Glucose-dependent insulinotropic polypeptide induces cytokine expression, lipolysis, and insulin resistance in human adipocytes

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Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone released from enteroendocrine K cells in response to oral ingestion of fat or glucose (34). GIP is best known for its stimulation of insulin release from pancreatic β-cells in a glucose-dependent manner (11), transducing its biological effects via G protein-coupled receptors (GPCR) (39). Depending on experimental conditions in vitro, GIP also influences lipid metabolism in rodent (64) and human adipocytes (61) via lipogenesis (36) and lipolysis (22, 25, 38). In vivo, treatment with GIP impairs insulin sensitivity in rodents (3, 37) and augments postprandial hyperglycemia in patients with type 2 diabetes (7). Conversely, ablation of GIP signaling protects against diet-induced obesity and insulin resistance (18, 19, 42). Circulating GIP levels are increased in obesity and type 2 diabetes in animal models and humans (17, 60). Taken together, this points to a possible “diabetogenic” effect of GIP that is supposed to be mediated by GIP effects on adipocytes. Chronic high-fat diet, obesity, and type 2 diabetes are associated not only with a disturbance in gut hormone regulation but also with a state of local and systemic low-grade inflammation (55). Thereby, adipose tissue has emerged as a major source of cytokines like interleukin (IL)-6, IL-1β, and tumor necrosis factor-α (TNF-α) (28, 58), implicated in lipolysis (31, 50) and insulin resistance (30, 53). Lipopolysaccharide (LPS), which is increased postprandially and after oral fat intake (1), is triggering this activation of the innate immune system (4). Adipose tissue is also an important source of IL-1 receptor antagonist (IL-1Ra), an endogenous inhibitor of IL-1 action, in obese rodents and humans (33). Serum levels of IL-1Ra are markedly increased in obesity (32, 40) and are considered as predictor of type 2 diabetes (6).

Although GIP is implicated in the etiology of diet-induced obesity and related insulin resistance (29), effects of GIP on human adipocytes in the context of inflammation have not been studied. Therefore, we investigated the effect of GIP in human preadipocyte-derived adipocytes in vitro on expression and secretion of cytokines as well as its impact on lipolysis and glucose uptake.

MATERIALS AND METHODS

Human adipocyte culture and treatment. Human adipocyte tissue samples from subcutaneous abdominal fat depots were obtained from nine patients (age 50.3 ± 18.0 yr, BMI 30.4 ± 8.3 kg/m²), 4 nonobese (3 nondiabetic, 1 diabetic), 5 obese (1 diabetic, 4 nondiabetic) undergoing elective visceral surgery for nonmalignant, noninflammatory reasons or bariatric surgery. The study was approved by the local ethics committee, and informed consent was obtained from every patient. Stromal vascular cells were isolated as previously described (16) and expanded in DMEM-F12 containing 10% fetal calf serum (FCS), 5 ng/ml 2-fibroblast growth factor (FGF) (all from Lonza, Verviers, Belgium), sodium pyruvate, and penicillin-streptomycin (both from Invitrogen, Basel, Switzerland) until confluence was reached. Differentiation into adipocytes was induced by incubating confluent cells in DMEM-F12 containing 3% FCS, 50 mg/ml gentamicin, and 30 mM HEPES (all from Invitrogen) and supplemented as follows: 250 μM 3-isobutyl-1-methylxanthine (IBMX), 1 μM dexamethasone, 0.2 nM 3,5,3′-triiodo-L-thyrione, 100 mM l-ascorbic acid, 8 μg/ml insulin, 15 mM β-mercaptoethanol (all from Sigma-Aldrich, Buchs, Switzerland), 5 μM transferrin (Merck, Dülmen, Germany), 100 nM insulin (Novo Nordisk, Köthen, Switzerland), and 1 μM rosiglitazone (GlaxoSmithKline, Worthing, UK).
State of differentiation was evaluated by accumulation of lipid droplets using an inverted microscope (Zeiss, Feldbach, Switzerland). After 14 days of differentiation, cells were washed twice with phosphate-buffered saline (PBS) and incubated in 5 mM glucose-DMEM-phosphate-buffered saline (PBS) and incubated in 5 mM glucose-DMEM.

Fig. 1. Treatment with glucose-dependent insulinotropic polypeptide (GIP) induces mRNA expression of IL-6, IL-1β, and IL-1 receptor antagonist (IL-1Ra) in a dose- and time-dependent manner. A: for dose-response experiments, differentiated human preadipocyte-derived adipocytes were treated with GIP (10 pM–100 nM) for 1 h. B: for time course experiments, differentiated human preadipocyte-derived adipocytes were treated with GIP (1 nM) for indicated periods of time. For all experiments, total RNA was isolated from each sample, and mRNA expressions of IL-6, IL-1β, and IL-1Ra were determined by real-time PCR and normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) expression levels. Results are expressed in arbitrary units (AU) with the control value taken as 1 and are means ± SE of 3–6 independent experiments, each carried out in triplicate. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

RNA isolation and quantitative analysis of mRNA expression of preadipocyte-derived adipocytes. RNA was isolated using TRIzol (Sigma-Aldrich) following the manufacturer’s protocol. Total RNA (1 μg) was subjected to reverse transcription-PCR, and cDNA was subjected to quantitative real-time PCR analysis as previously described (51). Optimal sets of primers yielding short PCR products suitable for SYBR-Green detection were designed using the Probe Finder (Roche Applied Bioscience) website (http://qpcr2.probefinder.com). The oligonucleotide primers used were as follows (forward and reverse, respectively): 5′-TCT CTA GAA CGA ATT GAC AAA CAA A-3′ and 5′-GCT GCT TTC ACA CAT GTT ACT CTT G-3′ (IL-6), 5′-AGC TGA TGG CCC TAA ACA GA-3′ and 5′-CACT CGT CTT TTC ACA CAT GTT ACT CTT G-3′ (IL-1β), 5′-TGG TCA GGG TTT TAC TAC CTA GAG ATT CGT CAG ACG TGG AT-3′ (IL-1Ra), 5′-TCC GAG GAA TGG TCA GGG TTT TAC TAC CTA GAG ATT CGT CAG ACG TGG AT-3′ (IL-1β), 5′-TGG TCA GGG TTT TAC TAC CTA GAG ATT CGT CAG ACG TGG AT-3′ (IL-1Ra), 5′-CTC AAC TGG ACA TCA CAA A-3′ and 5′-AGG TCC TAG TGT TCC CTT CTC-3′ (IL-6).

Fig. 2. Classical inflammatory and lipolytic pathways are involved in GIP-mediated cytokine mRNA expression. Differentiated human preadipocyte-derived adipocytes were treated with or without PKA inhibitor H-89 (20 μM; A), IKKβ inhibitor sc-514 (100 μM; B), lipase inhibitor orlistat (100 μM; C), or IL-1Ra (1 μg/ml; D) 1 h prior to and during stimulation with GIP (1 nM, 1 h). A: PKA phosphorylation (p) status of (Ser/Thr)PKA substrate (F) upon treatment with GIP (1 nM) for 1 h with and without pretreatment of H-89 (20 μM) or sc-514 (100 μM) for 1 h. Quantitative analysis of IκBα protein and pSer/Thr)PKA substrate levels are given as fold of basal and were corrected for potential loading variations using β-actin. Data (G and H) are means ± SE of 3–4 independent experiments each carried out in duplicate. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control; †P < 0.001, ‡P < 0.01, ††P < 0.001 vs. GIP (1 nM, 1 h).
EFFECTS OF GIP ON HUMAN ADIPOCYTES

A

IL-1β mRNA expression (Fold of control)

IL-6 mRNA expression (Fold of control)

IL-1Ra mRNA expression (Fold of control)

B

Sc-514 GIP 1 nM

H-89 GIP 1 nM

C

Sc-514 GIP 1 nM

Orlistat GIP 1 nM

D

Sc-514 GIP 1 nM

H-89 GIP 1 nM

E

IB : IκBα

IB : β-Actin

F

IB : p(Ser/Thr)PKA substrate

IB : β-Actin

GIP 1 nM H-89 Sc-514

GIP 1 nM H-89 Sc-514

GIP 1 nM H-89 Sc-514

GIP 1 nM H-89 Sc-514

GIP 1 nM H-89 Sc-514

GIP 1 nM H-89 Sc-514

GIP 1 nM H-89 Sc-514
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Fig. 3. GIP potentiates LPS-, IL-1β-, and TNFα-induced IL-6 and IL-1Ra secretion. To elicit the effect of GIP in an inflammatory background, differentiated human preadipocyte-derived adipocytes were serum starved for 2 h and incubated with LPS (100 ng/ml; A and D), IL-1β (20 ng/ml; B and E), and TNFα (10 ng/ml; C and F) with or without GIP (1 nM, 100 nM) for 24 h. Protein concentrations of IL-6 (A–C) and IL-1Ra (D–F) were measured in cell culture supernatants. Protein levels are expressed in AU with the control value taken as 1 and are means ± SE of 3–4 independent experiments, each carried out in duplicate or triplicate. C and F insets: induction of protein secretion levels of IL-6 (inset C) and IL-1Ra (inset F) upon 1 and 100 nM GIP treatment expressed in arbitrary units, with protein secretion levels induced by TNFα alone taken as 1 and are means ± SE of 3 independent experiments, each carried out in triplicate; n.s., not significant. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

GTA TG T-3’ and 5’-TGC TCG TCT CAC CAC AAA C-3’ (GLUT1), 5’-CCC CCT CAG CAG CGA GTG A-3’ and 5’-GCA CCG CCA GGA CAT TGT TG-3’ (GLUT4), 5’-TCA GGC AGT ATA ATC CAA AGA TGG T-3’; 5’-AGT CGT GCT TAT ATC CAA CAC TTC-3’ hypoxanthine-guanine phosphoribosyltransferase (HPRT). Each cDNA sample tested for quantitative gene-expression analysis was subjected to HPRT mRNA analysis. Results are expressed as the ratio of the respective gene of interest mRNA and HPRT mRNA threshold values. The reaction volume was 25 μl, and the conditions were set as suggested by the manufacturer.

Fig. 4. GIP-induced lipolysis involves the classical lipolytic PKA/HSL pathway. Differentiated human preadipocyte-derived adipocytes were serum starved in 5 mM glucose-DMEM-F12 for 2 h and treated in the same medium under serum-free, albumin-free conditions with 1 nM GIP for up to 24 h (A), with and without forskolin (20 μM) for 6 h (B), and with and without preincubation with 20 μM H-89 (C) and 1 μM KT5720 (D) or 100 μM orlistat (E) for 1 h and then treated with 1 nM GIP for 6 h. Glycerol release was measured in cell culture medium as an index of lipolysis. Data are expressed in AU with the control value taken as 1 and are means ± SE of 2–3 independent experiments, each carried out in triplicate. Immunoblots showing phosphorylation status of Ser552 and protein content of HSL upon treatment with GIP (1 nM) and forskolin (20 μM) for 6 h. F: quantitative analysis of p(Ser552)HSL protein levels given as fold of basal and corrected for potential loading variations using HSL. Immunoblots showing phosphorylation status of Ser552 and protein content of HSL upon treatment with 1 nM GIP for 6 h with and without pretreatment of 100 μM orlistat. G: quantitative analysis of p(Ser552)HSL protein levels given as fold of basal and corrected for potential loading variations using HSL. Data in F are means ± SE of 3, data in G are means of 2 independent experiments, each carried out in duplicate. Differentiated human adipocytes were serum starved for 1 h in Krebs-Ringer buffer (KRB) with 0.1% fatty acid-free BSA and 2 mM glucose before treatment in the same KRB with 1 nM GIP and/or 20 μM forskolin for up to 6 h (H–M). Glycerol (H–J), and FFA (K–M) content was measured in the supernatant after indicated time points. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control; 1P < 0.05; **P < 0.01 vs. GIP (1 nM, 6 h).

Western blot analysis. Cells were harvested in a 20 mM Tris-HCl (pH 7.5) lysis buffer containing 1% Triton X-100, 150 mM NaCl, 10% glycerol, protease inhibitor cocktail, Na3VO4, NaF, PMSF, and EDTA and incubated on ice for 10 min. Cell lysates were cleared by centrifugation (10,000 g, 10 min), sample buffer as well as reducing agent (NuPAGE, Invitrogen) were added, and lysates were heated up to 70°C for 10 min and centrifuged afterward. Protein concentrations of lysates were determined with a BCA protein assay kit from Thermo Fisher Scientific (Lausanne, Switzerland). Lysates containing equal amounts of proteins were subjected to 4–12% NuPAGE Bis-Tris gels.
RESULTS

GIP induces cytokine gene expression in differentiated human preadipocyte-derived adipocytes in a dose- and time-dependent fashion. Treatment of differentiated preadipocyte-derived adipocytes with 1 nM GIP for 1 h increased IL-6, IL-1β, and IL-1Ra mRNA expression 4.2 ± 0.3-, 1.6 ± 0.1-, and 7.2 ± 1.1-fold, respectively (Fig. 1A). While mRNA expression of IL-6 and IL-1β was already maximally induced at 1 nM GIP, IL-1Ra mRNA expression was induced maximally up to 23.5 ± 3.9-fold at 100 nM GIP after 1 h (Fig. 1A). In contrast, mRNA expressions of TNFα, IL-8, and MCP-1 were not altered (data not shown). On the basis of the dose-response studies, we used 1 nM GIP for time course experiments. IL-6 mRNA expression was induced 2.6 ± 0.5-fold at 1 h and decreased to basal level thereafter (Fig. 1B), whereas IL-1β and IL-1Ra were maximally increased after 4 h (Fig. 1B). Since serum components like antibodies might nonspecifically activate GPCRs (20), additional serum-free experiments were performed to ensure specificity of the GIP effect. Serum-free conditions revealed an even more pronounced effect of GIP, inducing IL-6 mRNA 7.7 ± 1.0-fold (P < 0.001 vs. control) at 1 h, IL-1β mRNA to a maximum of 2.5 ± 0.2-fold (P < 0.001 vs. control) at 6 h, and IL-1Ra mRNA 12.5 ± 1.7-fold (P < 0.001 vs. control) at 6 h (data not shown).

To elaborate the specificity of the GIP effect, cells were preincubated with the competitive GIP-R antagonist GIP(6–30) (100 nM-10 µM) 15 min prior to GIP stimulation. Pretreatment with GIP(6–30) inhibited GIP-induced IL-6, IL-1β, and IL-1Ra mRNA expression (data not shown). In undifferentiated preadipocytes, the lack of GIP-R expression (57) was mirrored by the absence of a GIP effect on IL-6, IL-1β, or IL-1Ra gene expression (data not shown).

Although differentiated preadipocyte-derived adipocytes were cultured from donors differing in phenotype, we observed no differences in cell shape or responsiveness to GIP or insulin treatment in preadipocyte-derived adipocytes from obese vs. lean and diabetic vs. nondiabetic patients.

PKA, IKKβ/NF-κB, and IL-1R activation are involved in GIP-induced cytokine expression. Next, we investigated the underlying mechanisms of GIP-induced cytokine expression. Pretreatment with the PKA inhibitor H-89 reduced GIP-stimulated expression of IL-6 and of IL-1β to basal levels and IL-1Ra mRNA expression by 74% (Fig. 2A). Similar results were found using a different PKA inhibitor, KT5720 (data not shown). Preincubation with the IKKβ inhibitor sc-514 reduced GIP-induced IL-6 gene expression by 61% and IL-1Ra gene expression by 61%, whereas IL-1β mRNA expression was not significantly altered (Fig. 2B). Similar results were obtained using another IKKβ inhibitor, IMD0354 (data not shown). Preincubation with IL-1Ra reduced GIP-induced IL-6 mRNA expression by 68%, whereas IL-1β and IL-1Ra were not altered (Fig. 2C). Interestingly, preincubation with the lipase inhibitor olisstat reduced GIP-induced mRNA expression of IL-6 by 78%, IL-1β to basal level, and IL-1Ra by 80% (Fig. 2D). Indicative for NF-κB pathway activation by GIP, we found that GIP reduced IkBα protein expression by 20% (Fig. 2E), which was prevented by pretreatment with sc-514 (Fig. 2E) but not by preincubation with H-89 (Fig. 2F). Investigating a possible link between the PKA and the NF-κB pathway, we also measured phosphorylation status of (Ser/Thr) PKA sub-
strate. p(Ser/Thr) PKA substrate was induced 1.4 ± 0.08-fold by GIP, which was prevented by preincubation with H-89 or sc-514 (Fig. 2F).

**GIP potentiates LPS-, IL-1β-, and TNFα-induced IL-6 protein secretion.** In contrast to the effect of GIP at mRNA level, treatment with GIP alone for up to 24 h failed to induce IL-6 or IL-1Ra protein release (data not shown). GIP at 1 nM and 100 nM potentiated IL-6 protein secretion 1.7- and 2.3-fold in the presence of LPS, 1.8- and 1.8-fold in the presence of IL-1β, and 1.4- and 2.3-fold, respectively, when coincubated with TNFα (Fig. 3, A–C). IL-1Ra protein secretion was induced 6.6 ± 1.0-fold by LPS alone and 7.8 ± 1.5- and 9.2 ± 2.1-fold in the presence of 1 nM and 100 nM GIP, respectively (Fig. 3D). IL-1Ra was induced 4.0 ± 0.6-fold by IL-1β alone and 6.6 ± 1.3- and 6.5 ± 1.5-fold when coincubated with 1 nM and 100 nM GIP, respectively (Fig. 3E). TNFα alone induced IL-1Ra secretion 80 ± 30.2- and 82 ± 31.0-fold, respectively (Fig. 3F). The amount of IL-6 and IL-1Ra protein secretion induced by TNFα alone differed greatly between

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**Fig. 5. GIP-induced lipolysis additionally involves IKKβ/NF-κB and MAPK modules as well as IL-1 receptor activation.** Differentiated human preadipocyte-derived adipocytes were serum starved for 2 h and preincubated with 100 μM sc-514 (A), 1 μM IMD0354 (B), 1 μg/ml IL-1Ra (C), 10 μM U-0126 (D), 500 nM SP-600125 (E), or 10 μM SB-203580 (F) for 1 h and then treated with 1 nM GIP for 6 h. Glycerol release was measured in cell culture medium as an index of lipolysis. Data are expressed in AU with the control value taken as 1 and are means ± SE of 2–3 independent experiments, each carried out in triplicate.

**G:** immunoblots showing phosphorylation status of Ser552 and protein content of β-actin upon treatment with GIP (1 nM) for 1 h with or without preincubation with H-89 (10 μM) or sc-514 (100 μM) for 1 h. Data are means ± SE of 3 independent experiments, each carried out in duplicate. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control; tP < 0.05; *P < 0.01; tttP < 0.001 vs. GIP (1 nM, 6 h or 1 h as indicated).
First, we investigated the effect of GIP on lipolysis under serum- and albumin-free conditions. GIP induced glycerol release 1.3 ± 0.2-fold (Fig. 4A) already after 1 h. The maximum stimulatory effect was seen after 8 h with a 1.6 ± 0.4-fold increase. Thereby, the prolipolytic effect of GIP was comparable to the effect of forskolin, a strong inducer of lipolysis, after a stimulatory period of 6 h (Fig. 4B). Coincubation with GIP and forskolin together did not result in an augmented glycerol release compared with forskolin alone (Fig. 4B). Pretreatment with the PKA inhibitors H-89 and KT5720 completely abolished GIP-induced glycerol release (Fig. 4C and D), whereas basal glycerol levels were not significantly affected. The involvement of lipase was investigated using the lipase inhibitor orlistat. GIP-induced glycerol release was prevented by pretreatment with orlistat (Fig. 4E).

We did not detect any FFA release upon GIP or forskolin under albumin-free conditions (data not shown). In addition, we investigated underlying mechanisms of GIP-induced lipolysis. GIP induced phosphorylation of HSL at Ser552 1.7 ± 0.1-fold. Treating cells at the same conditions with forskolin revealed a 2.0 ± 0.2-fold induction of phosphorylation of Ser552 HSL (Fig. 4F). GIP-induced phosphorylation of Ser552 HSL was prevented by orlistat (Fig. 4G).

Performing experiments in the presence of 0.1% fatty acid-free BSA revealed a more potent induction of lipolysis upon GIP and forskolin with a maximal 3.9 ± 0.48-fold increase in glycerol secretion upon forskolin and a 2.4 ± 0.38-fold increase upon GIP treatment after 3 h (Fig. 4I). Thereby, GIP slightly reduced forskolin-induced glycerol release after 1 h and 3 h, although statistical significance was not reached (Fig. 4, H and I). However, in the presence of BSA, forskolin stimulated FFA release 2.8 ± 0.52-fold after 3 h (Fig. 4L) and 4.7 ± 0.81-fold after 6 h (Fig. 4M), whereas no FFA release was detected upon GIP treatment at any time point investigated.

**IKKβ/NF-κB and IL-1R activation, JNK, and the MAPK1/2 module are also involved in GIP-induced lipolysis.** Pretreatment with the IKKβ inhibitors sc-514 and IMD0354 significantly reduced GIP-induced glycerol release (Fig. 5, A and B). Furthermore, IL-1Ra was able to block lipolysis induced by GIP (Fig. 5C). The MAPK1/2 inhibitor U-0126 and the JNK inhibitor SP-600125 inhibited GIP-induced glycerol release (Fig. 5, D and E), whereas pretreatment with the p38 MAPK inhibitor SB-203580 did not result in a significant reduction of GIP-induced lipolysis (Fig. 5F). The involvement of PKA as well as IKKβ/NF-κB in GIP-induced lipolysis was further demonstrated by the ability of H-89 and sc-514 to inhibit GIP-induced phosphorylation of Ser552 HSL (Fig. 5G).

**Long-term treatment with GIP impairs insulin-induced glucose uptake possibly via impaired GLUT4 translocation.** Treatment of adipocytes with 1 nM GIP alone slightly increased glucose uptake 1.1 ± 0.03- and 1.2 ± 0.06-fold after 6 and 24 h, respectively (Fig. 6A). Coincubation with GIP for 20 min did not increase insulin-induced glucose uptake at insulin concentrations ranging from 1 nM to 100 nM (Fig. 6B). However, prolonged pretreatment with GIP for 6 h dose-dependently reduced insulin-stimulated glucose uptake by 67% at 1 nM GIP and by 74% at 100 nM GIP (Fig. 6C). Time-response experiments with 1 nM GIP revealed a reduction of insulin-induced glucose uptake by 64% after 2 h, by 77% after 4 h, and by 59% after 6 h (Fig. 6D). Pretreatment with the GIP-R antagonist GIP(6–30) 15 min prior to stimulation with 1 nM GIP for 6 h completely prevented GIP-induced insulin resistance, indicating specificity (Fig. 6E). Neither pretreatment with the PKA inhibitor H-89, the IKKβ inhibitor sc-514, the IL-1 receptor antagonist IL-1Ra nor a blocking anti-IL-6 receptor antibody or orlistat was effective to protect from GIP-induced insulin resistance (data not shown). Investigating intracellular mechanisms of insulin signaling, we found no significant reduction of pAkt (Ser473) or protein content of Akt either after 6 h GIP treatment (Fig. 6F) or after 24 h (data not shown). Treatment with GIP for 6 h reduced IRS-1 mRNA level by 52% (Fig. 6G) but only slightly decreased protein expression of IRS-1 (Fig. 6H).

To further unravel the mechanism underlying GIP-induced insulin resistance, we studied the GIP effect on GLUT1 and GLUT4. GIP treatment slightly increased GLUT1 mRNA expression 1.5 ± 0.18-fold after 6 h (Fig. 6G), whereas GLUT4 mRNA expression tended to be reduced over time (Fig. 6G). Further investigating GLUT4 translocation to the PM revealed an impairment of insulin-induced GLUT4 translocation upon 1 nM GIP treatment for 6 h (Fig. 6I). Interest-
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**A**

**Glucose uptake (Fold of control)**

- GIP 1 nM (hrs) - 6 24

**B**

**Glucose uptake (Fold of control)**

- GIP 1 nM - + - +
- **Log10[Ins] (M) -9 -8 -7**

- Insulin 100 nM + + + +

**C**

**Glucose uptake (Fold of control)**

- GIP 1 nM - + - +
- **Log10[GIP] (M) -11 -10 -9 -8 -7**

**D**

**Glucose uptake (Fold of control)**

- GIP 1 nM (hrs) - - 1 2 4 6

**E**

**Glucose uptake (Fold of control)**

- GIP 1 nM - + - +
- **Insulin 100 nM + + + +**

**F**

**IB : p(Ser473)Akt**

**IB : Akt**

**G**

**IRS-1 mRNA expression (Fold of control)**

- GIP 1 nM (6h) - - + +

**H**

**IRS-1 protein level (Fold of control)**

- GIP 1 nM (hrs) - 6

**I**

**GLUT-4 mRNA expression (Fold of control)**

- GIP 1 nM (6h) - - + +

**GLUT-4 protein level (Fold of control)**

- GIP 1 nM (6h) - - + +
ingly, GIP alone slightly induced GLUT4 translocation to the PM, although statistical significance was not reached (Fig. 6f).

DISCUSSION

Ablation of GIP receptor activation protects against diet-induced obesity and insulin resistance (19, 42), pointing to an important role of GIP in adipose tissue. Investigating the effects of GIP in nonmodified preadipocyte-derived human adipocytes revealed an inflammatory and prolipolytic response along with a mild increase in basal glucose uptake but impaired insulin sensitivity after prolonged exposure.

The pattern of cytokines induced was characterized by increased expression of IL-1β, IL-6, and IL-1Ra mRNA, while expression of TNFα, IL-8, and MCP-1 mRNA remained unaffected. In our study, GIP induced IL-6 mRNA expression showed a short lasting peak at 1 h, which is in line with a recent report demonstrating GIP-induced cytokine expression in a GIP receptor-overexpressing 3T3-cell line (47). In contrast to our findings, Nie Y et al. (47) observed the induction of TNFα and MCP-1 along with IL-6 in GIP receptor-overexpressing 3T3 cells. The differences in the cytokine expression pattern are most probably attributable to the different cell models used. Although GIP induced cytokine mRNA expression in our cells, protein secretion of IL-6 and IL-1Ra upon GIP was observed only in the presence of IL-1β, TNFα, or LPS. Full activation of cytokine processing and release often requires two separate hits, most probably to prevent overwhelming inflammation (15, 54). IL-6 mRNA is unstable and degraded with a half-life of about 30 min (48). This can be overcome by IL-1 or LPS (43, 65), which stabilize IL-6 mRNA (9, 46, 62), leading to translation and protein secretion of IL-6. Similarly, the presence of IL-1β or IL-1α is needed for IL-1Ra secretion in human adipocytes (8, 32). The synergistic stimulation of IL-6 and IL-1Ra released by GIP in the presence of IL-1β, LPS, or TNFα is of physiological relevance, since, in the setting of obesity, levels of IL-1β and TNFα are locally elevated in adipose tissue, inducing low-grade inflammation (27, 28). Of note, LPS levels are elevated postprandially and chronically after high-fat diet (4, 14). In the settings of low-grade inflammation, GIP might further promote chronic inflammation in adipose tissue while in a noninflamed environment as in the fasting state, and in metabolically healthy individuals GIP might not exert any substantial inflammatory activity.

In addition, our data provide evidence that GIP is a regulator of IL-1Ra in human adipocytes. In line with this observation, elevated plasma levels of GIP and IL-1Ra have been observed in obesity (40, 60), which drop after intestinal bypass surgery (40, 52).

GIP activates cyclic AMP and its downstream signaling module PKA via GPCR (10, 38). In line with our previous finding that GIP increases cAMP in human adipocytes (57), inhibition of PKA abolished GIP-induced expression of IL-6, IL-1β, and IL-1Ra. In addition, GIP has been shown to activate the NF-κB pathway in a GIP receptor-overexpressing 3T3 cell line (47). Accordingly, in our human adipocytes, GIP reduced IkBα protein expression that was prevented by an IKKβ inhibitor as was GIP-induced cytokine expression. Both PKA inhibitors H-89 and KT5720 prevented GIP-induced cytokine mRNA expression. Using an antibody directed against p(Ser/Thr)PKA substrate, indicative of the activation of PKA, revealed an inhibitory effect of sc-514 on PKA substrate phosphorylation. Therefore, we conclude that GIP-induced PKA activation is partly dependent on NF-κB pathway activation in our experimental setting, indicative of a close interaction of the PKA and the NF-κB pathway.

Increased lipolysis is a feature of inflamed adipose tissue in obesity. Lipolysis is induced by cytokines like IL-1β, IL-6, and TNFα (31, 53), while FFAs emerging from lipolysis induce cytokine expression in adipocytes in a positive feedback loop. It has been demonstrated that, when released in parallel to glycerol upon lipolysis in adipocytes, FFAs leave the adipocyte to be then taken up again and used for reesterification (12). The induction of lipolysis by GIP in adipocytes has been previously reported (38). For the first time, we demonstrated that GIP-induced lipolysis involves phosphorylation of HSL at Ser552 in human adipocytes that is sensitive to orlistat. Furthermore, we showed that both H-89 and sc-514 inhibited GIP-induced lipolysis at the level of glycerol release as well as at the level of (Ser552)HSL phosphorylation, indicative of a close link between the PKA and the IKKβ/NF-κB pathways in GIP-induced lipolysis. The close interaction between lipolysis and inflammation was also demonstrated by the finding that GIP-induced cytokine expression was inhibited by orlistat. Investigating other cellular stress-activated kinases using specific inhibitors revealed that MAPK1/2 and JNK are also involved in lipolysis stimulated by GIP in human adipocytes. However, our results did not point to an involvement of p38 MAPK, since SB-203580 prevented neither GIP-induced glycerol release nor GIP-induced phosphorylation of (Ser552)HSL (data not shown). In line with the observations by Nie Y et al. (47), showing that the IKKβ/NF-κB module and stress kinases as well as the PKA pathway are partially involved in GIP-induced cytokine expression in 3T3 adipocytes, our results indicate the involvement of numerous signaling pathways in GIP-induced lipolysis. This might be explained by a prolipolytic effect of cytokines that are released upon GIP accompanying the raw GIP effect and leading to the activation of several different signaling pathways. The observation that GIP-induced lipolysis was partially reduced by IL-1Ra strengthens this hypothesis.

While the induction of glycerol release upon GIP was in line with previous reports (22, 38), the low lipolytic response upon forskolin in our cell system was most probably related to our experimental design lacking albumin supplementation (2, 21). Performing our experiments in the presence of albumin revealed a higher prolipolytic response upon forskolin and GIP. In line with previous reports (22), GIP slightly reduced forskolin-stimulated glycerol release, although significance was not reached. While forskolin induced FFA release, no FFA release was detected upon GIP treatment, indicating that lipolysis induced by GIP is accompanied by FFA reesterification, in line with previous observations (22). However, it might also be possible that the decrease in extracellular FFA levels in parallel to an increase in glycerol levels results from an increase in fatty acid oxidation (59). While GIP is inducing lipolysis and glycerol release in the absence of insulin, GIP-induced lipolysis is inhibited in the presence of insulin (38). This might explain why in vivo studies in humans, conducted in the presence of insulin, did not show a prolipolytic GIP effect (23).

The vicious cycle of lipolysis and cytokine expression contributes to insulin resistance in adipose tissue (31). While
IL-1β induces insulin resistance in adipocytes (30), the role of IL-6 is unclear. On the one hand, IL-6 is upregulated in adipose tissue in obesity and has been shown to induce insulin resistance (35); on the other hand, IL-6 is secreted from contracting skeletal muscle during exercise (49) and has been shown to improve whole body insulin sensitivity (5, 26) and to induce GLP-1 production (13). Since GIP induced the expression of the cytokines IL-6 and IL-1β as well as lipolysis, we investigated the effects of short- and long-term GIP treatment on insulin-induced glucose uptake. Although a short time exposure to GIP has been shown to augment insulin-stimulated glucose uptake in 3T3 adipocytes in vitro (56), chronic GIP treatment in vivo induces insulin resistance (3). In our experimental setting, GIP alone slightly induced glucose uptake in human adipocytes after 6 and 24 h albeit to a lower extent than previously described in murine adipocytes (56). Interestingly and in line with previous reports (44, 56), GIP alone tended to increase GLUT4 translocation. We found no additive effect of GIP on insulin-induced glucose uptake in the short term, in line with the results reported by Mohammad et al., demonstrating an augmenting effect of GIP on insulin-induced glucose uptake only at insulin concentrations up to 0.2 nM, whereas at insulin concentrations of 1 nM or higher, treatment with GIP did not augment the insulin effect (45). In addition, Mohammad et al. showed that GIP acts as an insulin sensitizer and has no significant effect on adipocyte glucose uptake in the absence of insulin in the short term. In line with observations in vivo (3, 7, 37) and in vitro (47), prolonged GIP treatment for up to 6 h inhibits insulin-induced glucose uptake. Regarding the underlying mechanisms of GIP-induced insulin desensitization, we detected a clear impairment of insulin-induced GLUT4 translocation to the PM upon long-term treatment with GIP, providing a reasonable mechanism for GIP-induced insulin resistance in human adipocytes. Accordingly, we found a tendency to decreased mRNA GLUT4 levels and a significant increase in GLUT1 upon long-term GIP treatment. Interestingly, GIP treatment for up to 6 h did not affect Akt phosphorylation at Ser473 in line with previous reports (47). Although GIP induced a downregulation of IRS-1 mRNA over time, IRS-1 protein levels were not significantly altered, indicating that IRS-1 is not essentially involved in GIP-induced insulin resistance.

A recent study in GIP-overexpressing mice reported reduced adipose tissue macrophage infiltration and expression of genes involved in inflammatory signaling (35). This is in apparent contrast to our data showing GIP-induced cytokine expression. However, GIP-overexpressing mice showed markedly reduced fat mass and body weight due to reduced energy intake (35). Therefore, it is difficult to assess whether reduced inflammation was due to a direct GIP effect or indirectly via body weight decrease in this study.

In summary, we report that GIP induces inflammation and lipolysis in human adipocytes and that the classical inflammatory and lipolytic signaling pathways are closely linked. Furthermore, we have found that GIP substantially potentiates the inflammatory effects of LPS, IL-1β, and TNFα in human adipocytes. Importantly, we have demonstrated that long-term GIP treatment impairs insulin-induced glucose uptake in human adipocytes, possibly via inhibition of GLUT4 translocation to the plasma membrane. These results demonstrate that, besides its known metabolic function, GIP is able to directly modulate inflammation and insulin sensitivity in human adipocytes. This may play a particular role in patients with type 2 diabetes, where GIP levels are increased, and, furthermore, adipocytes remain sensitive to GIP effects while the insulinotropic activity of GIP is blunted (41). The ability of GIP to stimulate cytokines in an already inflamed environment and to induce insulin resistance should be taken into consideration in the pharmacological approach of GIP analogs for treatment of patients with obesity and type 2 diabetes.

REFERENCES


AUTHOR CONTRIBUTIONS


DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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AUTHOR CONTRIBUTIONS


REFERENCES


AJP-Endocrinol Metab • doi:10.1152/ajpendo.00100.2012 • www.ajpendo.org
32. Mohammad S, Ramos LS, Buck J, Levin LR, Rubin F, McGraw TE. Gastric inhibitory peptide controls adipose insulin sensitivity via activa-


