Glucose-dependent Insulinotropic Peptide Stimulates Glucagon-like Peptide 1 Production by Pancreatic Islets via Interleukin-6, Produced by β Cells

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Short title: GIP induces GLP-1 via IL-6

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Abstract

Background & Aims: Glucose-dependent insulinotropic peptide (GIP) induces production of interleukin-6 (IL6) by adipocytes. IL6 increases production of glucagon-like peptide (GLP1) by L cells and α cells, leading to secretion of insulin from β cells. We investigated whether GIP regulates GLP1 and glycemia via IL-6.

Methods: We obtained samples of human pancreatic islets and isolated islets from mice; human α cells and β cells were sorted by flow cytometry and incubated with GIP. Islets were analyzed by quantitative PCR and immunohistochemistry. BKS.Cg-Deck7m+/+ Leprdb/J db/db mice (diabetic mice) and db/+ mice, as well as C57BL/6J IL6-knockout mice (IL6-KO) and C57BL/6J mice with the full-length Il6 gene (controls), were fed a chow or a high-fat diet; some mice were given injections of recombinant GIP, IL6, GLP, a neutralizing antibody against IL6 (anti-IL6), lipopolysaccharide, and/or interleukin-1β (IL1B). Mice were given a glucose challenge and blood samples were collected and analyzed.

Results: Incubation of mouse and human pancreatic α cells with GIP induced their production of IL6, leading to production of GLP1 and insulin secretion from pancreatic islets. This did not occur in islets from IL6-KO mice or incubated with anti-IL6. Incubation of islets with IL1B resulted in IL6 production but directly reduced GLP1 production. Incubation of mouse islets with the SGLT2 inhibitor dapagliflozin induced production of GLP1 and IL6. Injection of control mice with GIP increased plasma levels of GLP1, insulin, and glucose tolerance; these effects were amplified in mice given lipopolysaccharide but reduced in IL6-KO mice or in mice given anti-IL6. Islets from diabetic mice had increased levels of IL1B and IL6, compared with db/+ mice, but injection of GIP did not lead to production of GLP1 or reduce glycemia.

Conclusions: In studies of pancreatic islets from humans and mice, we found that GIP induces production of IL6 by α cells, leading to islet production of GLP1 and insulin. This process is regulated by inflammation, via IL1B, and by SGLT2. In diabetic mice, increased islet levels of IL6 and IL1B might increase or reduce production of GLP1 and affect glycemia.

KEY WORDS: mouse model, hormones, immune regulation, incretin
Introduction

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP)-1 are incretin hormones stimulating insulin secretion in response to food intake. GIP is secreted from enteroendocrine K-cells located in the upper-gut and GLP-1 from L-cells located in the lower-gut. GLP-1 is cleaved from its precursor peptide proglucagon by prohormone convertase (PC) 1/3 while processing of the same precursor peptide by PC2 is yielding glucagon. Accordingly, GLP-1 can also be detected in pancreatic α-cells. GIP and GLP-1 enhance glucose-stimulated insulin secretion, inhibit pancreatic β-cell apoptosis and promote β-cell proliferation in addition to extrapancreatic effects. Pancreatic α-cells express GIP receptors and GIP directly induces glucagon secretion from α-cells, while GLP-1 inhibits glucagon release. Furthermore, GIP stimulates GLP-1 secretion from intestinal L-cells in an upper-to-lower gut interaction, however the mediators of this stimulation are unknown.

The incretin effect of GIP is blunted in patients with diabetes mellitus while GLP-1 remains active. However, the ability of GIP to stimulate glucagon is retained in the diabetic state. In line, exogenous infusion of GIP worsens postprandial hyperglycemia in patients with type 2 diabetes. Therefore incretin-based therapies for type 2 diabetes have focused on GLP-1 and not on GIP.

Multiple mechanisms underlie insulin resistance and defective insulin secretion in type 2 diabetes. Most of the factors involved in this development include an inflammatory response. Consequently, anti-inflammatory drugs are under development for the treatment of patients with this condition. Interestingly, in addition to its incretin effect, GIP also induces an inflammatory response in human adipocytes characterized by the induction of cytokines including Interleukin (IL)-1β and IL-6. Furthermore, IL-6 stimulates insulin from β-cells, glucagon from α-cells and GLP-1 secretion from L- and α-cells. Thereby, IL-6 increases GLP-1 production from α-cells through increased proglucagon and PC 1/3 expression.

Therefore, we hypothesized that GIP may stimulate GLP-1 release via IL-6 and that this effect may be impacted by the prevailing inflammation in diabetes.
Research Design and Methods

Human pancreatic islets

Human islets were isolated in the islet transplantation centres of Lille (France) from cadaveric pancreata in accordance with the local Institutional Ethical Committees. Human islets were provided by the islet for research distribution program through the European Consortium for Islet Transplantation, under the supervision of the Juvenile Diabetes Research Foundation (31-2008-416). Islets were cultured as previously described 27.

Mouse pancreatic islets

Mouse islets were isolated as previously described 27.

Fluorescence-activated cell sorting

Human islet cells were sorted by FACS after labelling with Newport Green using a FACS Vantage (Becton Dickinson, USA) as described before 28 yielding β-cell-enriched (84-89% insulin positive) and α-cell-enriched (63-92% glucagon positive) cell fractions.

Animal studies

All animal experiments were conducted according to the Swiss Veterinary Law and Institutional Guidelines and were approved by the Swiss Authorities of the cantonal veterinary office according to the Swiss Law.

Statistics

Data were analyzed using one-way ANOVA or unpaired t-test with Bonferroni correction and presented as mean ± SEM. Analysis was performed using GraphPad Prism 5 (Graphpad Software Inc., San Diego, CA, USA). p<0.05 was considered statistically significant. n indicates the number of biological replicates per treatment condition for in vitro experiments or number of mice per treatment group for in vivo experiments.
**Results**

**GIP induces glucagon and GLP-1 release in human and mouse pancreatic islets**

Treatment of human pancreatic islets with GIP increased mRNA expression of *proglucagon*, *Pcsk1*, coding for PC1/3 and *Pcsk2*, coding for PC2, already after 1 h while expression of *somatostatin* was not altered (Fig. 1A). GLP-1 protein release was increased 6 h and 24 h after incubation with GIP while glucagon release was enhanced only after 24 h and insulin tended to increase only after 6 h (Fig. 1B). Although GIP significantly induced GLP-1 secretion already at 1 nM (data not shown) we chose 100 nM GIP as working concentration for our *in vitro* experiments to allow for a robust induction of GLP-1. Next, we FAC-sorted human islet cells providing β- and α-cell-enriched fractions. As expected, insulin release was higher in the β-cell than in the α-cell enriched fraction while GLP-1 and glucagon release were mainly detected and induced in the α-cell enriched fraction by GIP (Fig. S1A and Fig. 1C).

According to the upregulation of GLP-1 by GIP in the α-cell enriched fraction, GIP induced mRNA expression of *Pcsk1* specifically in sorted human α-cells (Fig. 1D). Similar to human islets, treating mouse islets with GIP for 24 h increased active GLP-1 and glucagon release (Fig. 1E). Finally, we compared the ability of GIP to induce GLP-1 and glucagon secretion during incubation at low (2.8 mM) versus high (16.7 mM) glucose concentrations in isolated human islets. GIP-induced GLP-1 secretion was comparable during low and high glucose incubations, while GIP-induced glucagon secretion robustly only at 2.8 mM glucose (Fig. 1F).

**GIP induces IL-6 release in human and mouse pancreatic α-cells**

Treatment of human islets with GIP for 1 h increased mRNA expression of *IL-6*, *IL-1β* and non-significantly of *TNFa* (Fig. 2A). Furthermore, IL-6 protein release was increased in human and mouse islets upon GIP treatment (Fig. 2B).

We next investigated the cellular source of IL-6 in islets using FAC-sorted human α- and β-cell enriched fractions treated with GIP for 6 h. mRNA expression of *IL-6* was higher at baseline and stimulated by GIP in the α-cell enriched fraction (Fig. 2C, left graph). The *IL-6 receptor* (*IL-6R*) was also predominantly expressed in the α-cell enriched fraction but not regulated by GIP (Fig. 2C, middle graph). In contrast, the pan-macrophage marker *CD68* was equally detectable in α- and β-cell enriched fractions (Fig. 2C, right graph). In line with the mRNA expression data, IL-6 protein release was much higher in the α- compared to the β-cell-enriched fraction (Fig. 2D). Interestingly,
GIP-induced IL-6 release tended to be increased in the α-cell-enriched fraction while it was decreased in the β-cell-enriched fraction (Fig. 2E). To confirm the cellular source of IL-6 in islets, mouse islets were treated with GIP or IL-1β and then dispersed into single cells prior to fixation. Subsequent immunocytochemistry revealed GIP-induced IL-6 staining only in glucagon-positive cells, while IL-1β increased IL-6 expression also in glucagon-negative cells (Fig. 2F and Fig. S2).

**GIP-induced GLP-1, glucagon and insulin secretion is partly IL-6-dependent and modified by IL-1β in human and mouse islets**

We next investigated if GIP-induced GLP-1, glucagon and insulin secretion in pancreatic islets is promoted by IL-6. Indeed, in mouse islets GIP-induced GLP-1 and glucagon release was diminished by a neutralizing anti-mouse IL-6 antibody (IL-6AB) compared to the isotype control (Fig. 3A). Consistently, GIP-induced GLP-1 and glucagon secretion was reduced in mouse islets isolated from IL-6 knockout (IL-6KO) mice compared to wild-type controls (Fig. 3B, left graph). Similarly, pretreatment of human islets with an anti-human IL-6 receptor antibody (IL-6RAB) abolished GIP-induced secretion of GLP-1 and glucagon (Fig. 3B, right graph). The specificity of the antibody was confirmed by blocking recombinant IL-6-induced GLP-1 secretion (Fig. S3).

Since we observed that GIP induced IL-1β, IL-6 and GLP-1 in islets and because IL-1β stimulates islet-derived IL-6⁹, we tested whether IL-1β potentiates the GIP effects in islets in an IL-6-dependent manner. Pretreating islets from wild-type mice for 4 h with IL-1β strongly increased GIP-induced GLP-1 secretion while this effect was abolished in islets from IL-6KO mice (Fig. S4A). Of note, IL-1β treatment alone for 4 h did not affect GLP-1 release in mouse islets (Fig. S4A). In human islets IL-1β alone robustly induced IL-6 secretion after 6 h and 24 h (Fig. S4B). However, GLP-1 secretion was not altered after 6 h (Fig S4C, left graph) and was even reduced after 24 h of IL-1β treatment (Fig. S4C, middle graph). In contrast, treatment with an IL-1 receptor antagonist for 24 h showed a tendency to increase GLP-1 secretion (Fig. S4C, right graph).

Next we tested whether IL-6 mediates GIP-induced GLP-1, glucagon and insulin secretion during acute glucose stimulation. Therefore, pancreatic islets were incubated for 1 h at low glucose (2.8 mmol/L) alone followed by 1 h at high glucose (16.7 mmol/L) in the presence and absence of GIP and IL-6AB or IL-6RAB, respectively. In that
setting, GIP increased the stimulatory index (ratio of high to low glucose incubations) of GLP-1, glucagon and insulin in human (Fig. 3C) and mouse (Fig. 3D, E) islets during high glucose incubation. This GIP effect was partly reduced by IL-6AB or IL-6RAB, respectively, (Fig 3C, D) or in islets isolated from IL-6KO mice (Fig. 3E). Of note, in contrast to GIP, GLP-1-induced insulin secretion during high glucose incubation was higher in islets from IL-6 deficient than wild-type mice (Fig. S5).

In summary GIP-induced GLP-1, glucagon and insulin secretion in islets is partly dependent on IL-6. Furthermore GIP-induced GLP-1 secretion is potentiated by IL-1β pretreatment in an IL-6-dependent manner, while chronic IL-1β alone impairs GLP-1 secretion in pancreatic islets.

The adenylyl cyclase/cAMP/proteinkinase A (PKA) pathway and sodium glucose transporter (SGLT) 2 are involved in GIP-induced IL-6 and GLP-1 secretion from pancreatic islets

To investigate the underlying mechanisms of islet-derived IL-6 and GLP-1 secretion, we hypothesized that GIP-induced IL-6 and GLP-1 release is mediated by the adenylyl cyclase/cAMP/PKA pathway, which is activated by GIP in pancreatic α-cells. Indeed, forskolin, a direct activator of adenylyl cyclase activity, robustly stimulated the release of IL-6 and GLP-1 (Fig. 4A). Furthermore, preincubation of isolated mouse islets with the PKA-inhibitor H-89 abolished GIP-induced GLP-1 production (Fig. 4A). Since it has recently been reported that SGLT2 inhibition induces glucagon secretion from pancreatic α-cells, we further investigated if SGLT2-inhibition also plays a role in α-cell-derived IL-6 and GLP-1 release. Slc5a1, encoding SGLT1, expression was higher in the α- than in the β-cell fraction of sorted human islets, and was not affected by GIP treatment (Fig. S1B and C). Slc5a2, encoding for SGLT2, expression was not detectable in FACS-sorted cultured α- or β-cell fractions. However, both were detectable in whole human islets and treatment with GIP or IL-6 for 24 h decreased mRNA expression of Slc5a2 but not of Slc5a1 (Fig. 4B). We then investigated if SGLT2 inhibition stimulates GLP-1 secretion from pancreatic islets. Indeed, treatment of isolated mouse islets with the SGLT2 inhibitor dapagliflozin induced both GLP-1 and IL-6 release (Fig. 4C, left graphs). Strikingly, stimulation of pancreatic islets with GIP in the presence of dapagliflozin
resulted in a robust potentiation of GLP-1 secretion that exceeded the additive effect of the single agents by far (Fig. 4C, right graph).

**IL-6 also promotes GIP-induced GLP-1 release via increased glucose uptake in GLUTag cells**

GIP stimulates GLP-1 from enteroendocrine L-cells \(^\text{14}\). To investigate if IL-6 also promotes intestinal GIP-induced GLP-1 secretion we used the mouse L-cell line GLUTag. Exposure to IL-6 dose dependently increased the glucose-stimulated release of GLP-1 and potentiated the effect of GIP (Fig. S6A). Furthermore, treatment with IL-6 for 2 h resulted in a tendency to increased glucose uptake (Fig. S6B). To test whether the potentiating effect of IL-6 on GIP-induced GLP-1 release was mediated by glucose uptake via SGLT1, we used the inhibitor phlorizin. In the presence of phlorizin, the IL-6 effect on GIP-induced GLP-1 secretion was significantly reduced (Fig. S6C). To assess the effect of IL-1\(\beta\), we first tested for expression of IL-6 by GLUTag cells, since IL-1\(\beta\) effects on GIP-induced GLP-1 in islets were partly mediated by IL-6. In GLUTag cells, endogenous IL-6 could not be detected with and without IL-1\(\beta\) stimulation (data not shown). Treatment of GLUTag cells with IL-1\(\beta\) for 24 h decreased GIP-induced GLP-1 release during glucose stimulation (Fig. S6D). Furthermore, IL-1\(\beta\) reduced chronic GLP-1 release and decreased GLP-1 content (Fig. S6D). The impairment in GLP-1 production was not due to changes in cell viability, since IL-1\(\beta\) did not induce GLUTag cell death (data not shown). Furthermore, IL-6 increased and IL-1\(\beta\) reduced mRNA expression of *Slc5a1* in GLUTag cells (Fig. S6E). In summary, these results suggest that in GLUTag cells IL-6 potentiates the GIP effect on GLP-1 release via SGLT-1-mediated glucose uptake. This effect is impaired by IL-1\(\beta\).

**GIP effects on glucose metabolism are partly IL-6 dependent**

Next, we asked if GIP also induces GLP-1 via IL-6 *in vivo*. To enable detection of active GLP-1 in the plasma, mice were pretreated with the dipeptidylpeptidase IV-inhibitor sitagliptin 30 minutes before GIP or saline administration. Intraperitoneal (i.p.) injection of GIP at a dose of 70 pmol/g in C57BL/6 mice together with glucose (2 g/kg i.p.) at time point 0 min increased GLP-1 and insulin secretion leading to an improved glucose tolerance and induced systemic IL-6 production (Fig. 5A). To investigate if GIP also stimulates GLP-1 in the absence of a glucose bolus we injected GIP alone and found again a decrease in blood glucose along with a robust increase in GLP-1 plasma levels
while insulin secretion was not significantly affected (Fig. 5B). To unravel if the ability of GIP to induce GLP-1 differs according to the route of glucose application, we tested the effects of GIP during an oral glucose tolerance test (OGTT) compared to an intraperitoneal GTT (IPGTT). GIP-induced GLP-1 secretion was comparable during OGTT and IPGTT as was the improvement in glucose tolerance upon GIP treatment in the different experimental settings (Fig. 5C). Therefore, endogenous incretin secretion upon oral glucose administration does not further promote the stimulatory effects of GIP on GLP1 release. However, GIP-induced insulin secretion was more pronounced during IPGTT than OGTT, most likely due to the overall higher plasma glucose values following an intraperitoneal compared to an oral glucose administration (Fig. 5C). We then investigated if GIP also induces glucagon secretion during an IPGTT. In contrast to plasma GLP-1, which peaked 10 min after GIP and glucose injection, administration of GIP together with glucose induced glucagon only after 30 min, possibly reflecting a counter-regulatory response to hypoglycemic range (Fig. S7). Next we injected GIP in IL-6KO and WT control mice. IL-6 deficiency strongly reduced the GIP effect on glucose tolerance, GLP-1 and insulin secretion (Fig. 5D). Administration of IL-6 30 min before GIP and glucose injection partially restored GIP effects on GLP-1 secretion as well as on glucose tolerance in IL-6KO mice (Fig. 5E). In contrast, administration of IL-6 in wild-type mice did not impact on GIP effects on GLP-1, insulin and glycaemia (Fig. S9A). To further confirm our genetic data, we used a pharmacologic approach and injected wild type mice with a neutralizing IL-6AB 6 h before the administration of GIP and found GIP-induced GLP-1 and insulin secretion to be significantly reduced (Fig. 5F).

To trigger endogenous IL-6 levels we applied lipopolysaccharide (LPS), which markedly increased circulating IL-6 levels (Fig. S8A). Injection of LPS 4 h before the administration of GIP and glucose robustly potentiated GIP effects on glucose tolerance, GLP-1 and insulin secretion in wild-type mice (Fig. S8B). However, when injecting IL-6KO mice with LPS (Fig. S8C) or injecting mice that where pretreated with IL-6AB 2 h before LPS administration (Fig. S8D), LPS-potentiation of GIP effects was lost. Overall these findings suggest that GIP effects on GLP-1 and insulin secretion as well as glycemic control in vivo are partly dependent on functional IL-6 signaling.

**GLP-1 effects on glucose control are preserved and even enhanced in IL-6-deficient mice**
Next we tested if the lack of GIP efficacy in IL-6 deficient mice was specific to GIP or due to a general unresponsiveness to incretins. In contrast to GIP, GLP-1 showed a stronger insulin stimulation capacity in IL-6KO versus wild type mice (Fig. 6A) in accordance with our ex-vivo islet experiment (Fig. S5). However in normal C57BL/6 mice GIP and GLP-1 at equimolar concentrations had a comparable effect on glucose tolerance and insulin secretion (Fig. 6B). In contrast, GLP-1 at equimolar amounts to GIP displayed a much stronger effect on glucose clearance and insulin secretion in IL-6KO mice (Fig. 6C). In conclusion, the impaired incretin effect on glucose tolerance and insulin secretion in IL-6-deficient mice seems to be selective for GIP and compensated for by an oversensitivity to GLP-1.

GIP effects are differentially modulated by IL-6 and IL-1β in murine models of diabetes and diet-induced obesity

In patients with type 2 diabetes, the incretin effect of GLP-1 is maintained while the glucose lowering effect of GIP is impaired. We hypothesized that the impaired glucose-lowering ability of GIP may be due to a lack of GIP-induced GLP-1 in the context of diabetes-induced inflammation. We chose db/db mice as a model to study GIP effects in type 2 diabetes, since these mice develop obesity and severe type 2 diabetes early in life. In 6-8 week old diabetic db/db mice GIP neither improved glucose tolerance nor GLP-1 secretion compared to age-matched db/+ control mice while the insulin-potentiating effect of GIP remained intact (Fig. 7A). However, IL-6 injection 30 min before GIP rescued GIP-induced GLP-1 secretion and glucose tolerance in db/db mice (Fig. 7B), whereas in db/+ mice GIP effects on glucose control and GLP-1 secretion were not affected by IL-6 (Fig. S9B). Interestingly, IL-6 alone had no effect on intraperitoneal glucose tolerance, GLP-1 or insulin levels, neither in db/db nor in db/+ mice (Fig. 7B and S9B). In contrast to diabetic db/db mice, in pre-diabetic high-fat diet (HFD)-fed obese mice GIP effects on glucose tolerance were still present and GIP-induced GLP-1 and insulin secretion was even enhanced (Fig. 7C). To investigate if the enhanced GIP effect on insulin and GLP-1 secretion was also present in mice with impaired glucose tolerance due to chronic inflammation, lean C57Bl/6 were treated with daily injections of IL-1β for two weeks before an IPGTT with GIP. IL-1β treatment alone impaired glucose tolerance and tended to decrease active GLP-1 and insulin production, while IL-1β in combination with GIP enhanced GLP-1 and insulin secretion (Fig. 7D). To unravel the differences
leading to an altered GIP sensitivity in islets from diabetic db/db versus HFD obese or chronically IL-1β-treated mice

we examined expression of IL-1β and IL-6 in islets isolated from these different mouse models ex vivo. IL-1β and IL-6 mRNA expression were massively increased in db/db versus db/+ islets (Fig. 7E, left graph) while this upregulation was much lower in islets from HFD- versus chow diet fed mice (Fig. 7E, middle graph) and in islets from mice injected for 14 days with IL-1β versus saline (Fig. 7E, right graph). Overall, excessive and chronic inflammation may prevent additional GIP-induced IL-6 production in pancreatic islets of db/db mice and thereby blunt GIP effects on GLP-1.
Discussion

In the present study we provide evidence that GIP-induced GLP-1 secretion is partly mediated and promoted by IL-6 derived from pancreatic islets. In human and mouse islets GIP stimulated IL-6 exclusively in α-cells followed by an increased GLP-1 secretion. In the absence of IL-6, the ability of GIP to induce GLP-1 and insulin was impaired. This identifies IL-6 as an essential factor promoting GIP action on pancreatic islets and reveals a yet unknown intra-islet GIP-GLP-1 interaction. In islets, IL-1β robustly stimulated IL-6, and potentiated GIP-induced GLP-1 in an IL-6-dependent manner. However, and in contrast to GIP, IL-1β-induced IL-6 was not limited to α-cells and, in the absence of GIP, IL-1β impaired islet- as well as GLUTag cell-derived GLP-1 secretion. Therefore, IL-1β indirectly stimulates GLP-1 via IL-6 while it is directly inhibiting GLP-1 secretion.

GLP-1 and glucagon have opposite effects on glucose regulation and a priori it appears surprising that GIP induces both. However, the glucagonotropic effect of GIP is modulated by the prevailing glycaemia and in vivo is present only at glucose levels below a certain threshold \(^{32}\). Accordingly, GIP robustly induced glucagon secretion only at 2.8 mM glucose \textit{in vitro} and during lowered plasma glucose concentrations \textit{in vivo}. This is in line with recent studies pointing to the physiological importance of GIP-induced glucagon secretion for restoration of plasma glucose levels during hypoglycemia \(^{32,33}\). In contrast to GIP-induced glucagon secretion, GIP stimulated GLP-1 release also during high glucose concentrations \textit{in vitro} and \textit{in vivo}.

GIP strongly induces cAMP in pancreatic alpha cells \(^{10}\) and transcription of the proglucagon gene is regulated by cAMP \(^{34}\). Accordingly, it was reported that GIP-induced glucagon secretion from pancreatic islets is mediated via the cAMP/PKA pathway \(^{13}\). In line, we now demonstrate that GIP-induced GLP-1 secretion in islets is dependent on the adenylyl cyclase/cAMP/PKA signaling module. Thereby the finding that GIP stimulated mRNA expression of \textit{proglucagon} as well as \textit{Pcsk1} and \textit{Pcsk2} suggests that GIP not only stimulates the secretion but also enhances the synthesis of GLP-1 and glucagon.

Bonner C et al. have recently reported that SGLT2 regulates glucagon secretion from pancreatic α-cells \(^{31}\). Here we show that GIP-induced IL-6 and GLP-1 release from pancreatic islets is also SGLT2 dependent. In line with Bonner C et al. \(^{31}\) we found a much higher expression of \textit{Slc5a1} in the α- compared to the β-cell fraction of FACS-sorted human islets. However we found no detectable expression of \textit{Slc5a2} mRNA in human sorted α- or β-cells, possibly
due to our cell sorting and culture procedure or, because, in contrast to Bonner C et al., we did not pre-amplify the mRNA prior to qPCR. Indeed, both Slc5a1 and Slc5a2 mRNA were well detectable in whole human islets. Furthermore GIP and IL-6 inhibited Slc5a2 but not Slc5a1 expression and SGLT2 inhibition with dapagliflozin stimulated GLP-1 and IL-6 secretion from pancreatic islets. In line, recent observations revealed increased GLP-1 plasma levels upon SGLT2 inhibition in humans. Overall, this points to a possible additional mechanisms explaining the beneficial effect of SGLT2 inhibition in patients with type 2 diabetes.

Local GLP-1 production in pancreatic α-cells is induced by muscle-derived IL-6 during exercise and in response to hyperglycemia. In the present study we identified GIP as an additional factor inducing IL-6 from human and mouse pancreatic α-cells and thereby promoting local GLP-1 production. These findings confirm in human islets our previous observation in mice that IL-6 regulates GLP-1 from α-cells. Supporting our observations on the cellular source of IL-6, a recent publication reported developmental up-regulation of IL-6 expression in pancreatic α-cells of neonatal rats.

In vivo, GIP stimulated GLP-1, insulin and IL-6 secretion and improved glucose tolerance. However, these effects were strongly diminished in IL-6-deficient mice and in the presence of a blocking IL-6 antibody, further indicating that IL-6 is essential for the glucose regulatory action of GIP. In contrast to GIP-induced GLP-1 secretion, GIP was still able to stimulate insulin secretion in IL-6 deficient mice, although to a lesser extent than in wild-type control mice. The lowered insulin secretion capacity of GIP in the absence of IL-6 can be explained by the lack of GLP-1 stimulation.

Interestingly and in contrast to GIP, GLP-1 effects on glucose control and insulin secretion were preserved and even enhanced in IL-6 deficient mice in vivo and in isolated islets from these mice. The GLP-1 oversensitivity of islets in IL-6 deficient mice might thereby compensate for the impaired islet responsiveness to GIP, in line with an enhanced GLP-1 efficacy in GIP receptor-deficient mice. Furthermore, we found, that, in contrast to GLP-1-induced suppression of food intake in the brain, GLP-1-dependent insulin secretion from pancreatic islets is not dependent on IL-6.
The fact that GIP induced GLP-1 in vivo is in apparent contrast with findings by Nauck and colleagues, reporting no increase in plasma GLP-1 following GIP infusion \(^{18}\). However, in the present study active plasma GLP-1 was measured in mice while in the study by Nauck et al. total (active and degraded) GLP-1 was evaluated in humans.

GIP-induced glucose control and insulin secretion is preserved in prediabetic obese subjects \(^{40}\). Accordingly, GIP effects on GLP-1 and insulin secretion as well as glycemic control were retained and even enhanced in prediabetic high-fat diet-induced obese mice and in mice injected with IL-1\(\beta\) for two weeks, possibly reflecting an adaptive mechanism. However, in established diabetes the glucose-lowering effect of GIP is impaired \(^{16-19}\). Accordingly, diabetic db/db mice were resistant to the glucose-lowering action of GIP and showed an impaired GIP-induced GLP-1 secretion. However, exogenous IL-6 administration was able to restore GIP effects on glycaemia and GLP-1 secretion in db/db mice. To examine possible intra-islet factors explaining this impairment we measured expression levels of IL-1\(\beta\) and IL-6 in mouse islets isolated from the three different mouse models. We found a much higher intra-islet IL-1\(\beta\) and IL-6 expression in islets from db/db compared to islets from high-fat diet fed or chronically IL-1\(\beta\)-treated mice. Massive upregulation of IL-6 in pancreatic islets from db/db mice might have impaired the ability of GIP to further induce IL-6 expression in \(\alpha\)-cells resulting in a blunted GIP effect on GLP-1. On the other hand, direct inhibition of GLP-1 secretion by IL-1\(\beta\) may be responsible for the blunted GIP effect on GLP-1. In support of this hypothesis, it has been shown that pharmacological normalization of plasma glucose reduces IL-1\(\beta\) expression in islets of diabetic animals \(^{41}\) and restores GIP effects on glucose control in patients with type 2 diabetes \(^{42, 43}\).

In a recent preliminary clinical study, we observed that anti-IL-1\(\alpha\) antibodies may improve glycaemia in patients with type 2 diabetes \(^{44}\). It remains to be investigated whether IL-1\(\alpha\) also negatively impacts GLP-1 secretion.

Overall our results point to a physiological significance of IL-6 as an \(\alpha\)-cell-derived factor promoting GIP-induced GLP-1 and insulin secretion as well as glucose control. It further supports the concept that the immune system plays an integral role in the regulation of metabolism in physiology and pathology.

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References


Figure legends

Figure 1. GIP induces GLP-1 in human and mouse pancreatic islets

(A) mRNA expression of proglucagon prohormone convertase (Pcsk1), Pcsk2 and somatostatin of cultured human islets incubated for 1 h with GIP or solvent (DMSO 0.1%; control) (n=12, 3 separate islet preparations done in quadruplicates).

(B) GLP-1, glucagon and insulin release from cultured human islets exposed for 6 h or 24 h to GIP or control (n=12-20, 3-5 separate islet preparations done in quadruplicates).

(C) GLP-1 and glucagon release from FACS-sorted human α-cell-enriched fraction exposed for 6 h to GIP or control (n=14-17, 5 separate islet preparations done in duplicates, triplicates or quadruplicates).

(D) mRNA expression of Pcsk1 in cultured human FACS-sorted human β- and α-cell-enriched fractions exposed for 6 h to GIP or control (n=11-12, 4 separate islet preparations done in duplicates, triplicates or quadruplicates).

(E) GLP-1 and glucagon release from cultured mouse islets exposed for 24 h to GIP or control (n=20, 5 separate islet preparations done in triplicates or quadruplicates).

(F) GLP-1 and glucagon release from cultured human islets exposed for 1 h to GIP or control (n=16, 4 separate islet preparations done in quadruplicates).

Bar graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 determined by ONE way ANOVA and Bonferroni correction (A) or by unpaired t-test (B-F).

Figure 2. GIP induces IL-6 in human and mouse pancreatic α-cells

(A) mRNA expression of cytokines of cultured human islets incubated for 1 h with GIP or control (n=12, 3 separate islet preparations done in quadruplicates).

(B) IL-6 release from cultured human and mouse islets exposed for 6 h to GIP or control (n=16 and n=20, 4 and 5 separate islet preparations done in quadruplicates, respectively).

(C) mRNA expression of IL-6, IL-6 receptor (IL-6R) and CD68 in FACS-sorted human β-cell- and α-cell-enriched fractions exposed for 6 h to GIP or control (n=11-14, 4-5 separate islet preparations done in duplicates, triplicates or quadruplicates).

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(D) IL-6 release from FACS-sorted human β-cell- and α-cell-enriched fractions (n=11-14, 4-5 separate islet preparations done in duplicates, triplicates or quadruplicates).

(E) IL-6 release from FACS-sorted human β-cell- and α-cell-enriched fractions exposed for 6 h to GIP or control (n=11-14, 4-5 separate islet preparations done in duplicates, triplicates or quadruplicates).

(F) Immunocytochemistry of IL-6 in red, glucagon in green and DAPI in blue in dispersed mouse islet cells following exposure to GIP or IL-1β or control for 1 h (representative images out of 3 independent experiments). The scale bar indicates 322 nm.

Bar graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 and tP < 0.05 determined by ONE way ANOVA and Bonferroni correction (A, C) or by unpaired t-test (B, E, F).

Figure 3. GIP-induced GLP-1, glucagon and insulin secretion is IL-6 dependent in human and mouse islets

(A) GLP-1 and glucagon release from cultured mouse islets exposed for 6 h to GIP or control alone or with a neutralizing anti-mouse IL-6AB or isotype control antibody (IgG1) (n=16, 4 separate islet preparations done in quadruplicates).

(B) GLP-1 and glucagon release from cultured wild type (WT) and IL-6-deficient (IL-6KO) mouse islets exposed to GIP or control (left graph) and in cultured human islets exposed to GIP or control alone or with a neutralizing anti-human IL-6RAB (right graph) (left graph: n=12, 3 separate islet preparations done in quadruplicates, right graph: n=16, 4 separate islet isolations done in quadruplicates).

(C) Stimulatory index (ratio of high to low glucose incubation) of GLP-1, glucagon and insulin release from human islets incubated sequentially for 1 h at low (2.8 mmol/L) glucose alone followed by 1 h at high (16.7 mmol/L) glucose alone or in the presence of GIP or control with and without IL-6RAB (n=12-16, 3 separate islet isolations done in quadruplicates).

(D) Stimulatory index of GLP-1, glucagon and insulin release from mouse islets incubated sequentially for 1 h at low (2.8 mmol/L) glucose alone followed by 1 h at high (16.7 mmol/L) glucose alone or in the presence of GIP or control with and without IL-6AB (n=20, 5 separate islet isolations done in quadruplicates).
Sequentially for 1 h at low (2.8 mmol/L) glucose alone followed by 1 h at high (16.7 mmol/L) glucose alone or in the presence of GIP or control (n=11, 3 separate islet isolations done in triplicates or quadruplicates).

Bar graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.001 determined by ONE way ANOVA and Bonferroni correction.

Figure 4. The adenylyl cyclase/cAMP/PKA pathway and SGLT2 are involved in GIP-induced IL-6 and GLP-1 secretion from pancreatic islets

(A) IL-6 and GLP-1 release from cultured mouse islets exposed for 24 h to forskolin or control or to GIP or control alone or with H-89 (n=10, 3 separate islet preparations done in triplicates or quadruplicate).

(B) Slc5a1 and Slc5a2 mRNA expression in cultured human islets exposed for 24 h to GIP or IL-6 or control (n=10-12, 3 separate islet preparations done in triplicates or quadruplicates).

(C) IL-6 and GLP-1 release from cultured mouse islets exposed for 24 h to dapagliflozin or GIP or control (n=6-10, 3 separate islet preparations done in triplicates or quadruplicate).

Bar graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.001 determined by unpaired t-test (A and C two left graphs) or by ONE way ANOVA and Bonferroni correction (A and C right graph; B).

Figure 5. GIP effects on glucose metabolism are IL-6 dependent

(A) Plasma glucose, GLP-1 and insulin levels in C57BL/6N mice upon a single bolus of GIP (7 or 70 pmol/g) or saline with glucose i.p. (n=9, 2 independent cohorts) and plasma IL-6 levels in C57BL/6N mice upon a bolus of GIP (70 pmol/g) or saline with glucose i.p. (n=10, 2 independent cohorts).

(B) Plasma glucose, GLP-1 and insulin levels in C57BL/6N mice upon a bolus of GIP or saline i.p. (n=9-11, 1 cohort)

(C) Plasma glucose, GLP-1 and insulin levels in C57BL/6N mice upon a bolus of GIP or saline with glucose i.p. (IPGTT) or oral (OGTT) (n=11-12, 1 cohort, cross-over design).

(D) Plasma glucose, GLP-1 and insulin levels in IL-6 deficient (IL-6KO) and wild-type (WT) control mice upon a bolus of GIP or saline with glucose i.p. (n=19-22, 2 independent cohorts).
(E) Plasma glucose, GLP-1 and insulin levels in IL-6KO mice that received a bolus of IL-6 (400 ng/mouse, i.p.) 30 min before a single bolus of GIP or saline with glucose i.p. (n=9-11, 2 independent cohorts).

(F) Plasma glucose, GLP-1 and insulin levels in C57BL/6N mice that received IL-6AB (500 µg/mouse, i.p.) 6 h before a bolus of GIP or saline with glucose i.p. (n=11-17, 3 independent cohorts).

Graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 determined by ONE way ANOVA and Bonferroni correction comparing GIP to saline, IL-6+GIP to saline, IL-6AB+GIP to saline, respectively and *P < 0.05, **P < 0.01, ***P < 0.001 comparing WT/GIP to IL-6KO/GIP, and IL-6AB+GIP to GIP, respectively.

**Figure 6. GLP-1 effects on glucose control are preserved and even enhanced in IL-6-deficient mice**

(A) Plasma glucose and insulin levels in in WT and IL-6KO mice upon a bolus of GLP-1 or saline with glucose i.p. (n=3-4, 1 cohort).

(B) Plasma glucose and insulin levels in C57BL/6N mice upon a bolus of GIP, GLP-1 or saline with glucose i.p. (n=6-7, 1 cohort).

(C) Plasma glucose and insulin levels in IL-6KO mice upon a bolus of GIP or GLP-1 or saline with glucose i.p. (n=7-9, 1 cohort).

Graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 determined by ONE way ANOVA and Bonferroni correction comparing GIP to saline, GLP-1 to saline, GLP-1 to saline, respectively and *P < 0.05, **P < 0.01, ***P < 0.001 comparing WT/GLP-1 to IL-6KO/GLP-1, and IL-6KO/GLP-1 to IL-6KO/GIP, respectively.

**Figure 7: GIP effects in murine models of diabetes, diet-induced obesity and cytokine-induced glucose intolerance**

(A) Plasma glucose, GLP-1, insulin and IL-6 levels in diabetic db/db and db/+ control mice upon a bolus of GIP or saline with glucose i.p. (n=12, 1 cohort).

(B) Plasma glucose, GLP-1 and insulin levels in diabetic db/db mice that received a IL-6 (400 ng/mouse, i.p.) injection 30 min before a bolus of GIP or saline with glucose i.p. (n=5-6, 1 cohort).
(C) Plasma glucose, GLP-1 and insulin levels in high-fat diet (HFD)-fed and chow diet (CD)-fed control mice upon a bolus of GIP or saline with glucose i.p. (n=9-10, 1 cohort).

(D) Plasma glucose, GLP-1 and insulin levels in C57BL/6N mice, that received daily injections with IL-1β (5 µg/kg, i.p.) or saline for 14 d before a bolus of GIP or saline with glucose i.p. (n=5, 1 cohort).

(E) mRNA expression of IL-1β and IL-6 in isolated islets from diabetic db/db and db/+ control mice (left graph, n=4-5, 1 cohort), from mice that were for 29-32 weeks on high-fat or chow control diet (middle graph, n=12-13, 3 independent cohorts) and from mice after daily injections with IL-1β (5 µg/kg) or saline i.p. for 14 d (right graph, n=3-4, 1 cohort).

Bar graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 determined by ONE way ANOVA.

Graphs are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 determined by ONE way ANOVA and Bonferroni correction comparing GIP to saline, GIP/IL-6 to saline, HFD/saline to CD/Saline, HFD/GIP to HFD/saline, CD/GIP to CD/saline, GIP to PBS, chronic IL-1β to PBS, chronic IL-1β/GIP to PBS, chronic IL-1β/GIP to chronic IL-1β, respectively and 1P < 0.05, 2P < 0.01, 3P < 0.001 comparing GIP/IL-6 to GIP, HFD/GIP to CD/GIP, and chronic IL-1β/GIP to GIP, respectively.
Figure 1: GIP induces GLP-1 and glucagon in human and mouse islets

A) mRNA

B) Glucagon

C) Active GLP-1

D) mRNA

E) Glucagon

F) Active GLP-1

Human islets

Human alpha cell-enriched fraction

Sorted human islet-cell fractions

Mouse islets

Human islets

Sorted human islet-cell fractions
Figure 2: GIP induces IL-6 in human and mouse pancreatic α-cells

A. Human islets

B. Human islets

C. Mouse islets

D. Sorted human islet cell-fractions

E. Sorted human islet cell-fractions

F. Control

GIP 100nM 1h

IL-1β 1ng/mL 1h

Sorted human islet cell-fractions

6 h

0
1
2
3
4

IL-6 (pg/mL)

0
1
2
3
4

IL-6 (fold of control)

0
0.5
1.0
1.5
2.0
2.5

Control

GIP 100nM

DAPI

Glucagon

IL-6

DAPI Glucagon

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Figure 3: GIP-induced GLP-1, glucagon and insulin secretion is IL-6 dependent in human and mouse islets

A

Mouse islets

B

Mouse islets

Human islets

C

Human islets

D

Mouse islets

E

Mouse islets

**MANUSCRIPT ACCEPTED**
Figure 4: The adenylyl cyclase/cAMP/PKA pathway and SGLT2 are involved in GIP-induced IL-6 and GLP-1 secretion.
Figure 5: GIP effects on glucose metabolism are IL-6 dependent

(A) 
Bars  IL-6KO GLP-1 IPGTT 19.05.14+12.05.14+06.06.13
Glucose (mmol/L)
Insulin (pg/mL)
Active GLP-1 (pg/mL)

(B) 
Bars  IL-6KO GLP-1 IPGTT 19.05.14+12.05.14+06.06.13
Glucose (mmol/L)
Insulin (pg/mL)
Active GLP-1 (pg/mL)

(C) 
IPGTT
OGTT

(D) 
Bars  IL-6KO GLP-1 IPGTT 19.05.14+12.05.14+06.06.13
Glucose (mmol/L)
Insulin (pg/mL)
Active GLP-1 (pg/mL)

(E) 
Bars  IL-6KO GLP-1 IPGTT 19.05.14+12.05.14+06.06.13
Glucose (mmol/L)
Insulin (pg/mL)
Active GLP-1 (pg/mL)

(F) 
Bars  IL-6KO GLP-1 IPGTT 19.05.14+12.05.14+06.06.13
Glucose (mmol/L)
Insulin (pg/mL)
Active GLP-1 (pg/mL)
Figure 6: GLP-1 effects on glucose control are preserved and even enhanced in IL-6 deficient mice
Figure 7: GIP effects in murine models of diabetes, diet-induced obesity and cytokine-induced glucose intolerance.