next, experiments in GLUTag cells that were incubated with

supernatant harvested from isolated adipocytes were performed to further explore the proposed adipo-enteroendocrine axis driven by leptin. As shown in Fig. 4C, GLP-1 release was significantly blunted in GLUTag cells incubated with supernatants collected from adipocytes of HFD-fed gp130$^{\Delta adipo}$ mice. Of note, leptin concentration was reduced in such supernantants as shown above (Fig. 3D). Importantly, leptin neutralization reversed observed difference on GLP-1 release in GLUTag cells incubated with supernatant collected from adipocytes (Fig. 4D) suggesting a critical role of leptin in the observed effect. In parallel, *Pcsk1* expression was reduced (Fig. 4E) in GLUTag cells incubated with supernatants collected from adipocytes of HFD-fed gp130$^{\Delta adipo}$ mice, an effect that was reversed upon leptin neutralization (Fig. 4F). Likewise, expression of *Pcsk1* was reduced in the ileum of HFD-fed knockout mice (Fig. 4G). Collectively, our data suggest that IL-6-type cytokine signaling in adipocytes promotes intestinal GLP-1 release leptin-dependently, probably by stimulating *Pcsk1* expression.

Discussion

The present study suggests that adipocyte-specific IL-6-type cytokine signaling promotes leptin-mediated GLP-1 release from enteroendocrine cells in obesity thereby improving glucose-stimulated insulin release and glucose tolerance (Fig. 5). Such notion is based on the following findings: i. gp130 depletion specifically in adipocytes impaired glucose tolerance and reduced circulating leptin, GLP-1 and insulin levels in HFD-fed mice; ii. GLP-1 secretion is reduced leptin-dependently from enteroendocrine cells incubated with supernatant collected from gp130-depleted adipocytes.

Reduced GLP-1 release from enteroendocrine cells in adipocyte-specific gp130 knockout mice may result from reduced *Pcsk1* expression, the gene encoding PC1/3 controlling GLP-1 production (10; 13). Leptin was previously reported to enhance *Pcsk1* expression in neuronal cells (30). In HFD-fed gp130$^{\Delta adipo}$ mice, intestinal *Pcsk1* expression was lower compared to control littermates and was associated with significantly lower circulating leptin levels. Moreover, supernatant collected from isolated adipocytes regulated *Pcsk1* expression in GLUTag cells in a leptin-dependent manner, and recombinant leptin stimulated *Pcsk1* expression in GLUTag cells as well as in ileum explants of HFD-fed mice. Of note, the role of intestinal GLP-1 in glucose homeostasis has recently been questioned suggesting that pancreatic GLP-1 may be more important for glucose homeostasis (14). Indeed, we did not find diminished GLP-1 release from islets isolated from HFD-fed knockout mice. Hence, our data indicate that reduced intestinal rather than pancreatic GLP-1 release affects glucose-stimulated insulin secretion and, consequently, glucose homeostasis in gp130$^{\Delta adipo}$ mice. Although we cannot rule out a role of pancreatic GLP-1, our data clearly support an important role of intestinal GLP-1 production in glucose homeostasis. In support of an involvement of the incretin system in the observed metabolic phenotype in HFD-fed gp130$^{\Delta adipo}$ mice, insulin release and content were not reduced in islets of HFD-fed knockout mice *ex vivo*. In fact, insulin content was rather elevated in islets of gp130$^{\Delta adipo}$ mice, potentially mirroring reduced (GLP-1-stimulated) insulin secretion *in vivo*. GLP-1 sensitivity of isolated islets was similar between HFD-fed control and knockout mice suggesting that reduced circulating GLP-1 levels rather than GLP-1 resistance of *β*-cells cause impaired insulin secretion in knockout mice. In support of such notion, administration of oral glucose, which induces circulating GLP-1 levels in contrast to intraperitoneal glucose

injection, exacerbated the difference in glucose tolerance between knockout and control mice.

Lack of IL-6-type cytokine signaling in adipocytes blunts circulating leptin levels independently of fat pad mass (4). Interestingly, reduced circulating leptin levels in knockout mice were paralleled by reduced leptin release from but similar mRNA expression in adipocytes indicating that gp130 depletion affects leptin secretion but not leptin transcription. Accordingly, it has been suggested that circulating signals regulate leptin on a post-transcriptional level, whereas the long-term nutritional status affects leptin mRNA expression (31). In the past, several IL-6-type cytokines were suggested to affect leptin production or were proposed as potential targets to treat obesity (5-8; 28). Here, we suggest that IL-6 contributes to obesity-induced leptin release from adipocytes, adding another metabolic function to the pleiotropic actions of IL-6 (32-34). In fact, among all tested IL-6-type cytokines only IL-6 significantly increased leptin release from isolated adipocytes. Consistently, treatment of HFD-fed C57BL/6 mice with an anti-IL-6 receptor antibody reduced circulating leptin levels (35). Clearly, we cannot rule out that other IL-6-type family members and/or other molecules binding gp130 such as superantigens (36) contribute to reduced circulating leptin levels in knockout mice. In addition, compensatory changes in the production of other adipose-derived factors in knockout mice may have contributed to reduced leptin levels.

Despite impaired glucose tolerance, insulin sensitivity was improved in HFD-fed $gp130^{\Delta adipo}$ mice. This observation may be explained by reduced portal FFA flux in knockout mice leading to blunted hepatic insulin resistance and steatosis (4). Hence, IL-6-cytokine signaling in adipocytes may promote both protective as well as harmful effects on glucose metabolism (Fig. 5). Physiologically, increased IL-6-mediated release

of FFA and leptin from adipocytes in obesity may be interpreted as an attempt to reduce lipid stores by increasing lipolysis (FFA release) and by lowering lipid accumulation via leptin-mediated reduction of food intake. Notably, the observed leptin-mediated effect of IL-6 on circulating GLP-1 levels may be additive to the direct effect of IL-6 on GLP-1 release from enteroendocrine cells (26).

Of note, reduced circulating leptin levels in knockout mice did not significantly affect body weight (4), suggesting similar central leptin action between control and knockout mice. In agreement, food intake was similar between HFD-fed $gp130^{F/F}$ and $gp130^{\Delta adipo}$ littermates. Such finding may suggest that high fat diet feeding induces a central leptin resistance, while leptin-mediated signaling in the periphery (e.g. in intestinal cells) is less affected (37; 38). Accordingly, leptin induced *Pcsk1* expression in the ileum of HFD-fed mice. Alternatively, decreased leptin passage across the blood-brain-barrier in obese mice and/or local GLP-1 production in the brain (10; 37) may lead to unaffected GLP-1 levels in the brain of knockout mice. Clearly, further studies are needed to shed more light on involved mechanisms. In addition, adipocyte-specific depletion of gp130 in female mice may result in a different metabolic phenotype, as leptin secretion and the potency of GLP-1-function are sex-dependent (39).

In conclusion, we identify a novel adipo-enteroendocrine axis driven by IL-6 in the regulation of glucose-stimulated insulin release and glucose tolerance in obesity. Such axis may sensitize pancreatic β-cells to glucose and, thus, counteract adipose tissue-induced insulin resistance (4; 40). Moreover, it may offset carbohydrate and/or leptin resistance of enteroendocrine cells resulting in augmented GLP-1 levels in obesity (9; 41-43).

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Author Contributions

SW and DK conceived the study and wrote the paper. SW, CIL, MB, FI and FCL performed the experimental work. WM provided gp130$^{\Delta adipo}$ mice and gave conceptual advice. MYD gave conceptual advice. All authors contributed to discussion and reviewed/edited manuscript. DK is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interests' statement

All authors state no conflict of interest.

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Figure Legends

Figure 1 Impaired glucose tolerance in HFD-fed gp130$^{\Delta adipo}$ mice

Intraperitoneal glucose tolerance (ipGTT) (**A** and **B**) and insulin tolerance (ipITT) (**C** and **D**) tests were performed in chow and HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ littermate mice. n=6-10 for chow; n=21-31 for HFD. (**E**) Circulating insulin levels were assessed after intraperitoneal glucose injection in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=10-14. (**F**) Insulin release was assessed in isolated islets of HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice stimulated with 2.8 mM (basal) or 16.7 mM (glucose) glucose. n=6-11 biological replicates à 25 pooled islets isolated from HFD-fed gp130$^{F/F}$ (n=4 mice) and gp130$^{\Delta adipo}$ (n=3 mice). Values are expressed as mean ± SEM. F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adipo}$. *p < 0.05, **p < 0.01, ***p < 0.001 (ANOVA).

Figure 2 Reduced circulating GLP-1 levels in obese gp130$^{\Delta adipo}$ mice

(**A** and **B**) Oral glucose tolerance (oGTT) test was performed in chow and HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=4-5 for chow; n=7 for HFD. Circulating insulin (**C**) and GLP-1 (**D**) levels were assessed after oral glucose administration in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=6-8. (**E** and **F**) Oral glucose tolerance test was performed in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice 15 minutes after intraperitoneal injection of 25 nM/kg BW exendin 9-39. n=6-8. (**G**) Intraperitoneal glucose (ipGTT) tolerance test was performed in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice 1 minute after intraperitoneal injection of 25 nM/kg BW exendin 9-39. n=14-15. (**H**) Insulin release was assessed in isolated islets of HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice stimulated without (basal) or with 100 nM GLP-1. n=6 biological replicates à 25 pooled islets isolated from HFD-fed

gp130$^{F/F}$ (n=4 mice) and gp130$^{\Delta adipo}$ (n=3 mice). Values are expressed as mean ± SEM. F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adipo}$. *p < 0.05, **p < 0.01, ***p < 0.001 (ANOVA).

Figure 3 IL-6 signaling in adipocytes contributes to obesity-induced circulating leptin levels

(**A**) GLP-1 release was determined from ileum explants harvested from HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=3. (**B**) Leptin concentration was determined in the circulation of chow and HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=5-15. Leptin release from (**C**) and mRNA expression in (**D**) epididymal adipocytes isolated from chow and HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=3-8. (**E**) Leptin content was determined in epididymal adipocytes isolated from HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=6-7. (**F**) Leptin release of epididymal adipocytes isolated from chow-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice stimulated with different IL-6-type cytokines. n=4-11. Values are expressed as mean ± SEM. F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adipo}$. $^{\#}$p=0.098, *p < 0.05, **p < 0.01, ***p < 0.001 (student's *t* test or ANOVA).

Figure 4 Leptin induces GLP-1 release and Pcsk1 expression in enteroendocrine cells

Pcsk1 mRNA expression was determined in GLUTag cells (**A**) or in ileum explants harvested from HFD-fed gp130$^{F/F}$ mice (**B**) treated with or without recombinant leptin. n=8-9 for GLUTag cells; n=3 for explants. GLP-1 release was determined from GLUTag cells treated with supernatant released from adipocytes isolated from HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice treated without (**C**) (n=6-7) or with (**D**) (n=3) neutralizing leptin antibody. Pcsk1 expression was determined in GLUTag cells treated with supernatant

released from adipocytes isolated from HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice treated without (**E**) (n=7) or with (**F**) (n=3) neutralizing leptin antibody. (**G**) Pcsk1 mRNA expression in ileum of HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=6-7. Values are expressed as mean ± SEM (except for B, E and F where mRNA expression was normalized to untreated control or control mouse in each litter). F/F: gp130$^{F/F}$; ∆ad: gp130$^{\Delta adipo}$. $^{\#}p = 0.057$, $^{*}p < 0.05$ (Student's *t* test or one sample *t*-test). Pcsk1: proprotein convertase subtilisin/kexin type 1; SN: supernatant.

Figure 5 Suggested model

Obesity-induced rise in IL-6 increase the release of FFA and leptin from adipose tissue. In turn, increased FFA flux to the liver induces hepatic insulin resistance and steatosis (4). Moreover, elevated leptin release contributes to circulating GLP-1 and insulin levels, thereby counteracting (hepatic) insulin resistance.

Figure 1

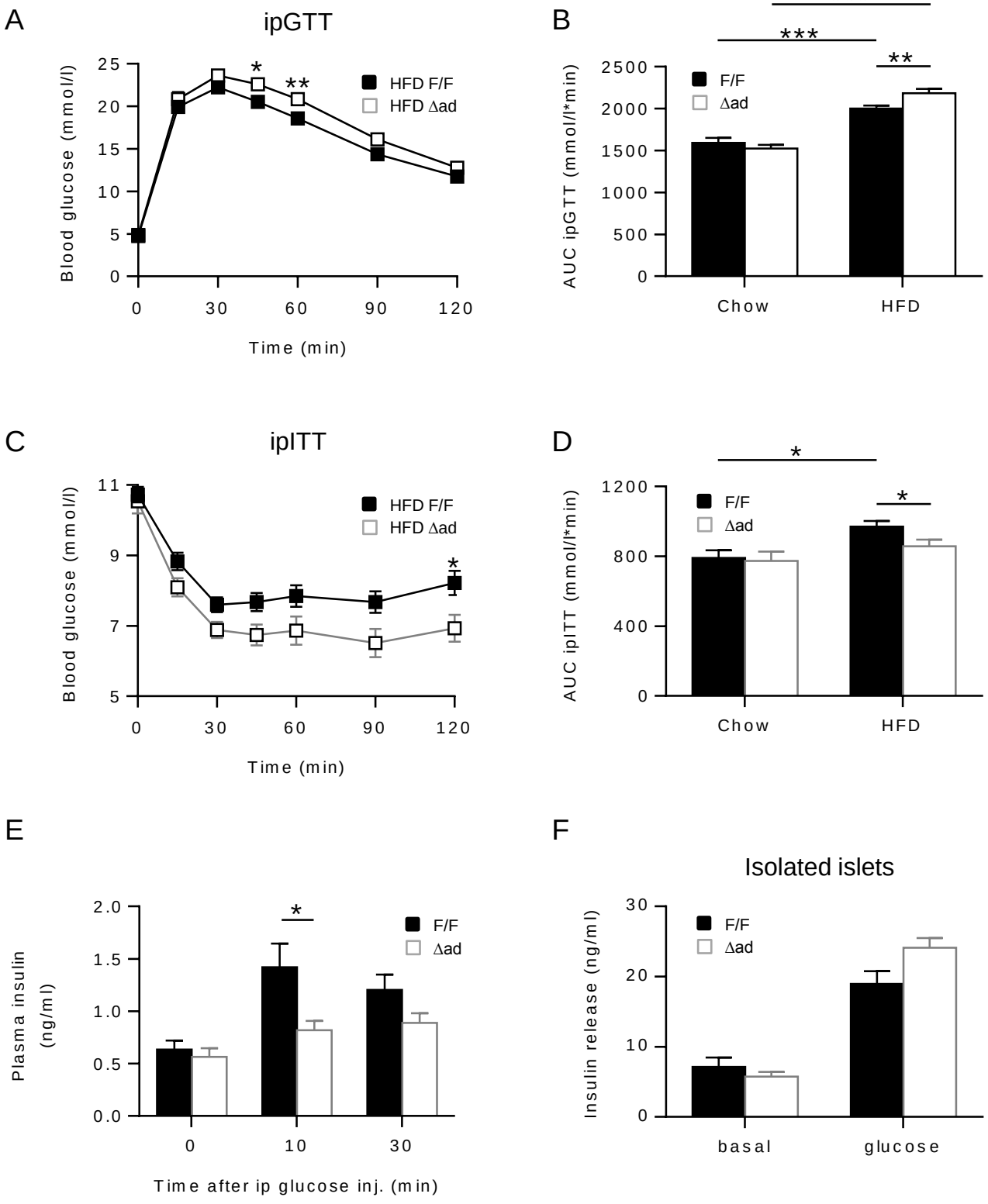
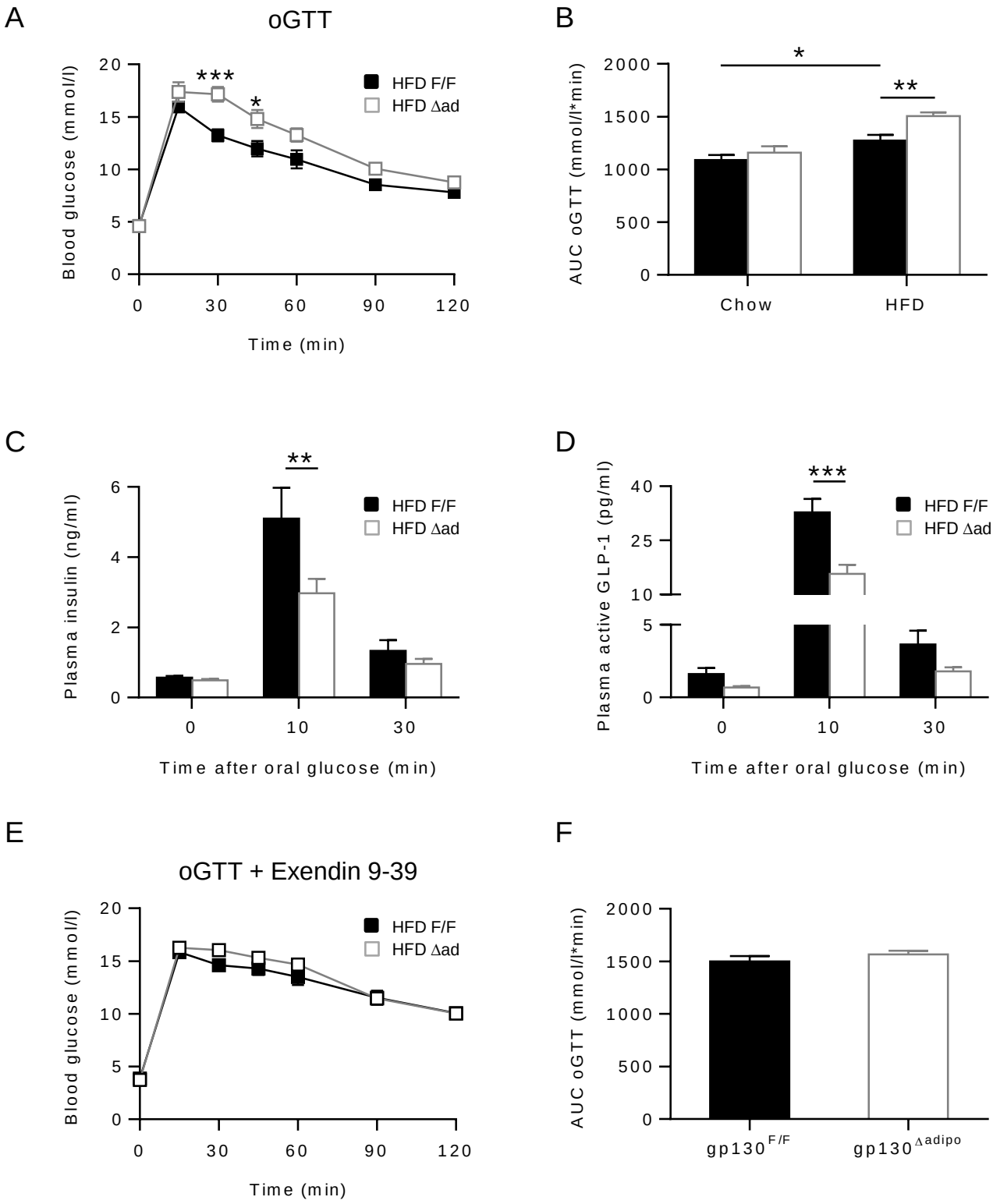
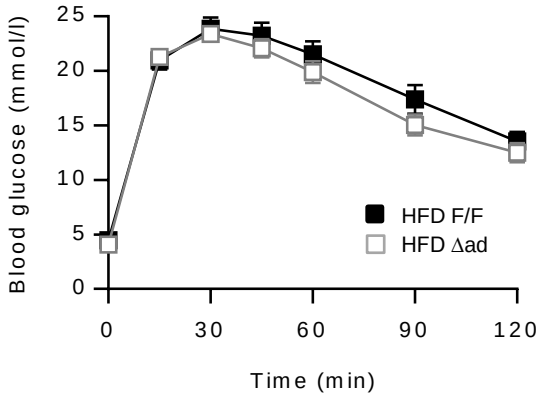

A
ipGTT
Blood glucose (mmol/l)
Time (min)
HFD F/F
HFD Δad
B
AUC ipGTT (mmol/l*min)
F/F
Δad
Chow
HFD
C
ipITT
Blood glucose (mmol/l)
Time (min)
HFD F/F
HFD Δad
D
AUC ipITT (mmol/l*min)
F/F
Δad
Chow
HFD
E
Plasma insulin (ng/ml)
Time after ip glucose inj. (min)
F/F
Δad
F
Isolated islets
Insulin release (ng/ml)
F/F
Δad
basal
glucose

Figure 2


A
oGTT
Blood glucose (mmol/l)
Time (min)
HFD F/F
HFD Δad
***
*
B
AUC oGTT (mmol/l*min)
Chow
HFD
*
**
C
Plasma insulin (ng/ml)
Time after oral glucose (min)
**
D
Plasma active GLP-1 (pg/ml)
Time after oral glucose (min)
***
E
oGTT + Exendin 9-39
F
gp130F/F
gp130Δadipo

G


ipGTT + Exendin 9-39
Blood glucose (mmol/l)
25
20
15
10
5
0
0
30
60
90
120
Time (min)
HFD F/F
HFD Δad

H

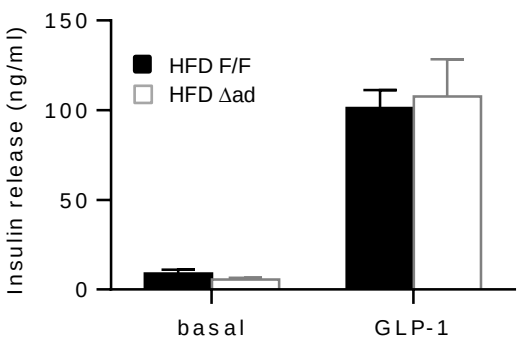

Isolated islets
Insulin release (ng/ml)
150
100
50
0
basal
GLP-1
HFD F/F
HFD Δad

Figure 3

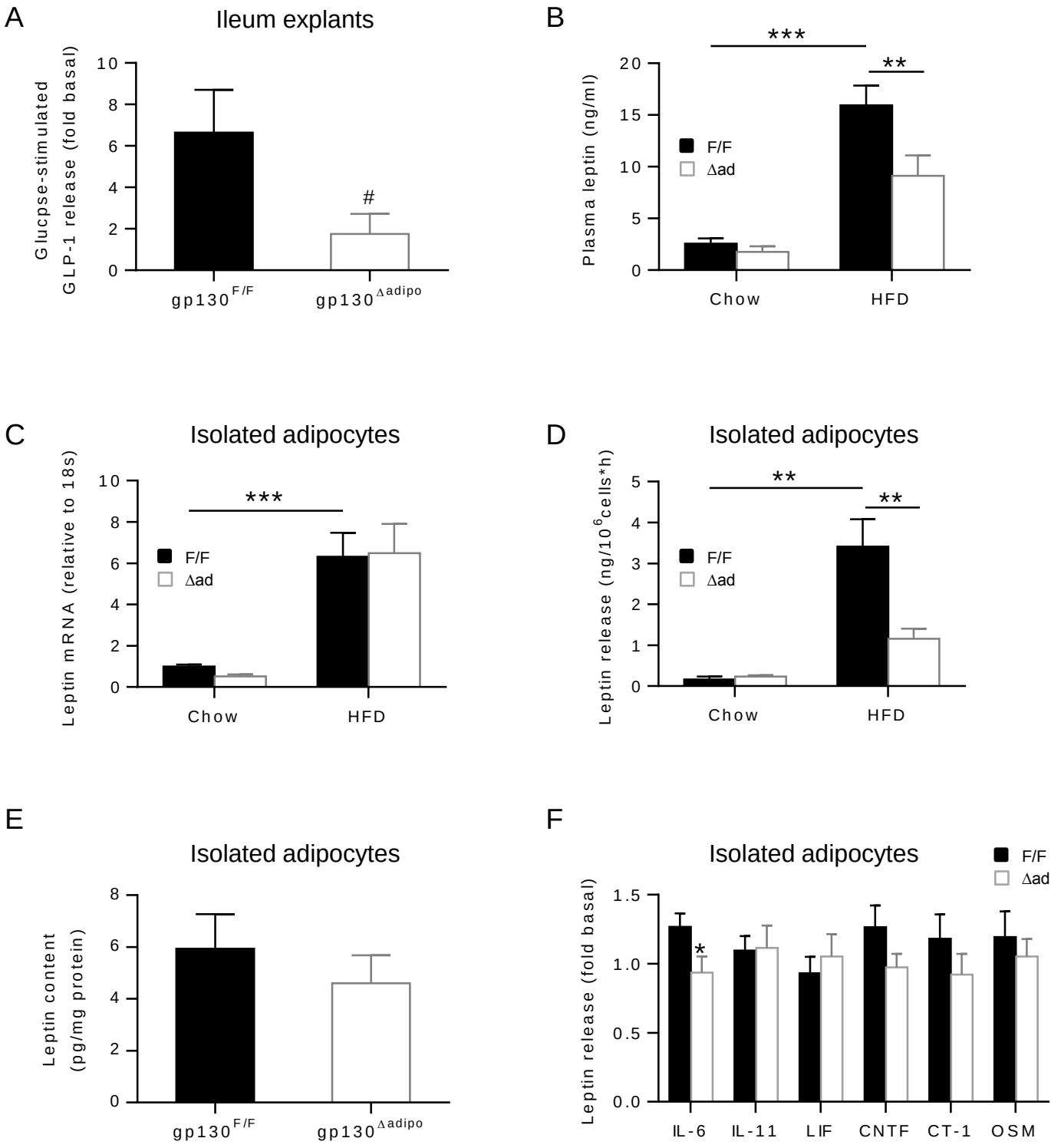
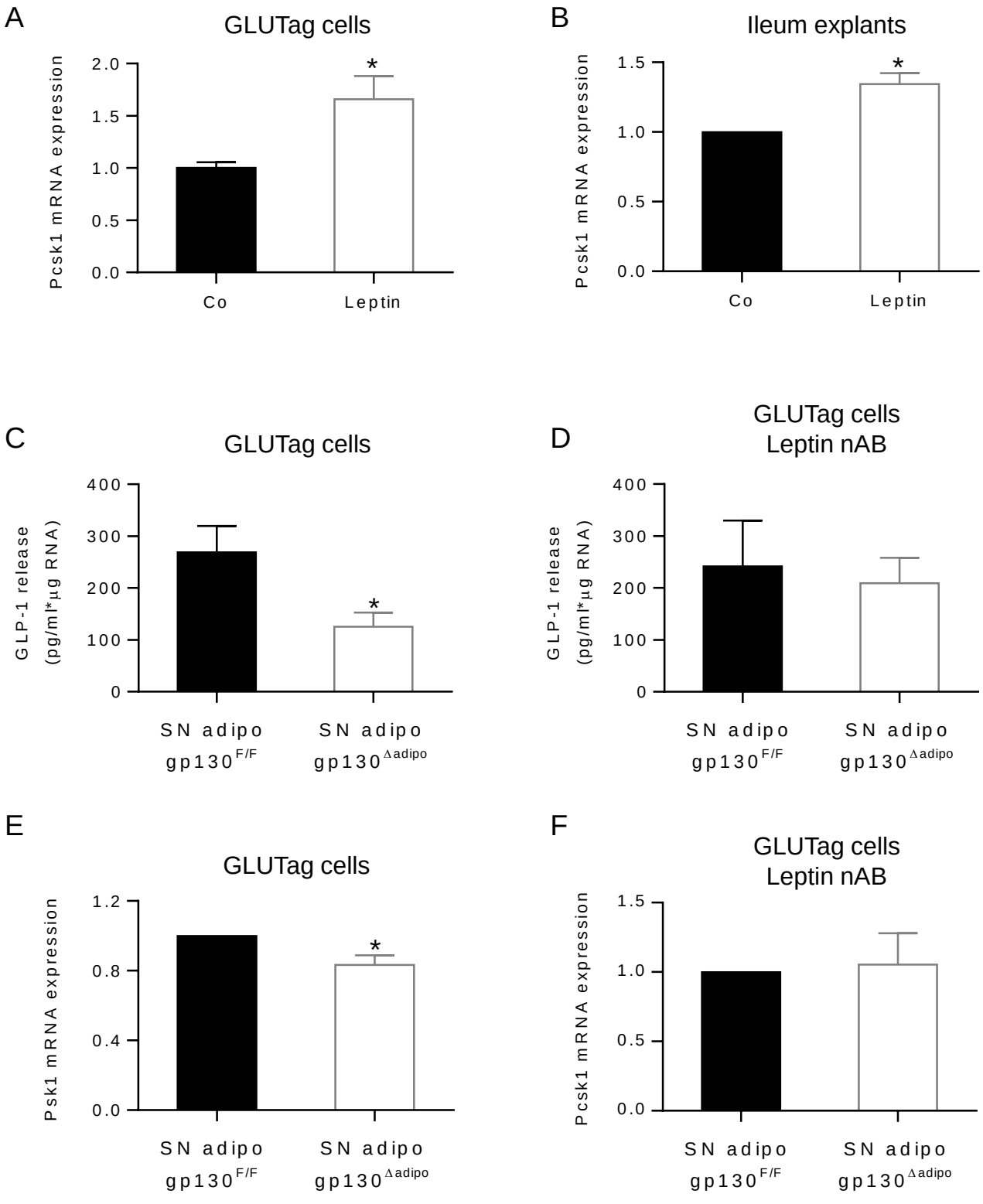

A
Ileum explants
Glucpse-stimulated GLP-1 release (fold basal)
10
8
6
4
2
0
#
gp130F/F
gp130Δadipo
B
***
**
Plasma leptin (ng/ml)
20
15
10
5
0
F/F
Δad
Chow
HFD
C
Isolated adipocytes
Leptin mRNA (relative to 18s)
10
8
6
4
2
0
***
F/F
Δad
Chow
HFD
D
Isolated adipocytes
Leptin release (ng/10^6cells*h)
5
4
3
2
1
0
**
**
F/F
Δad
Chow
HFD
E
Isolated adipocytes
Leptin content (pg/mg protein)
8
6
4
2
0
gp130F/F
gp130Δadipo
F
Isolated adipocytes
F/F
Δad
Leptin release (fold basal)
1.5
1.0
0.5
0.0
*
IL-6
IL-11
LIF
CNTF
CT-1
OSM

Figure 4


A
GLUTag cells
Pcsk1 mRNA expression
2.0
1.5
1.0
0.5
0.0
*
Co
Leptin
B
Ileum explants
Pcsk1 mRNA expression
1.5
1.0
0.5
0.0
*
Co
Leptin
C
GLUTag cells
GLP-1 release (pg/ml*µg RNA)
400
300
200
100
0
*
SN adipo gp130F/F
SN adipo gp130Δadipo
D
GLUTag cells
Leptin nAB
GLP-1 release (pg/ml*µg RNA)
400
300
200
100
0
SN adipo gp130F/F
SN adipo gp130Δadipo
E
GLUTag cells
Psk1 mRNA expression
1.2
0.8
0.4
0.0
*
SN adipo gp130F/F
SN adipo gp130Δadipo
F
GLUTag cells
Leptin nAB
Pcsk1 mRNA expression
1.5
1.0
0.5
0.0
SN adipo gp130F/F
SN adipo gp130Δadipo

G

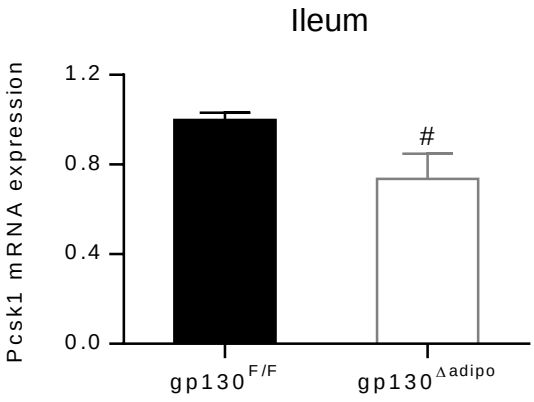
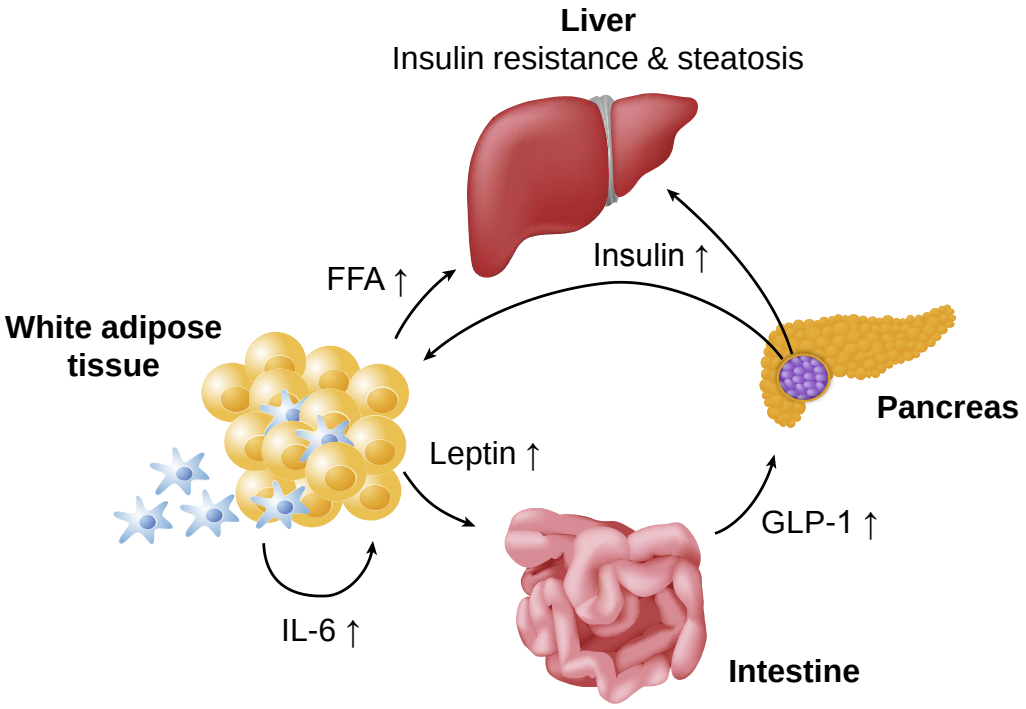
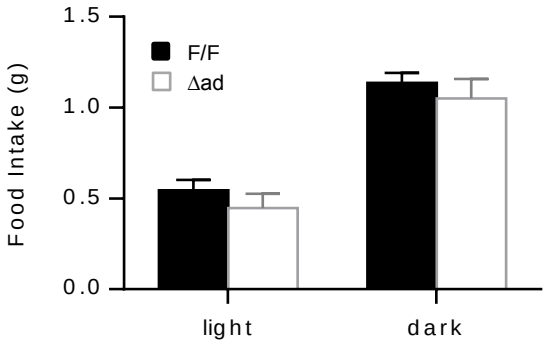

Ileum
Pcsk1 mRNA expression
1.2
0.8
0.4
0.0
#
gp130F/F
gp130Δadipo

Figure 5


Liver
Insulin resistance & steatosis
FFA ↑
Insulin ↑
White adipose tissue
Pancreas
Leptin ↑
GLP-1 ↑
IL-6 ↑
Intestine

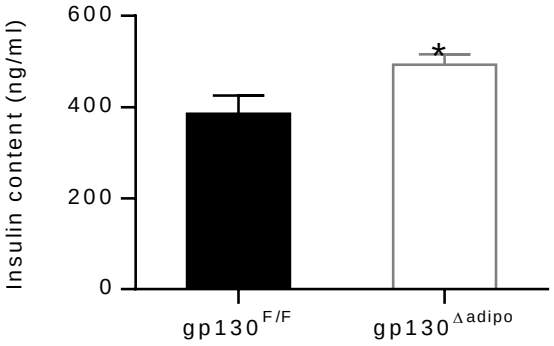
Supplemental Figure 1



Food intake in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice

Food intake was measured in a metabolic cage unit in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=5-7. Values are expressed as mean ± SEM.

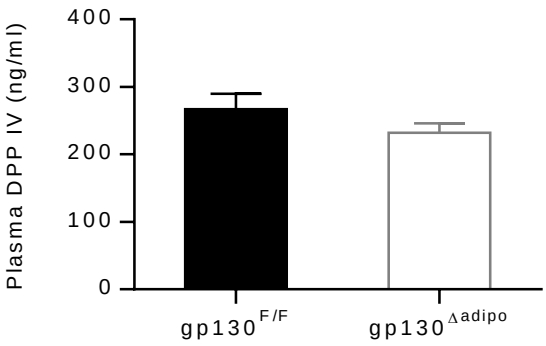
Supplemental Figure 2



Insulin content of islets isolated from HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice

Insulin content was determined in islets isolated from HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ littermate mice. n=9-12 biological replicates à 25 pooled islets isolated from HFD-fed gp130$^{F/F}$ (n=4 mice) and gp130$^{\Delta adipo}$ (n=3 mice). Values are expressed as mean ± SEM. *p < 0.05 (student's t test).

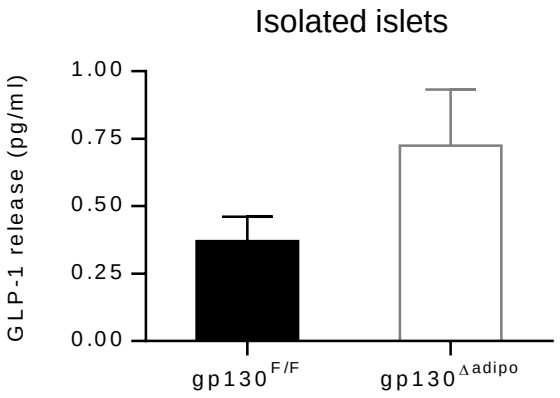
Supplemental Figure 3



Plasma DPP IV in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice

Circulating DPP IV levels were assessed in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=15-16. Values are expressed as mean ± SEM.

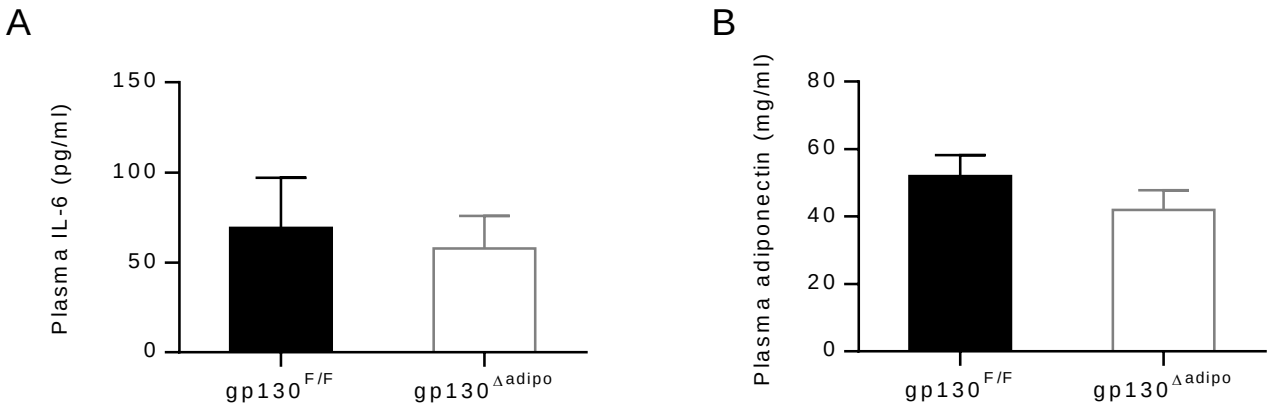
Supplemental Figure 4



GLP-1 release from isolated islet of HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice

GLP-1 release was assessed in isolated islets of HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. n=12 biological replicates à 25 pooled islets isolated from HFD-fed gp130$^{F/F}$ (n=4 mice) and gp130$^{\Delta adipo}$ (n=3 mice). Values are expressed as mean ± SEM.

Supplemental Figure 5



Plasma IL-6 and adiponectin in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice

Circulating IL-6 (**A**, n=5-6) and adiponectin (**B**, n=6) levels were assessed in HFD-fed gp130$^{F/F}$ and gp130$^{\Delta adipo}$ mice. Values are expressed as mean ± SEM.