Glucose-Dependent Insulinotropic Polypeptide (GIP) Induces Calcitonin Gene-Related Peptide (CGRP)-I and Procalcitonin (Pro-CT) Production in Human Adipocytes

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Context: Increased plasma levels of glucose-dependent insulinotropic polypeptide (GIP), calcitonin CT gene-related peptide (CGRP)-I, and procalcitonin (Pro-CT) are associated with obesity. Adipocytes express functional GIP receptors and the CT peptides Pro-CT and CGRP-I. However, a link between GIP and CT peptides has not been studied yet.

Objective: The objective of the study was the assessment of the GIP effect on the expression and secretion of CGRP-I and Pro-CT in human adipocytes, *CGRP-I* and *CT* gene expression in adipose tissue (AT) from obese *vs.* lean subjects, and plasma levels of CGRP-I and Pro-CT after a high-fat meal in obese patients.

Design and Participants: Human preadipocyte-derived adipocytes, differentiated *in vitro*, were treated with GIP. mRNA expression and protein secretion of CGRP-I and Pro-CT were measured. Human *CGRP-I* and *CT* mRNA expression in AT and CGRP-I and Pro-CT plasma concentrations were assessed.

Results: Treatment with 1 nM GIP induced *CGRP-I* mRNA expression 6.9 ± 1.0-fold ($P < 0.001$ *vs.* control) after 2 h and *CT* gene expression 14.0 ± 1.7-fold ($P < 0.001$ *vs.* control) after 6 h. GIP stimulated CGRP-I secretion 1.7 ± 0.2-fold ($P < 0.05$ *vs.* control) after 1 h. In AT samples of obese subjects, *CGRP-I* mRNA expression was higher in sc AT ($P < 0.05$ *vs.* lean subjects), whereas *CT* expression was higher in visceral AT ($P < 0.05$ *vs.* lean subjects). CGRP-I plasma levels increased after a high-fat meal in obese patients.

Conclusion: GIP induces *CGRP-I* and *CT* expression in human adipocytes. Therefore, elevated Pro-CT and CGRP-I levels in obesity might result from GIP-induced Pro-CT and CGRP-I release in AT and might be triggered by a high-fat diet. How these findings relate to the metabolic complications of obesity warrants further investigations. **(*J Clin Endocrinol Metab* 96: E297–E303, 2011)**

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone released from enteroendocrine K cells in response to oral nutrient ingestion, with fat being the main trigger (1). Obesity is associated with elevated fasting and postprandial GIP levels (2, 3). Ablation of GIP signaling protects against diet-induced obesity and insulin resistance in rodents (4–7). In addition to its role as an insulinotropic peptide (8), GIP acts on multiple target or-

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Abbreviations: AT, Adipose tissue; $[Ca^{2+}]_i$, intracellular calcium; CGRP, CT gene-related peptide; CT, calcitonin; GIP, glucose-dependent insulinotropic polypeptide; GIP-R, GIP receptor; GLP-1, glucagon-like peptide-1; GLUT, glucose transporter; HPRT, hypoxanthine-guanine phosphoribosyltransferase; LPS, lipopolysaccharide; PKA, protein kinase A; Pro-CT, procalcitonin.

gans via G protein-coupled receptors (9). Functional GIP receptors (GIP-Rs) are expressed by human adipocytes, using cAMP as second messenger (10). In rodent adipocytes, GIP directly influences fat metabolism and lipid accumulation (11). Therefore, GIP links diet-induced obesity and related metabolic dysfunctions.

Calcitonin (CT) peptides like CT gene-related peptide (CGRP)-I and procalcitonin (Pro-CT) are derived from the *CALC-I* gene by alternative splicing (12). They are expressed and released from adipose tissue (AT) during sepsis and systemic inflammation (13–15) and from adipocytes treated with inflammatory cytokines *in vitro* (14–16). Pro-CT is a widely used surrogate marker in bacterial infection and sepsis (17). In addition, Pro-CT is a marker of the chronic inflammatory activity of AT in women with the polycystic ovary syndrome (18), correlating with total fat mass, body fat distribution, and insulin resistance (18). Furthermore, a recent study reported an association of circulating Pro-CT concentrations with obesity and insulin resistance (19). Increased plasma levels of CGRP have been detected in obese *vs.* lean women in the fasting state (20) and after an oral high-fat load in lean subjects (20). In addition, CGRP induces insulin resistance in muscle and liver *in vitro* and *in vivo* (21–23), whereas other studies reported a direct effect of CGRP on pancreatic β-cells leading to impaired insulin secretion in humans (24), rats (25), and mice (26).

Because plasma levels of GIP (1) as well as Pro-CT (19) and CGRP (20) are elevated in the state of obesity and insulin resistance, we examined a potential link between GIP and the *CALC-I* gene derived peptides. We tested the hypothesis that GIP stimulates CGRP-I and Pro-CT expression and secretion in human adipocytes. We also evaluated *CGRP-I* and *CT* mRNA expression patterns in sc and visceral AT from obese *vs.* lean subjects and plasma levels of CGRP-I and Pro-CT after high-fat meal ingestion in morbidly obese patients.

Materials and Methods

Human AT sampling, adipocyte culture, and treatment

Human AT samples were obtained from patients undergoing elective surgery. All patients were operated for nonmalignant and noninflammatory diseases. The study was approved by the local ethical committee, and written informed consent was obtained from every patient. For AT mRNA expression studies, paired samples of sc and visceral AT were obtained from seven lean patients [three males and four females, aged 64.0 ± 15.0 yr; body mass index (BMI) 22.7 ± 2.0 kg/m^2; none of seven with diabetes mellitus type 2; C-reactive protein 11.8 ± 8.3 mg/liter] and 13 morbidly obese patients (four males and nine females, aged 41.8 ± 11.4 yr; BMI 43.2 ± 3.3 kg/m^2; three of 13 with diabetes mellitus type 2; C-reactive protein, 11.0 ± 6.1 mg/liter), respectively. Adipocyte-containing tissue was macroscopically dissected from nonadipose tissue and blood vessels and minced into very small pieces (5 μg). TRI reagent was added; the tissue was vortexed and needled with a syringe (1.2 × 40 mm). After centrifugation at 14,000 rpm for 10 min at 4 C, the fat layer was removed and the fluid phase was transferred into a fresh tube. mRNA was isolated as described below.

For *in vitro* cell culture experiments, the stromal vascular cell fraction was isolated from sc AT samples, cultured, and differentiated for 14 d as previously described (27, 28). After 14 d of differentiation, cells were washed twice with warm PBS and incubated in 5 mM glucose DMEM/F12 containing 3% fetal calf serum, supplemented with 50 μg/ml gentamycin, 15 nM HEPES (all from Invitrogen, Basel, Switzerland), 100 nM L-ascorbic acid, 8 μg/ml biotin, and 15 mM *d*-pantothenat (all from Sigma-Aldrich, Buchs, Switzerland) for 48 h. Thereafter the medium was renewed and adipocytes were exposed to human recombinant GIP (1–42) (Bachem AG, Bubendorf, Switzerland) for indicated time points (1–24 h) and concentrations (10 pM to 100 nM). Adipocytes were treated with human GIP-R antagonist GIP (6–30) (100 nM to 1 μM, preincubation 15 min; Bachem), the adenylyl cyclase activator forskolin (10 μM, 1 h; Merck Chemicals, Nottingham, UK), the protein kinase A (PKA) inhibitor H-89 (20 μM, preincubation 1 h; Merck, Dietikon, Switzerland), and the ionophor ionomycin (1 μM, preincubation 1 h; Sigma-Aldrich). Cells from passages 2 through 7 were used in all experiments.

RNA isolation and quantitative analysis of mRNA expression of preadipocyte-derived adipocytes and human AT samples

RNA was isolated, 1 μg total RNA was subjected to RT-PCR and cDNA was subjected to quantitative real-time PCR analysis as previously described (16). Optimal sets of primers yielding short PCR products suitable for SYBR-Green detection were designed using the Probe Finder (Roche Applied Bioscience; http://qpcr2.probefinder.com). The oligonucleotide primers used were: 5′-GTC ATT CCC TGG GTG ATC GT-3′, 5′-CCA CCA AAT GGC CTT GAC TT-3′ (*GIP-R*); 5′-CCC CCT CAG CAG CGA GTG A-3′, 5′-GCA CCG CCA GGA CAT TGT TG-3′ [glucose transporter (*GLUT*)-4]; 5′-TGC CCA TCC AAA AAG TCC A-3′, 5′-GAA GTC CAA ACC GGT GAC TTT CT-3′ (*leptin*); 5′-CCC AGA AGA GAG CCT GTG ACA-3′, 5′-CTT CAC CAC ACC CCC TGA TC-3′ (*CGRP-I*); 5′-GTG CAG ATG AAG GCC AGT GA-3′, 5′-TCA GAT TAC CAC ACC GCT TAG ATC-3′ (*CT*). Each cDNA sample tested for quantitative gene expression was also subjected to hypoxanthine-guanine phosphoribosyltransferase (*HPRT*)-mRNA analysis. Results are expressed as ratio of the respective gene of interest and *HPRT*-mRNA threshold values. Primers for *HPRT* were 5′-TCA GGC AGT ATA ATC CAA AGA TGG T-3′ and 5′-AGT CTG GCT TAT ATC CAA CAC TTC-3′. The conditions were set as suggested by the manufacturer.

cAMP measurement

Intracellular cAMP content was determined using the cAMP Glo assay kit (Promega, Dübendorf, Switzerland) with the following modifications to the conditions set by the manufacturer: cells were starved in serum free DMEM/F12 medium 24 h before treatment. After an incubation period, the supernatant was removed and the cells were harvested in cAMP-lysis buffer, pro-

vided by the manufacturer and scraped, and the lysate was subjected to a 96-well plate. A standard curve was performed with cAMP control and cAMP-lysis buffer both provided by the manufacturer.

Protein measurement in supernatants of preadipocyte-derived adipocytes

After treatment the supernatants were collected, centrifuged, and immediately stored at −70 C. Supernatants were either concentrated *in vacuo* at room temperature for 4 h (GIP treatment alone) or concentrated for 6 h (experiments with GIP and ionomycin). The CGRP-I and Pro-CT protein in cell supernatant was determined as previously described (16, 28).

Protein measurement in human plasma

EDTA plasma samples from a recently performed study (29) were kindly donated by the study investigators. Briefly, fasting EDTA plasma samples from 24 morbidly obese patients (four males and 20 females, aged 40.47 ± 10.3 yr; BMI 46.12 ± 6.5 kg/m^2) were obtained after an overnight fast of at least 10 h. After the fasting period, patients received a high-fat liquid test meal. We chose the time point of 180 min after meal ingestion for postprandial protein measurement. CGRP-I and Pro-CT protein were measured as described previously (16, 28).

Statistical analysis

Data were analyzed in triplicate using GraphPad Prism (Prism 4.0 for Windows, San Diego, CA) and expressed as means ± SEM unless indicated otherwise. For *in vitro* data, statistical significance was determined by ANOVA followed by Bonferroni's *post hoc* test for multiple comparison analysis or by the Mann-Whitney *U* test when only two groups were analyzed. For analysis of mRNA expression in human AT, we used the Mann-Whitney *U* test. Correlation analyses were performed by using Spearman rank correlation. For analysis of CGRP-I and Pro-CT protein concentrations in human plasma samples, we used the Wilcoxon signed rank test.

Results

Expression of functional GIP-R by differentiated human preadipocyte-derived adipocytes

GIP-R mRNA expression was induced 6846 ± 1255-fold at d 7 ($P < 0.01$ *vs.* preadipocytes) and 8196 ± 962.8-fold at d 14 ($P < 0.01$ *vs.* preadipocytes) during the differentiation process (Fig. 1A). This was paralleled by the induction of other adipocyte differentiation markers like *GLUT-4* and *leptin* (Fig. 1A). To further assess the functionality of the GIP-R in our human adipocytes, we measured GIP-induced cellular cAMP accumulation. Treatment of differentiated adipocytes with 1 and 100 nM GIP for 1 h in serum-free conditions produced a 7.1 ± 1.0-fold ($P < 0.01$ *vs.* control) and an 8.2 ± 1.4-fold ($P < 0.001$ *vs.* control) increase in cAMP accumulation, respectively (Fig. 1B). Treatment for 1 h with 10 μM forskolin served as a positive control and revealed a 9.8 ± 0.8-fold ($P <$ 0.001 *vs.* basal) induction of cAMP content (Fig. 1B). Preincubation with 1 μM of the GIP-R inhibitor GIP (6–30) reduced cAMP induction by GIP (1 and 100 nM) without reaching significance (Fig. 1B).

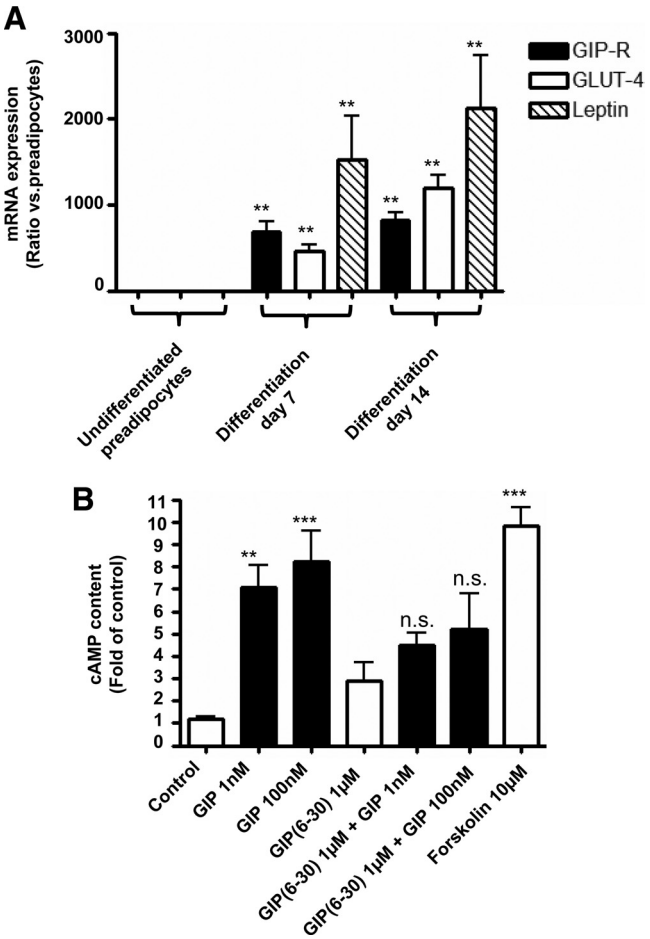

FIG. 1. Functional GIP-Rs are expressed by differentiated human preadipocyte-derived adipocytes. A, mRNA expression level of *GIP-R*, *GLUT-4*, and *leptin* in human preadipocyte-derived adipocytes were assessed by real-time PCR at d 0 (undifferentiated preadipocytes), d 7 and d 14 of differentiation. mRNA levels are normalized to *HPRT* expression levels. Results are expressed in arbitrary units with the preadipocyte values taken as 1 and are the means ± SEM of two independent experiments of two different donors each carried out in triplicate. **, $P < 0.01$ *vs.* preadipocyte expression level of the respective gene. B, Differentiated adipocytes were serum starved for 2 h and then treated for 1 h with GIP (1 nM, 100 nM) in the presence or absence of the GIP-R antagonist GIP (6–30) (1 μM, preincubation 15 min). Forskolin (10 μM) was added for 1 h and served as a positive control. Cellular cAMP content was determined using a luminescence assay. Results are expressed in arbitrary units with the control value taken as 1 and are the means ± SEM of three independent experiments all carried out in triplicate. **, $P < 0.01$; ***, $P < 0.001$ *vs.* control. n.s., Not significant *vs.* GIP 1 nM or GIP 100 nM.

GIP induced *CALC-I* gene expression in a dose- and time-dependent manner

Treatment of human preadipocyte-derived adipocytes with increasing concentrations of GIP from 1 pM to 100 nM for 1 h revealed a significant up-regulation of the *CALC-I*

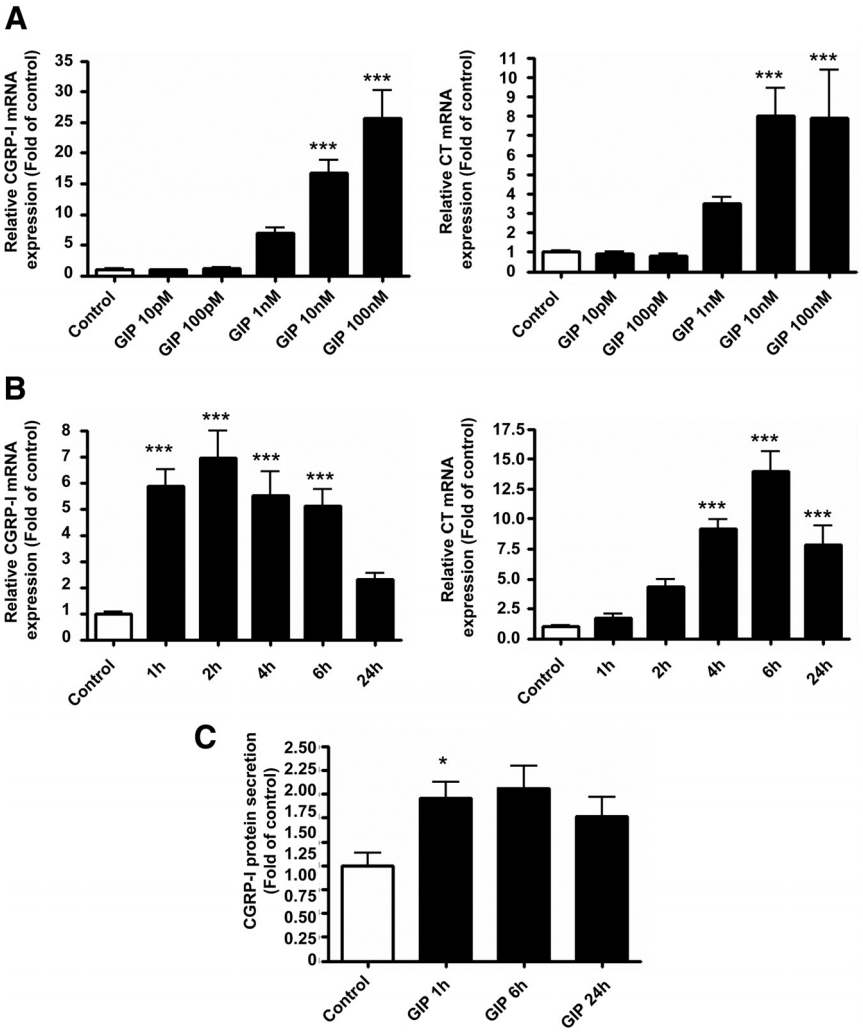

FIG. 2. GIP treatment induces *CGRP-I* and *CT* mRNA expression and CGRP protein release in human adipocytes. A, Differentiated human preadipocyte-derived adipocytes were treated with the indicated concentrations of GIP for 1 h. B, Differentiated human preadipocyte-derived adipocytes were treated with 1 nM GIP for the indicated period of time. mRNA expression was assessed by real-time PCR. mRNA level are shown as fold of control normalized to *HPRT* expression levels. Results are expressed in arbitrary units with the control value taken as 1 and are the means ± SEM of at least five independent experiments all carried out in triplicate. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ *vs.* control. C, Differentiated human adipocytes were treated with 1 nM GIP for 1 h (n = 3), 6 h (n = 2), or 24 h (n = 6). *CGRP-I* protein content in the cell supernatant was measured using a human-specific RIA kit. Results are expressed in arbitrary units with the control value taken as 1 and are the means ± SEM of indicated number (n) of experiments, carried out in duplicate. *, $P < 0.05$ *vs.* control.

gene products at 10 and 100 nM. *CGRP-I* mRNA expression was induced 16.7 ± 2.11-fold ($P < 0.001$ *vs.* control) at 10 nM GIP and 25.6 ± 4.7-fold ($P < 0.001$ *vs.* control) at 100 nM GIP (Fig. 2A). *CT* mRNA expression was maximally induced 8.0 ± 1.5-fold ($P < 0.001$ *vs.* control) at 10 nM GIP and 7.9 ± 2.4-fold ($P < 0.001$ *vs.* control) at 100 nM GIP (Fig. 2A). CGRP-II was induced in a similar pattern to CGRP-I, albeit to a much lower extent (data not shown). No significant change in mRNA expression levels was observed for other members of the *CALC* gene family, such as adrenomedullin or amylin (data not shown). Corresponding with barely detectable GIP-R expression in undifferentiated adipocytes, GIP treatment of preadipocytes did not result in a change in *CALC-I* gene expression levels (data not shown).

To assess the *CGRP-I* and *CT* mRNA expression pattern over time, differentiated adipocytes were treated with 1 nM GIP for 24 h. *CGRP-I* mRNA expression was induced 5.9 ± 0.7-fold ($P < 0.001$ *vs.* control) after 1 h, was maximally increased 6.9 ± 1.0-fold ($P < 0.001$ *vs.* control) after 2 h, and declined slowly thereafter to almost control level after 24 h (Fig. 2B). Up-regulation of *CT* mRNA expression was delayed compared with *CGRP-I* with a 9.2 ± 0.8-fold increase ($P < 0.001$ *vs.* control) after 4 h and a 14.0 ± 1.7-fold increase ($P < 0.001$) after 6 h. In contrast to *CGRP-I*, *CT* mRNA expression was still up-regulated by 7.9 ± 1.5-fold ($P < 0.01$ *vs.* control) after 24 h. Treatment with 1 nM GIP increased CGRP-I protein content in the medium by 1.7 ± 0.2-fold ($P < 0.05$ *vs.* control) already after 1 h (Fig. 2C), whereas Pro-CT secretion was not detectable after GIP treatment alone (data not shown).

GIP induced *CALC-I* gene expression was inhibited by the GIP-R inhibitor GIP (6–30)

The stimulatory effect of GIP was diminished or completely abolished by pretreatment with GIP (6–30). GIP-induced *CGRP-I* mRNA expression was reduced by 79% ($P < 0.01$ *vs.* GIP 1 nM, 1 h) at 1 μM GIP (6–30) and by 94% ($P < 0.001$ *vs.* GIP 1 nM, 1 h) at 10 μM GIP (6–30) (data not shown). GIP-stimulated *CT* mRNA expression was reduced by 96% ($P < 0.05$ *vs.* GIP 1 nM, 1 h) at 10 μM GIP (6–30) (data not shown).

The cAMP/PKA pathway and intracellular calcium ($[Ca^{2+}]_i$) are involved in GIP signal transduction

We next evaluated the signal transduction, which could be responsible for the observed effects of GIP. Addition of forskolin (10 μM) for 1 h potentiated the effect of GIP on *CGRP-I* and *CT* mRNA induction ($P < 0.001$ *vs.* GIP 1 nM, 1 h) and vice versa (data not shown), pointing to the crucial role of cAMP as second messenger. Inhibition of

PKA, the direct downstream signaling pathway module of cAMP, by H-89 (20 μM) efficiently blocked the GIP mediated stimulation of *CGRP-I* ($P < 0.001$ *vs.* GIP 1 nM, 1 h) and *CT* mRNA expression ($P < 0.01$ *vs.* GIP 1 nM, 1 h) (data not shown).

Because cAMP is often acting in synergism with $[Ca^{2+}]_i$ signaling, we tested the effect of ionomycin, a calcium-mobilizing agent. Treatment of differentiated adipocytes with ionomycin (1 μM, 6 h) potentiated the stimulatory effect of GIP on *CGRP-I* ($P < 0.001$ *vs.* GIP 1 nM, 6 h) and *CT* gene expression ($P < 0.01$ *vs.* GIP 1 nM, 6 h) (data not shown). Costimulation with GIP (1 nM) and ionomycin (1 μM) for 24 h induced secretion of CGRP-I 1.6 ± 0.1-fold ($P < 0.001$ *vs.* control) and Pro-CT 9.7 ± 1.4-fold ($P < 0.001$ *vs.* control) (data not shown).

CGRP-I and CT mRNA levels are altered in sc and visceral AT of obese subjects

Examining paired sc and visceral AT samples, we found increased *CGRP-I* mRNA expression in sc AT of morbidly obese patients ($P < 0.05$ *vs.* lean subjects), whereas *CGRP-I* gene expression in visceral AT was not significantly altered ($P = 0.2$ *vs.* lean subjects) (data not shown). In contrast, *CT* mRNA expression was not altered in sc AT ($P = 0.6$ *vs.* lean subjects) but was induced in visceral AT of morbidly obese patients (data not shown). Moreover, a positive correlation with BMI was found for *CGRP-I* mRNA expression ($n = 20$; $r = 0.54$; $P < 0.05$) and *CT* mRNA expression in visceral AT ($n = 20$; $r = 0.45$; $P < 0.05$). Correlation with BMI tended to be significant for *CGRP-I* mRNA expression in sc AT ($n = 20$; $r = 0.43$; $P = 0.06$) but not for sc *CT* mRNA expression.

Discussion

Our results demonstrate that GIP induces *CALC-I* gene expression and protein secretion of CGRP-I and Pro-CT in human adipocytes via GIP-R activation, involving the cAMP/PKA module and $[Ca^{2+}]_i$ signaling pathways. Furthermore, we found increased mRNA expression of *CGRP-I* and *CT* in sc and visceral AT samples of obese compared with lean control subjects. Additionally, CGRP-I protein content was increased and Pro-CT tended to be increased in plasma samples of obese patients after a high-fat meal ingestion.

In agreement with other human adipocyte models (10, 30), we found *GIP-R* mRNA to be barely expressed in undifferentiated primary human preadipocytes. Induction of *GIP-R* during differentiation was accompanied by expression of other adipocyte specific markers like *leptin*. Functional analysis of GIP-R in our differentiated adipocytes confirmed the results described for GIP-R in another human adipocyte model (10). However, and in contrast to Weaver *et al.* (10), who reported a peak of *GIP-R* mRNA expression at d 6–8 and a decreased expression to 50% at d 14 of differentiation in Simpson-Golabi-Behmel syndrome preadipocyte-derived adipocytes, we found near-to-maximal *GIP-R* mRNA expression levels already at d 7 of differentiation with a stable expression pattern up to d 14 of differentiation process in our human adipocytes. This discrepancy might most likely be attributed to differences in the primary preadipocytes used.

Treatment with GIP directly induced mRNA expression of *CGRP-I* and its splice variant *CT* in a dose-and time-dependent manner. Regarding possible signaling pathways transducing the GIP effect on *CGRP-I* and *CT* expression and secretion, we found the cAMP/PKA pathway as well as $[Ca^{2+}]_i$ to be involved. Both cAMP/PKA and $[Ca^{2+}]_i$ mediate lipopolysaccharide (LPS)-induced CGRP release in cultured neonatal rat dorsal root ganglion neurons (31). In accordance we recently demonstrated a crucial role for $[Ca^{2+}]_i$ in LPS-induced *CALC-I* gene expression and protein release of CGRP-I and Pro-CT in human adipocytes (16). In GIP-treated human adipocytes, only CGRP-I protein was secreted shortly after GIP treatment alone, whereas cotreatment with the ionophor ionomycin induced significant protein secretion of both CGRP-I and Pro-CT. This suggests that GIP-induced secretion of Pro-CT by adipocytes takes place only if cells are primed by other stimuli.

A recent study provided strong evidence for an association of Pro-CT plasma levels with obesity, insulin resistance, and the metabolic syndrome (19). Some studies provided further evidence for the detrimental effects of Pro-CT in states of systemic inflammation and sepsis (32, 33). The secretion of Pro-CT in the AT of obese patients might thereby further accelerate local proinflammatory activity in AT. Furthermore, CGRP-1 has been shown to directly induce insulin resistance (21–23, 34) and to impair glucose-induced insulin secretion (24, 35). Therefore, CGRP-I and Pro-CT might mediate GIP-induced insulin resistance in diet-induced obesity and related metabolic dysfunctions.

As a physiological link to our *in vitro* findings, human CGRP plasma levels increase after an oral fat load (20), which is also a trigger for GIP release (1). However, another study found no systemic increase of CGRP after oral fat ingestion (36). Due to the controversy of these reports, we measured CGRP-I and Pro-CT levels in plasma samples of 24 highly obese patients before and 180 min after ingestion of a high-fat test meal. We found an increase in CGRP-I plasma levels to 1.9 ± 0.3-fold ($P < 0.05$ *vs.* fasted) and a slight increase in Pro-CT levels to 1.1 ± 0.1-fold ($P = 0.07$ *vs.* fasted; data not shown). The finding

that the Pro-CT plasma levels were not significantly increased might be attributed to the fact that concentrations of this hormone were measured only at one time point (180 min) after meal ingestion. Pro-CT plasma levels after endotoxin injection have been reported to rise at the earliest within 3–6 h (37). Therefore, we might have missed a significant increase of Pro-CT concentrations at a later postprandial time point.

The induction of Pro-CT and CGRP by GIP in human adipocytes might be also of clinical relevance in another context. Glucagon-like peptide-1 (GLP-1), another incretin hormone, is used for the treatment of diabetes mellitus type 2. GLP-1 analogs have been shown to induce CT and CGRP expression and secretion in a murine C cell line (38). Furthermore, a long-acting GLP-1 receptor agonist, liraglutide, is associated with increased risk of thyroid C cell adenomas and carcinomas in rats and mice (39). Accordingly, the Food and Drug Administration requested long-term follow-up on rates of medullary cancer in patients treated with this drug. Dipeptidyl peptidase-IV inhibitors increase plasma levels of GLP-1 and GIP *in vivo* (40) and are widely used for the treatment of diabetes mellitus type 2. Our findings, that GIP is inducing *CALC-I* gene expression in human adipocytes, should further stimulate research investigating the effect of GIP in human thyroid C cells to cast light on any potential medullary thyroid carcinoma developing in patients treated with Dipeptidyl peptidase-IV inhibitors or newly developed GIP agonists/analogs.

In summary, our study shows that GIP is directly inducing CGRP-I and Pro-CT in human adipocytes. Together with the findings that *CGRP-I* and *CT* mRNA expression levels in visceral AT were positively correlated with BMI, these results provide strong evidence for a role of CGRP-I and Pro-CT as markers for adipocyte dysfunction in obesity.

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References

1. **Karhunen LJ, Juvonen KR, Huotari A, Purhonen AK, Herzig KH** 2008 Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. Regul Pept 149:70–78
2. **Flatt PR, Bailey CJ, Kwasowski P, Page T, Marks V** 1984 Plasma immunoreactive gastric inhibitory polypeptide in obese hyperglycaemic (ob/ob) mice. J Endocrinol 101:249–256
3. **Vilsbøll T, Krarup T, Sonne J, Madsbad S, Volund A, Juul AG, Holst JJ** 2003 Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. J Clin Endocrinol Metab 88:2706–2713
4. **Irwin N, McClean PL, Patterson S, Hunter K, Flatt PR** 2009 Active immunisation against gastric inhibitory polypeptide (GIP) improves blood glucose control in an animal model of obesity-diabetes. Biol Chem 390:75–80
5. **Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, Hiai H, Mizunoya W, Fushiki T, Holst JJ, Makino M, Tashita A, Kobara Y, Tsubamoto Y, Jinnouchi T, Jomori T, Seino Y** 2002 Inhibition of gastric inhibitory polypeptide signaling prevents obesity. Nat Med 8:738–742
6. **Gault VA, McClean PL, Cassidy RS, Irwin N, Flatt PR** 2007 Chemical gastric inhibitory polypeptide receptor antagonism protects against obesity, insulin resistance, glucose intolerance and associated disturbances in mice fed high-fat and cafeteria diets. Diabetologia 50:1752–1762
7. **McClean PL, Irwin N, Cassidy RS, Holst JJ, Gault VA, Flatt PR** 2007 GIP receptor antagonism reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat diet. Am J Physiol Endocrinol Metab 293: E1746–E1755
8. **Drucker DJ** 2003 Glucagon-like peptide-1 and the islet β-cell: augmentation of cell proliferation and inhibition of apoptosis. Endocrinology 144:5145–5148
9. **McIntosh CH, Widenmaier S, Kim SJ** 2009 Glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide; GIP). Vitam Horm 80:409–471
10. **Weaver RE, Donnelly D, Wabitsch M, Grant PJ, Balmforth AJ** 2008 Functional expression of glucose-dependent insulinotropic polypeptide receptors is coupled to differentiation in a human adipocyte model. Int J Obes (Lond) 32:1705–1711
11. **Kim SJ, Nian C, McIntosh CH** 2007 Activation of lipoprotein lipase by glucose-dependent insulinotropic polypeptide in adipocytes. A role for a protein kinase B, LKB1, and AMP-activated protein kinase cascade. J Biol Chem 282:8557–8567
12. **Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM** 1983 Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 304:129–135
13. **Müller B, Becker KL, Schächinger H, Rickenbacher PR, Huber PR, Zimmerli W, Ritz R** 2000 Calcitonin precursors are reliable markers of sepsis in a medical intensive care unit. Crit Care Med 28:977–983
14. **Linscheid P, Seboek D, Nylen ES, Langer I, Schlatter M, Becker KL, Keller U, Müller B** 2003 *In vitro* and *in vivo* calcitonin I gene expression in parenchymal cells: a novel product of human adipose tissue. Endocrinology 144:5578–5584

15. **Linscheid P, Seboek D, Schaer DJ, Zulewski H, Keller U, Müller B** 2004 Expression and secretion of procalcitonin and calcitonin gene-related peptide by adherent monocytes and by macrophage-activated adipocytes. Crit Care Med 32:1715–1721
16. **Radimerski T, Grisouard J, Timper K, Zulewski H, Christ-Crain M, Keller U, Muller B** 3 August 2010 Role of calcium in lipopolysaccharide-induced calcitonin gene expression in human adipocytes. Innate Immun 10.1177/1753425910377100
17. **Christ-Crain M, Jaccard-Stolz D, Bingisser R, Gencay MM, Huber PR, Tamm M, Müller B** 2004 Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. Lancet 363:600–607
18. **Puder JJ, Varga S, Kraenzlin M, De Geyter C, Keller U, Müller B** 2005 Central fat excess in polycystic ovary syndrome: relation to low-grade inflammation and insulin resistance. J Clin Endocrinol Metab 90:6014–6021
19. **Abbasi A, Corpeleijn E, Postmus D, Gansevoort RT, de Jong PE, Gans RO, Struck J, Hillege HJ, Stolk RP, Navis G, Bakker SJ** 2010 Plasma procalcitonin is associated with obesity, insulin resistance, and the metabolic syndrome. J Clin Endocrinol Metab 95:E26–E31
20. **Zelissen PM, Koppeschaar HP, Lips CJ, Hackeng WH** 1991 Calcitonin gene-related peptide in human obesity. Peptides 12:861–863
21. **Molina JM, Cooper GJ, Leighton B, Olefsky JM** 1990 Induction of insulin resistance *in vivo* by amylin and calcitonin gene-related peptide. Diabetes 39:260–265
22. **Yamaguchi A, Chiba T, Morishita T, Nakamura A, Inui T, Yamatani T, Kadowaki S, Chihara K, Fukase M, Fujita T** 1990 Calcitonin gene-related peptide and induction of hyperglycemia in conscious rats *in vivo*. Diabetes 39:168–174
23. **Kreutter DK, Orena SJ, Torchia AJ, Contillo LG, Andrews GC, Stevenson RW** 1993 Amylin and CGRP induce insulin resistance via a receptor distinct from cAMP-coupled CGRP receptor. Am J Physiol 264:E606–E613
24. **Ahrén B** 1990 Effects of galanin and calcitonin gene-related peptide on insulin and glucagon secretion in man. Acta Endocrinol (Copenh) 123:591–597
25. **Pettersson M, Ahrén B** 1988 Insulin and glucagon secretion in rats: effects of calcitonin gene-related peptide. Regul Pept 23:37–50
26. **Pettersson M, Ahrén B, Böttcher G, Sundler F** 1986 Calcitonin gene-related peptide: occurrence in pancreatic islets in the mouse and the rat and inhibition of insulin secretion in the mouse. Endocrinology 119:865–869
27. **Fischer M, Timper K, Radimerski T, Dembinski K, Frey DM, Zulewski H, Keller U, Müller B, Christ-Crain M, Grisouard G** 2010 Metformin induces glucose uptake in human preadipocyte-derived adipocytes from various fat depots. Diabetes Obes Metab 12:356–359
28. **Linscheid P, Seboek D, Zulewski H, Keller U, Müller B** 2005 Autocrine/paracrine role of inflammation-mediated calcitonin gene-related peptide and adrenomedullin expression in human adipose tissue. Endocrinology 146:2699–2708
29. **Peterli R, Wölnerhanssen B, Peters T, Devaux N, Kern B, Christoffel-Courtin C, Drewe J, von Flüe M, Beglinger C** 2009 Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial. Ann Surg 250:234–241
30. **Song DH, Getty-Kaushik L, Tseng E, Simon J, Corkey BE, Wolfe MM** 2007 Glucose-dependent insulinotropic polypeptide enhances adipocyte development and glucose uptake in part through Akt activation. Gastroenterology 133:1796–1805
31. **Hou L, Wang X** 2001 PKC and PKA, but not PKG mediate LPS-induced CGRP release and [Ca(2+)] (i) elevation in DRG neurons of neonatal rats. J Neurosci Res 66:592–600
32. **Becker KL, Nylén ES, White JC, Müller B, Snider Jr RH** 2004 Clinical review 167: procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab 89:1512–1525
33. **Nylen ES, Whang KT, Snider Jr RH, Steinwald PM, White JC, Becker KL** 1998 Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. Crit Care Med 26:1001–1006
34. **Kreutter D, Orena SJ, Andrews KM** 1989 Suppression of insulin-stimulated glucose transport in L6 myocytes by calcitonin gene-related peptide. Biochem Biophys Res Commun 164:461–467
35. **Ishizuka J, Greeley Jr GH, Cooper CW, Thompson JC** 1988 Effect of calcitonin gene-related peptide on glucose and gastric inhibitory polypeptide-stimulated insulin release from cultured newborn and adult rat islet cells. Regul Pept 20:73–82
36. **Pedersen-Bjergaard U, Høst U, Kelbaek H, Schifter S, Rehfeld JF, Faber J, Christensen NJ** 1996 Influence of meal composition on postprandial peripheral plasma concentrations of vasoactive peptides in man. Scand J Clin Lab Invest 56:497–503
37. **Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M, Bohuon C** 1994 Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 79:1605–1608
38. **Lamari Y, Boissard C, Moukhtar MS, Jullienne A, Rosselin G, Garel JM** 1996 Expression of glucagon-like peptide I receptor in a murine C cell line: regulation of calcitonin gene by glucagon-like peptide I. FEBS Lett 393:248–252
39. **Bjerre Knudsen L, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ, Gotfredsen C, Egerod FL, Hegelund AC, Jacobsen H, Jacobsen SD, Moses AC, Molck AM, Nielsen HS, Nowak J, Solberg H, Thi TD, Zdravkovic M** 2010 Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. Endocrinology 151:1473–1486
40. **McIntosh CH** 2008 Dipeptidyl peptidase IV inhibitors and diabetes therapy. Front Biosci 13:1753–1773