Glucose-Dependent Insulinotropic Polypeptide Reduces Fat-Specific Expression and Activity of 11β-Hydroxysteroid Dehydrogenase Type 1 and Inhibits Release of Free Fatty Acids

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Glucose-dependent insulinotropic polypeptide (GIP) has been suggested to have direct effects on nonislet tissues. GIP also reportedly increased glucose uptake and inhibition of lipolysis in adipocytes after inhibition of the intracellular cortisone-cortisol shuttle 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). We here analyzed whether GIP modifies lipid metabolism and further elucidated the relation between GIP, 11β-HSD1, and fatty acid metabolism. GIP reduced activity of 11β-HSD1 promoter constructs and the expression and activity of 11β-HSD1 in differentiated 3T3-L1 adipocytes in a time- and dose-dependent fashion. This was paralleled by a reduction of free fatty acid (FFA) release and a reduced expression of key enzymes regulating lipolysis in adipose tissue. Preinhibition of 11β-HSD1 completely abolished GIP-induced effects on FFA release. To investigate the acute effects of GIP in humans, a randomized clinical trial was performed. GIP lowered circulating FFAs compared with saline control and reduced expression and ex vivo activity of 11β-HSD1 and adipose triglyceride lipase expression in subcutaneous fat biopsies. Our data suggest that GIP reduces FFA release from adipose tissue by inhibition of lipolysis or by increased reesterification. This process appears to depend on a modification of 11β-HSD1 activity. In general, the presented data support that GIP has direct and insulin-independent effects on adipose tissue. *Diabetes* **61:292–300, 2012**

Postprandial metabolism is characterized by increased glucose uptake and inhibition of lipolysis. Both traits are predominantly mediated by peripheral effects of insulin. Numerous trials have established that incretins, namely, glucagon-like polypeptide 1 and glucose-dependent insulinotropic polypeptide (GIP), improve glucose-stimulated insulin secretion. However, recent studies also describe regulatory functions in many extrapancreatic tissues as insulin-independent effects of those hormones (1–3). Thus, long-term inactivation of GIP signaling in GIP receptor (GIPR) knockout mice resulted in animals resistant to high fat diet–induced obesity (4), and GIPR antagonism reversed obesity, insulin resistance, and associated metabolic disturbances in mice (5). An up-regulation of the GIPR has been demonstrated during differentiation of adipocytes, and an improved glucose uptake in GIP-treated adipocytes has been found (6). In comparison, an inhibition of lipolysis was demonstrated in vitro in adipocytes after exposure to GIP (7), and very recent human in vivo data support this effect (1,8). Asmar et al. (8) have suggested that GIP enhanced reesterification of free fatty acids (FFAs) under hyperinsulinemic, high-glycemic conditions in eight healthy and lean male subjects. Of interest, some reports demonstrate that obese individuals have elevated GIP levels (9). We therefore speculated that GIP might affect the basal lipolysis in obese individuals. Although not yet understood in detail, existing results suggest that peripheral GIP effects may contribute to the switch to anabolic metabolism in the postprandial situation independent of its insulinotropic effects. Although GIP-induced intracellular mechanisms in β-cells are increasingly well understood (10,11), the mechanisms of GIP-induced inhibition of lipolysis under basal conditions are less clear.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has been identified as a central hormonal mechanism in the regulation of glucose and lipid metabolism in adipose tissue (12–14). The enzyme is predominantly expressed in adipose tissue and hepatocytes and converts inactive 11-ketoglucocorticoids to the functionally active 11β-hydroxy derivates. Very similar to GIPR KO mice, 11β-HSD1 gene knockout mice exhibited improved glucose tolerance, reduced weight gain, and reduced fat accumulation in visceral fat depots under a high-fat diet (15). Selective 11β-HSD1 inhibitors lowered blood glucose levels and improved insulin sensitivity in mouse models of type 2 diabetes (16–18). In contrast, the fat-specific overexpression of 11β-HSD1 resulted in a phenotype comparable to metabolic syndrome (19). We therefore hypothesized that the insulin-independent effects of GIP might contribute to the postprandial fine-tuning of metabolism via an acute suppression of fat-specific 11β-HSD1 activity.

RESEARCH DESIGN AND METHODS

Cell culture and promoter constructs. Procedures inducing differentiation of 3T3-L1 fibroblasts and luciferase reporter plasmids containing the human 11β-HSD1 gene promoter were published previously (20). Briefly, differentiation of 3T3-L1 was obtained by 2 days' Dulbecco's modified Eagle's medium

(DMEM) supplemented with 10% FCS (Lot 764H, cortisol concentration 8 nmol/L; Biochrom AG, Berlin, Germany), 1 μmol/L insulin (Sigma-Aldrich Chemie GmbH, Munich, Germany), 500 μmol/L IBMX (Sigma-Aldrich Chemie GmbH), 1 μmol/L dexamethasone (Sigma-Aldrich Chemie GmbH), and 1 μmol/L rosiglitazone (Calbiochem, Merck KGaA, Darmstadt, Germany). The medium was subsequently changed to DMEM supplemented with 10% FCS, 1 μmol/L insulin, and 1 μmol/L rosiglitazone for another 2 days. Finally, the cells were cultured in DMEM with 10% FCS until days 8–10.

Within the promoter constructs, at least three base pairs (bp) of five putative transcription factor binding sites were mutated using the Stratagene Quik-Change Multi Site-Directed Mutagenesis Kit (Santa Clara, CA). Transfection of differentiated 3T3-L1 cells was performed using electroporation. The promoterless pGL3 basic vector or the promoter constructs were cotransfected with pRL-SV40 (Promega Corp., Madison, WI) for normalization. After transfection, media were changed to serum free, and cells were stimulated for 24 h with 1 nmol/L GIP (Sigma-Aldrich Chemie GmbH). Luciferase activity was determined as reported previously (21). Glucocorticoid concentrations in the basal media were standardized, and one batch of FCS was used for all experiments.

Analysis of mRNA expression. Differentiated cells were treated for 120 min (or as indicated for dose- and time-response experiments) with 1 nmol/L GIP or control. RNA from 3T3-L1 adipocytes was isolated using the ZR-96 Mini RNA Isolation I Kit (Zymo Research, Orange, CA). RNA of snap-frozen subcutaneous fat biopsies was extracted using Qiagen RNeasy spin columns of the Lipid Tissue RNA Kit (Qiagen, Hilden, Germany). RNA samples were stored at −80°C until assayed. Relative gene expression was determined by RT-PCR, and 16s ribosomal protein (16s) was used as reference gene. Primer sequences of analyzed genes will be provided by the authors on request. All assays were performed at least in triplicate.

Assessment of 11β-HSD1 activity. Quantification of 11β-HSD1 enzyme activity was measured as conversion of ^{3}H-cortisone to ^{3}H-cortisol. Briefly, differentiated 3T3-L1 and tissue biopsies were incubated with 0.5 μL ^{3}H-cortisone ([1,2(n)-^{3}H]-cortisone; Amersham, GE Healthcare, Freiburg, Germany), 100 nmol/L unlabeled cortisone, and 250 μmol/L NADPH$_2$. At 10 min before adding substance for measurement of enzyme activity, cells were stimulated as indicated. Cells were harvested with 0.05% SDS after 120 min. Steroids were extracted with ethyl acetate and separated by thin layer chromatography in dichloromethane/methanol (75+5). The individual steroid fractions were quantified using a β-counter (Wallac; PerkinElmer, Rodgau, Germany) as previously described (4).

Analysis of fatty acid metabolism. Differentiated 3T3-L1 cells were incubated with 2 μL of a 1:50 dilution of ^{3}H-oleic acid (Oleic Acid, [9,10-^{3}H(N)]; Perkin Elmer LAS, Waltham, MA). After 24-h incubation, cells were washed and stimulated in DMEM with 1 g/L glucose and 1 nmol/L GIP, 1 μmol/L carbenoxolone, 1 μmol/L cortisol, and 100 nmol/L insulin or coincubated with 1 nmol/L GIP plus 1 μmol/L carbenoxolone (Sigma-Aldrich Chemie GmbH). After 120-min stimulation, the supernatant was measured for ^{3}H release in a β-counter. Release of ^{3}H was adjusted for total protein content, which was determined using the Bradford method.

Lipid uptake. Differentiated 3T3-L1 cells were incubated with 2 μL of 1:50 dilution of ^{3}H-oleic acid (Perkin Elmer LAS) in DMEM with 1 g/L glucose and different stimulations as mentioned for lipolysis experiments for 120 min. Cells were washed twice with phosphate-buffered saline and lysed. Protein was determined using the Bradford method, and ^{3}H was determined in the lysates of cells in a β-counter.

Small interfering RNA experiments. Small interfering RNA (siRNA; HSD11B1 ON-TARGETplus SMARTpool, L-046349–01; ON-TARGETplus Nontargeting siRNA 2, D-001810–02) were obtained from Dharmagon (Thermo Fisher Scientific, ABgene House, Epsom, U.K.). Transfection of differentiated 3T3-L1 cells was performed using the AmaxaLine Nucleofector Kit L (Lonza, Cologne, Germany). After electroporation, cells were seeded in 12-well plates, and experiments were conducted after 48-h incubation.

Western blot. Protein concentration was determined using the trichloroacetic acid method (22). Western blots were performed as described previously (23). The antibodies for detection of adipose triglyceride lipase (ATGL), phosphorylated hormone-sensitive lipase (HSL), and HSL were obtained from Cell Signaling Technology (Danvers, MA), perilipin was purchased from Abnova Taiwan (Taipei City, Taiwan), and α-tubulin was obtained from Abcam plc (Cambridge, U.K.).

Subjects. A total of 11 apparently healthy male obese subjects were studied (BMI 33.5 ± 2.0 kg/m^2; age 48.4 ± 11.3; waist-to-hip ratio 1.01 ± 0.5). In each subject, a complete physical examination and evaluation of medical history, an oral glucose tolerance test with 75-g glucose (Roche Diagnostics, Mannheim, Germany) after overnight fasting, and blood measurements in the fasting state for standard laboratory and clinical chemistry evaluation were performed. Two of the subjects were under stable medication as a result of chronic hypothyroidism; both were euthyroid. All participants reported a stable body weight for at least 12 months. All subjects were instructed to avoid significant physical activity 3 days before the examination day.

The study was approved by the ethics committee of Brandenburg, Germany (AS 2(a)/2005). All individuals gave written informed consent prior to the study. The trial was registered at www.clinicaltrials.gov (NCT00774488).

Study design of clinical trial. All participants were overnight fasted on the study days. GIP or NaCl infusions were administered during a 240-min period in random order. The GIP infusion was administered at a rate of 2 pmol/kg/min, and the placebo infusion was performed with 0.9% physiological saline solution. Wash-out period between the two study days was at least 7 days.

Synthetic GIP was produced from PolyPeptide Laboratories GmbH (Wolfenbüttel, Germany) and was diluted in 0.9% physiological NaCl solution (24). Using a biopsy needle (14G; Somatex, Teltow, Germany), subcutaneous adipose tissue biopsies were obtained before ($t = 0$ min) and at the end of the infusion ($t = 240$ min). The two adipose tissue biopsies were taken from distinct sites at the level of the umbilicus. After removal, one part of the tissue samples was stored in an RNA protection buffer (RNAlater, Qiagen) and kept at 4°C until the procedure was finished. Another part of the fat biopsies was directly frozen in liquid nitrogen. Finally, all samples were stored at −80°C until enzyme activity measurement or RNA isolation procedures.

Serum FFA, free glycerol, insulin, and GIP enzyme-linked immunosorbent assay in human blood samples. Fatty acids and free glycerol in serum were determined using commercial assays (Biovision Inc., Mountainview, CA). Concentrations of insulin were determined in serum by using an insulin ELISA kit (Mercodia, Uppsala, Sweden) as described (25). Sensitivity of the insulin enzyme-linked immunosorbent assay was 1 mU/L (6 pmol/L), intra-assay coefficient of variation (CV) was 2.8–4.0%, and interassay CV was 2.6–3.6%.

Concentrations of total GIP were determined in plasma samples containing aprotinin (Trasylol; Bayer, Leverkusen, Germany) by using a human GIP (total) ELISA kit (Linco Research, St. Charles, MO). Sensitivity was 8.2 pmol/L, intra-assay CV was 3.0–8.8%, and interassay CV was 1.8–6.1%.

Statistical analysis. The IBM SPSS 18.0 package (IBM Deutschland, Ehningen, Germany) was used for statistical evaluation of data. Promoter activity data are presented as ratios of luciferase/Renilla and normalized to the activity of the promoterless pGL3 basic vector. Each measurement was performed in at least five replicates. The Mann-Whitney U test was used to analyze differences between groups. All time courses of Fig. 5 were analyzed by repeated-measures ANOVA.

The results were considered significant at two-sided $\alpha < 0.05$. Data are presented as the mean ± SEM unless stated otherwise.

RESULTS

GIP reduced 11β-HSD1 promoter activity in differentiated 3T3-L1 cells. GIP reduced 11β-HSD1 promoter activity in differentiated 3T3-L1 cells in all analyzed constructs (from −823 bp to 188 bp relative to transcription start; relative reduction: −42 to −30% vs. control; $P < 0.001$) (Fig. 1A), suggesting that at least one element responsive to GIP-dependent signaling is located within the proximal promoter region. An in silico analysis of the F1 fragment (using MatInspector [Genomatix, Munich, Germany] and Jaspar [http://jaspar.cgb.ki.se/]) suggested one putative cAMP-responsive element–binding protein 2 (CREB2) and four putative CCAAT/enhancer binding protein (C/EBP) binding sites. Mutation of these binding sites reduced the activity of the F1 fragment per se (Fig. 1B) and virtually abolished GIP-induced effects on promoter activity (Fig. 1C).

GIP reduced 11β-HSD1 expression and activity and lowered expression of ATGL and HSL in differentiated 3T3-L1 adipocytes. After 120-min GIP treatment, 11β-HSD1 mRNA expression was reduced in differentiated 3T3-L1 adipocytes by ~50% ($P < 0.001$). In comparison, GIP reduced 11β-HSD1 enzyme activity in differentiated 3T3-L1 cells to 71 ± 3% of control activity ($P = 0.01$) (Fig. 2A). The expression of ATGL and HSL, two key enzymes in the regulation of lipolysis, was reduced by 47% ($P < 0.01$) and 18% ($P < 0.05$), respectively, whereas other genes involved in fat metabolism, such as LPL, perilipin, and CD36, were not affected (Fig. 2B).

The GIP effect on 11β-HSD1 and ATGL was dose and time dependent. The calculated half-maximal effective concentration of GIP on expression of 11β-HSD1 and ATGL was between 0.2 and 0.25 nmol/L (Supplementary Fig. 1A). ATGL protein was decreased in GIP-treated 3T3-L1 cells to 70–80% (Supplementary Fig. 1B). Up to 50 nmol/L GIP, we
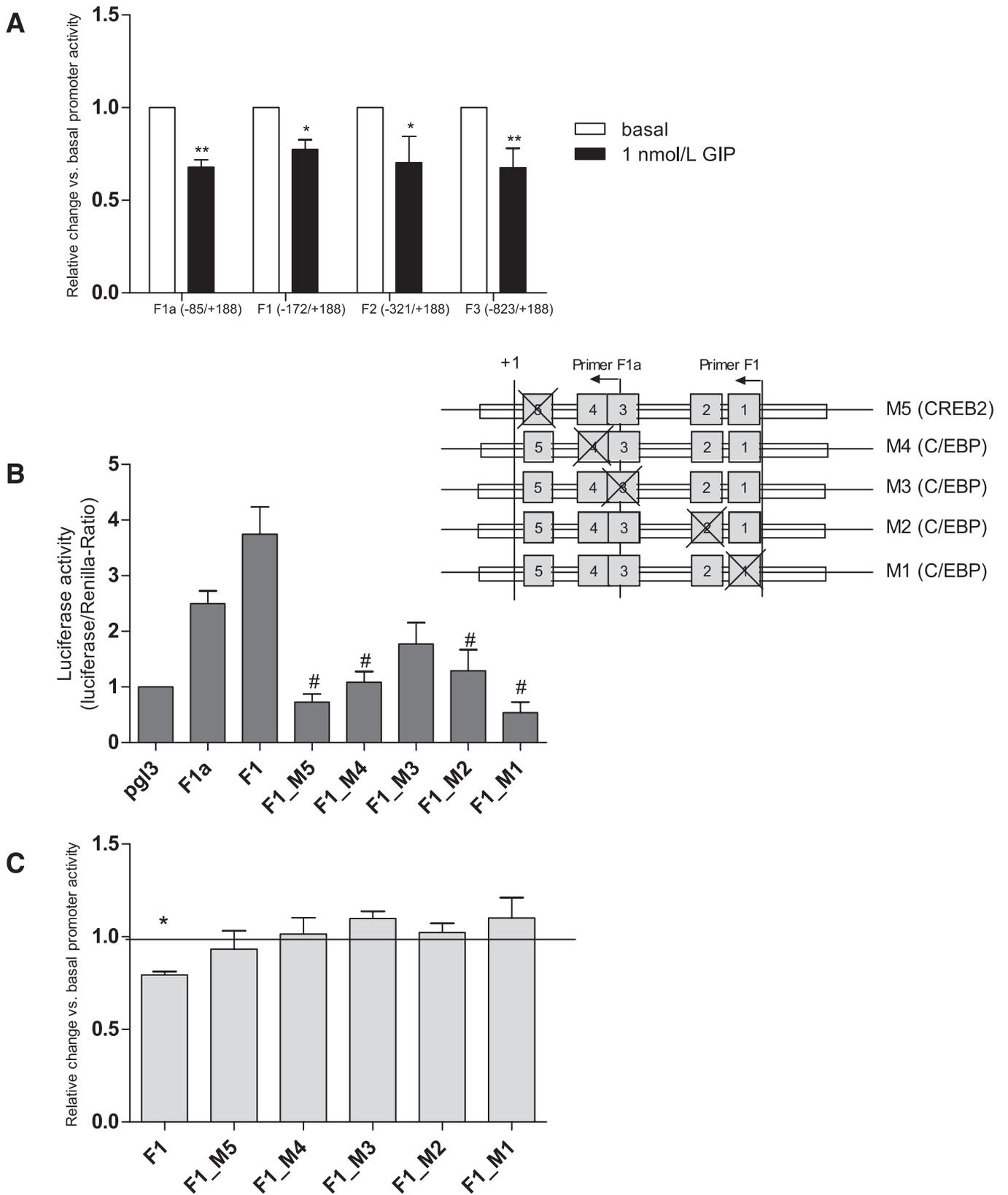
FIG. 1. *A*: Relative activity of human 11β-HSD1 promoter fragments (F1a–F3) in differentiated 3T3-L1 adipocytes under stimulation with 1 nmol/L GIP vs. unstimulated controls (set to 1). Basal luciferase activity of 11β-HSD1 promoter fragments (F1a–F1) and promoter fragments with mutations of different transcription factor binding sites (M1–M5) (*B*) and the relative luciferase activity under the stimulation of 1 nmol/L GIP (*C*). Data are mean ± SEM; **$P < 0.01$, *$P < 0.05$, #$P < 0.05$ mutation vs. F1.
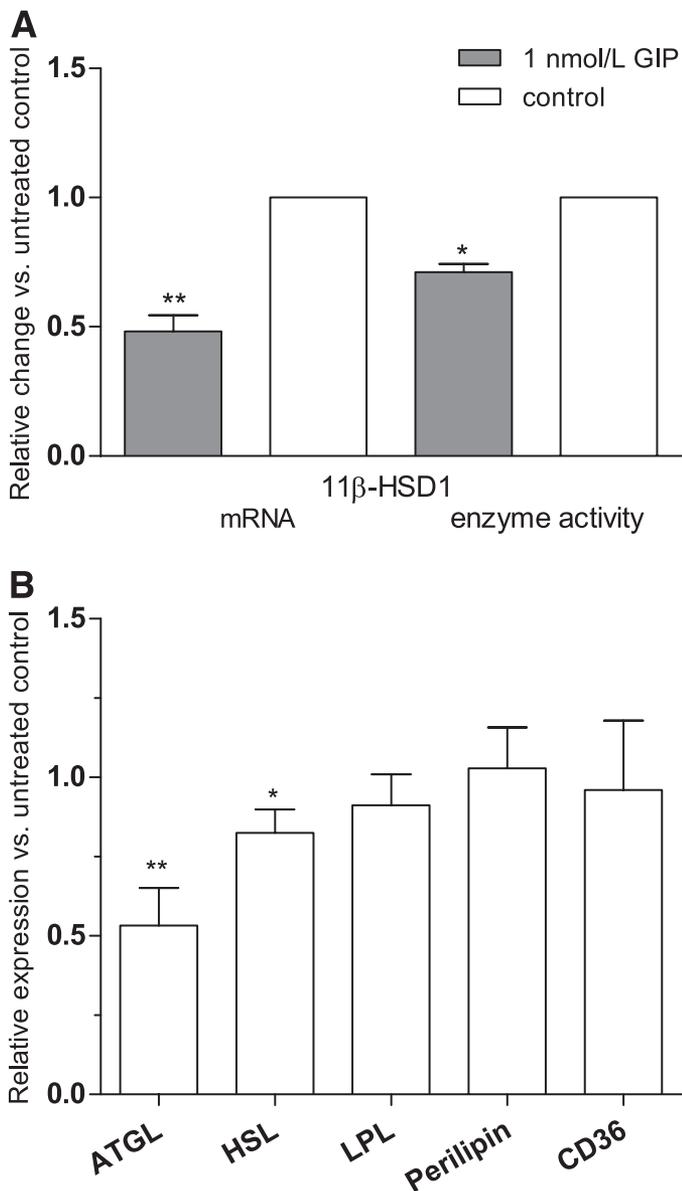
A

B

FIG. 2. GIP-induced effects on 11β-HSD1 expression and enzyme activity (*A*) and effects on lipolysis-related genes (ATGL, HSL, LPL, perilipin, and CD36) (*B*) in differentiated 3T3-L1 adipocytes after 120-min treatment with 1 nmol/L GIP. Data are mean ± SEM; *P < 0.05, **P < 0.01.

found no effect on the inhibiting phosphorylation (Ser565) of HSL or total HSL protein. Treatment of cells with 1 nmol/L GIP resulted in a significant reduction of 11β-HSD1 and ATGL expression after 30 min and a lowering of ATGL protein after ~60 min. This reduction was stable up to 6 h (Supplementary Fig. 2*A* and *B*).

GIP reduced FFA release in differentiated 3T3-L1 adipocytes in an 11β-HSD1–dependent fashion. GIP inhibited release of FFAs in differentiated 3T3-L1 adipocytes by ~17% (*P* < 0.05), whereas uptake of FFAs was not affected: GIP, −6.4 ± 6.1% (0.195 ± 0.103 pmol/L); insulin, 6.9 ± 5.5% (0.197 ± 0.102 pmol/L); and dexamethasone, −20.2 ± 10.3% (0.167 ± 0.078 pmol/L) compared with control (0.231 ± 0.133 pmol/L). Carbenoxolone reduced 11β-HSD1 activity by >95% and inhibited lipolysis by ~26% compared with controls (*P* < 0.001). It is interesting that cotreatment with carbenoxolone completely abolished GIP-induced effects (Fig. 3).

siRNA-induced reduction of 11β-HSD1 expression lowered ATGL expression and reduced FFA release in differentiated 3T3-L1 cells. siRNA-based knockdown reduced 11β-HSD1 expression after 48 h to 19.7 ± 3.7% versus scramble siRNA (*P* < 0.001). This was accompanied by a reduction of ATGL expression to 65.7 ± 7.8% (*P* < 0.001) (Fig. 4*A*). The release of FFAs from 3T3-L1 cells with 11β-HSD1 knockdown was also reduced to 67.1 ± 3.6% versus scramble siRNA (*P* < 0.001). There was no significant difference of 11β-HSD1 and ATGL expression, as well as lipolysis, between mock transfection and scramble siRNA. Most important, the GIP effect on FFA release was abolished after siRNA-induced knockdown of 11β-HSD1 (Fig. 4*B*).

GIP lowered circulating FFAs in vivo in a human randomized controlled trial. Clinical characteristics of study participants are summarized in Table 1. GIP infusion resulted in significantly increased GIP plasma concentrations (mean value 120 pmol/L at 4 h in the GIP infusion group; treatment vs. time interaction for absolute values *P* < 0.001) (Fig. 5*A* for relative changes). FFAs slightly increased during saline administration. In contrast, FFAs were significantly and time-dependently reduced during GIP infusion compared with baseline and compared with saline infusion (FFA–saline: baseline 0.015 ± 0.002, 120 min 0.024 ± 0.005, and 240 min 0.018 ± 0.002 mmol/L; GIP: baseline 0.026 ± 0.003, 120 min 0.022 ± 0.004, and 240 min 0.019 ± 0.002 mmol/L) (treatment vs. time interaction for absolute values *P* = 0.32) (Fig. 5*D* for relative changes). We also determined FFAs at early time points (30 and 60 min), which did not reveal significant differences between both groups for absolute or relative values. In both groups, a slight increase of free glycerol and triglyceride was found, which also was not significant compared with baseline and between-treatment groups (free glycerol–saline: baseline 0.131 ± 0.01, 120 min 0.147 ± 0.01, and 240 min 0.166 ± 0.02 mmol/L; GIP: baseline 0.133 ± 0.01, 120 min 0.144 ± 0.02,
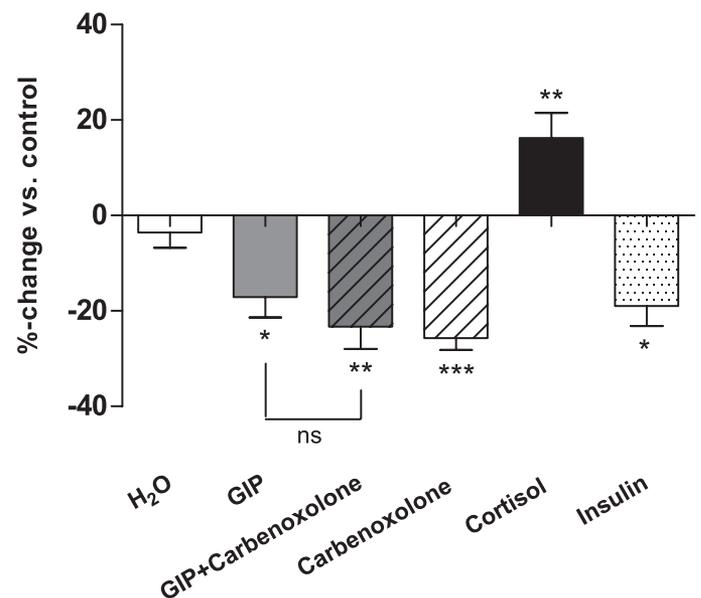

FIG. 3. GIP and carbenoxolone reduced FFA release in differentiated 3T3-L1 adipocytes but had no additive effect. Differentiated 3T3-L1 cells were treated for 120 min with 1 nmol/L GIP and/or 1 μmol/L carbenoxolone, and 1 μmol/L cortisol or 100 nmol/L insulin were used as positive or negative control, respectively. Data are mean ± SEM; *P < 0.05, **P < 0.01, ***P < 0.001.

A

B
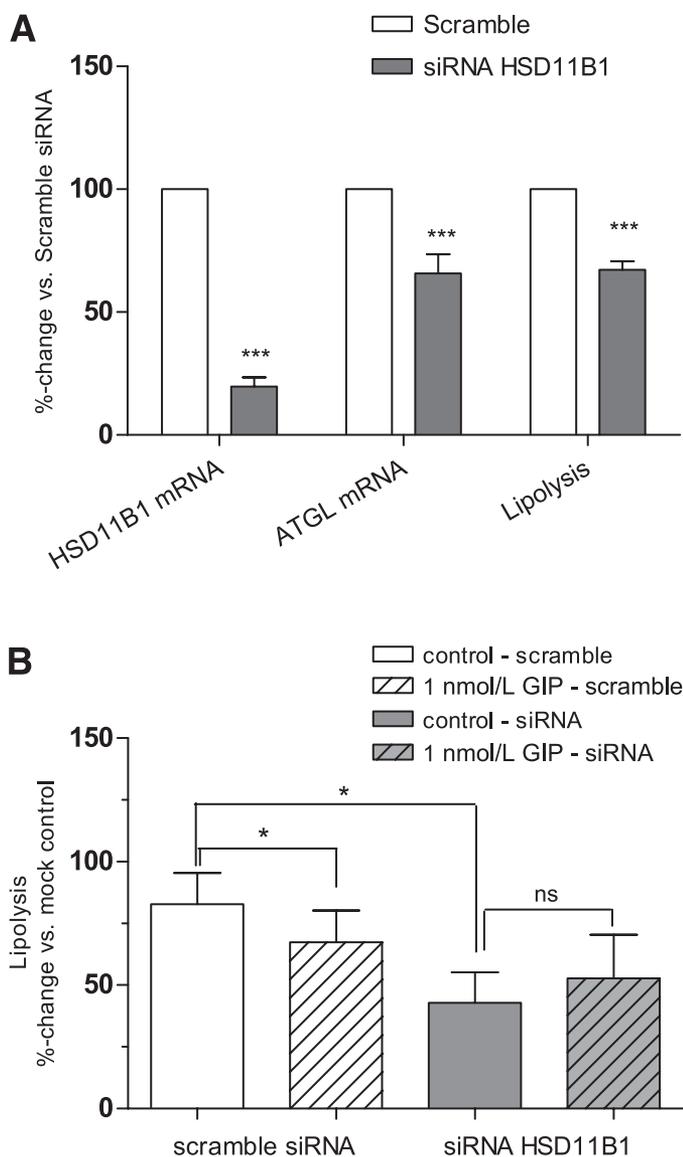

FIG. 4. siRNA knockdown of 11β-HSD1 in differentiated 3T3-L1 cells. Cells were treated with scramble siRNA or 11β-HSD1–specific siRNA and were harvested after 48 h (*A*) or treated for 120 min with 1 nmol/L GIP (*B*). Effects on FFA release and mRNA expression of lipolysis-related genes were calculated relative to the scramble siRNA. Data are mean ± SEM; *$P < 0.05$, ***$P < 0.001$.

TABLE 1
Baseline characteristics of participants

Characteristic	
N	11
Age (years)	48.4 ± 11.3
BMI (kg/m$^{2)}$)	33.5 ± 2
Weight (kg)	101.8 ± 6.6
Waist-to-hip ratio	1.01 ± 0.53
Fat mass (kg)	35.0 ± 7.2
Lean mass (kg)	66.8 ± 4.8
Triglyceride (mmol/L)	1.55 ± 0.65
Nonesterified fatty acid (mmol/L)	0.014 ± 0.007
Free glycerol (mmol/L)	0.124 ± 0.037
LDL cholesterol (mmol/L)	3.9 ± 0.75
HDL cholesterol (mmol/L)	1.2 ± 0.2
Cortisol (nmol/L)	159.8 ± 64.9
Fasting blood glucose (mg/dL)	89.5 ± 5.8
Blood glucose 120 min of OGTT (mg/dL)	118.0 ± 14.9
Fasting serum insulin (mU/L)	15.5 ± 9.8
Serum insulin 120 min (mU/L)	92.9 ± 59
Systolic blood pressure (mmHg)	130 ± 13.9
Diastolic blood pressure (mmHg)	80 ± 11.1

Data are mean ± SD. OGTT, oral glucose tolerance test.

GIP reduced 11β-HSD1 mRNA expression and enzyme activity and lowered the expression of lipolysis-related genes in human adipose tissue ex vivo. GIP treatment decreased the 11β-HSD1 mRNA in subcutaneous adipose tissue biopsies by ~20% compared with baseline (GIP 80.5 ± 6.7% vs. NaCl 107.7 ± 7.3%) (Fig. 6*B*). The ex vivo 11β-HSD1 enzyme activity also decreased in human adipose tissue by ~25% compared with baseline: enzyme activity–saline: 0 min 0.08 ± 0.006 pmol/mg vs. 240 min 0.078 ± 0.007 pmol/mg (N.S.); enzyme activity–GIP: 0 min 0.078 ± 0.008 pmol/mg vs. 240 min 0.058 ± 0.009 pmol/mg ($P < 0.05$) (Fig. 6*A*). ATGL expression was also reduced to 80.5 ± 6.7% ($P < 0.05$) after 240-min GIP infusion (Fig. 6*C*). HSL expression tended to be lower in GIP-treated individuals versus saline treatment, although this effect failed to be significant (Fig. 6*D*). LPL (Fig. 6*E*), fatty acid synthase, and resistin were not affected (fatty acid synthase, resistin, and perilipin data not shown).

DISCUSSION

The data presented herein suggest that GIP inhibits basal FFA release in adipocytes by direct peripheral effects. This appears to depend at least partially on an inhibition of the intracellular cortisone-cortisol shuttle 11β-HSD1.

Recent studies demonstrate upregulation of GIPRs during differentiation of adipocytes and an improved glucose uptake in GIP-treated adipocytes (1–3,6). An inhibition of FFA release was demonstrated in vitro in primary rat adipocytes (7). This and another study demonstrated an increased glycerol release under GIP exposure, suggesting that an increased reesterification of FFAs rather than an inhibition of lipolysis may be induced (7, 26). This finding is supported by in vivo studies, which reveal reduced circulating FFAs during GIP treatment in humans (8). Glucocorticoids and increased 11β-HSD1 activity have been shown to increase lipolysis in subcutaneous adipose tissue (27). Vice versa, an inhibition of 11β-HSD1 reduced lipolysis, suggesting that the GIP-induced inhibition of FFA release may be mediated by a reduction of 11β-HSD1 activity.
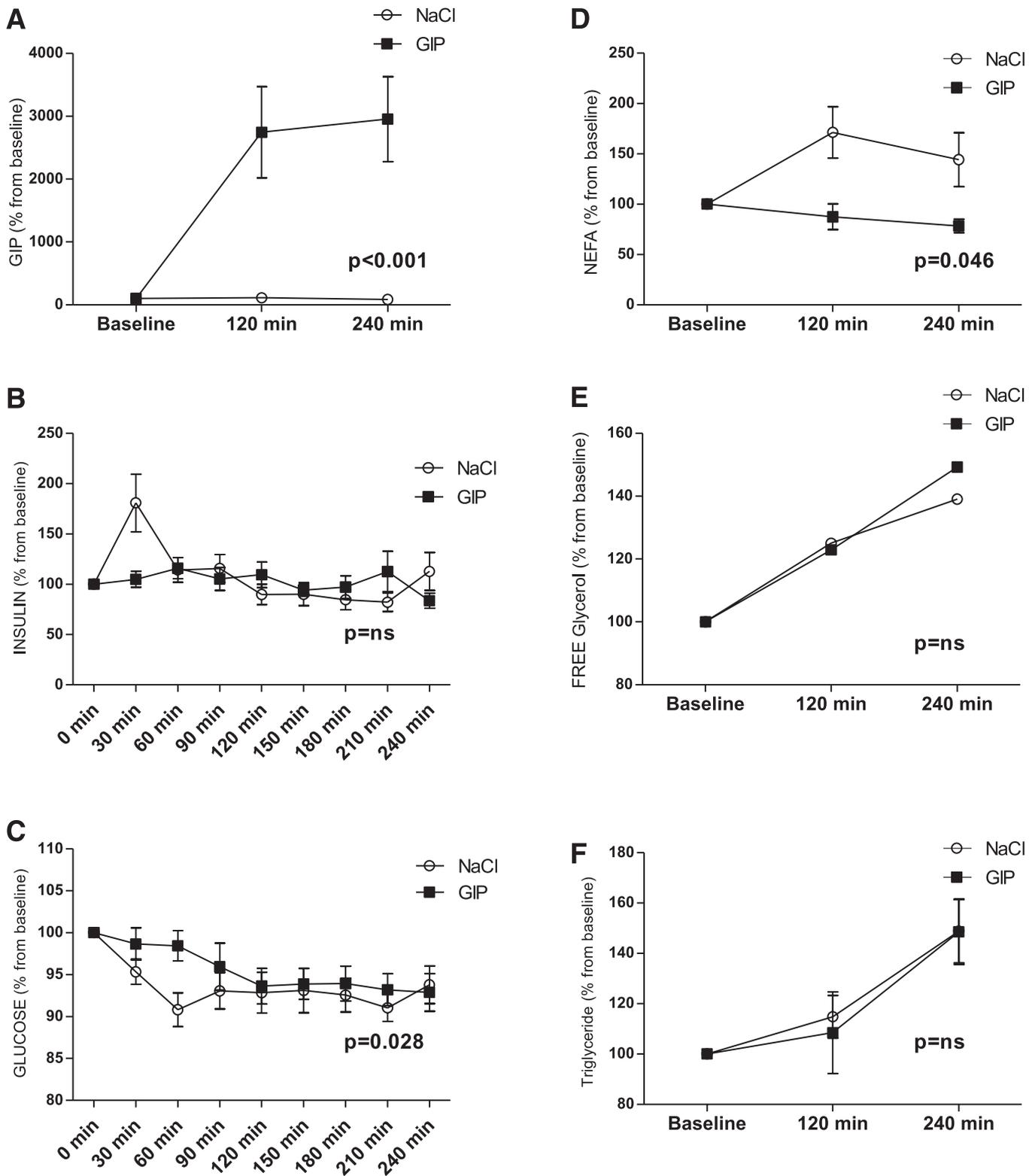
and 240 min 0.165 ± 0.01 mmol/L; triglyceride–saline: baseline 0.441 ± 0.029, 120 min 0.494 ± 0.033, and 240 min 0.634 ± 0.042 mmol/L; GIP: baseline 0.450 ± 0.044, 120 min 0.466 ± 0.063, and 240 min 0.618 ± 0.027 mmol/L) (treatment vs. time interaction for absolute values $P = 0.98$ and 0.83, respectively) (Fig. 5*E* and *F* for relative changes). Time courses of insulin and glucose were as follows: insulin–saline: 0 min 9.84 ± 1.60, 30 min 15.95 ± 2.50, 60 min 11.38 ± 2.12, 120 min 8.48 ± 1.47, and 240 min 11.92 ± 3.50 mU/L; GIP: 0 min 9.42 ± 1.58, 30 min 9.94 ± 2.07, 60 min 11.67 ± 2.70, 120 min 10.49 ± 2.34, and 240 min 8.05 ± 1.67 mU/L; glucose–saline: 0 min 88.6 ± 2.0, 30 min 84.5 ± 2.1, 60 min 80.2 ± 1.1, 120 min 81.9 ± 1.5, and 240 min 82.9 ± 1.9 mmol/L; GIP: 0 min 90.6 ± 1.3, 30 min 89.5 ± 2.2, 60 min 89.2 ± 2.0, 120 min 84.8 ± 2.0, and 240 min 84.2 ± 2.4 mmol/L (treatment vs. time interaction for absolute values $P = $ N.S. and 0.034, respectively) (Fig. 5*B* and *C* for relative changes).

FIG. 5. Metabolic changes of GIP or saline in euglycemic healthy overweight individuals (n = 11) relative to individual baseline value. A: Time course of GIP concentrations (P < 0.001). B: Time course of circulating insulin (P = N.S.). C: Time course of blood glucose levels (P = 0.028). D: Time course of FFAs (P = 0.046); NEFA, nonesterified fatty acid. E: Time course of free glycerol concentrations (P = N.S.). F: Time course of circulating triglycerides (P = N.S.). Data are mean ± SEM; P values are reported for treatment vs. time interaction (repeated-measures ANOVA).

We found a GIP-induced inhibition of 11β-HSD1 promoter constructs in differentiated 3T3-L1 cells. The data were consistent with a model of at least one element within the proximal 11β-HSD1 promoter being responsive to GIP-mediated repression, although additional GIP response elements in more distal regions upstream of the transcription start may exist. In silico analysis of the 11β-HSD1 promoter revealed putative CREB2 and C/EBP binding sites in the proximal 11β-HSD1 promoter region, and recent data support that CREB2 is involved in GIP-induced lipolysis (28).
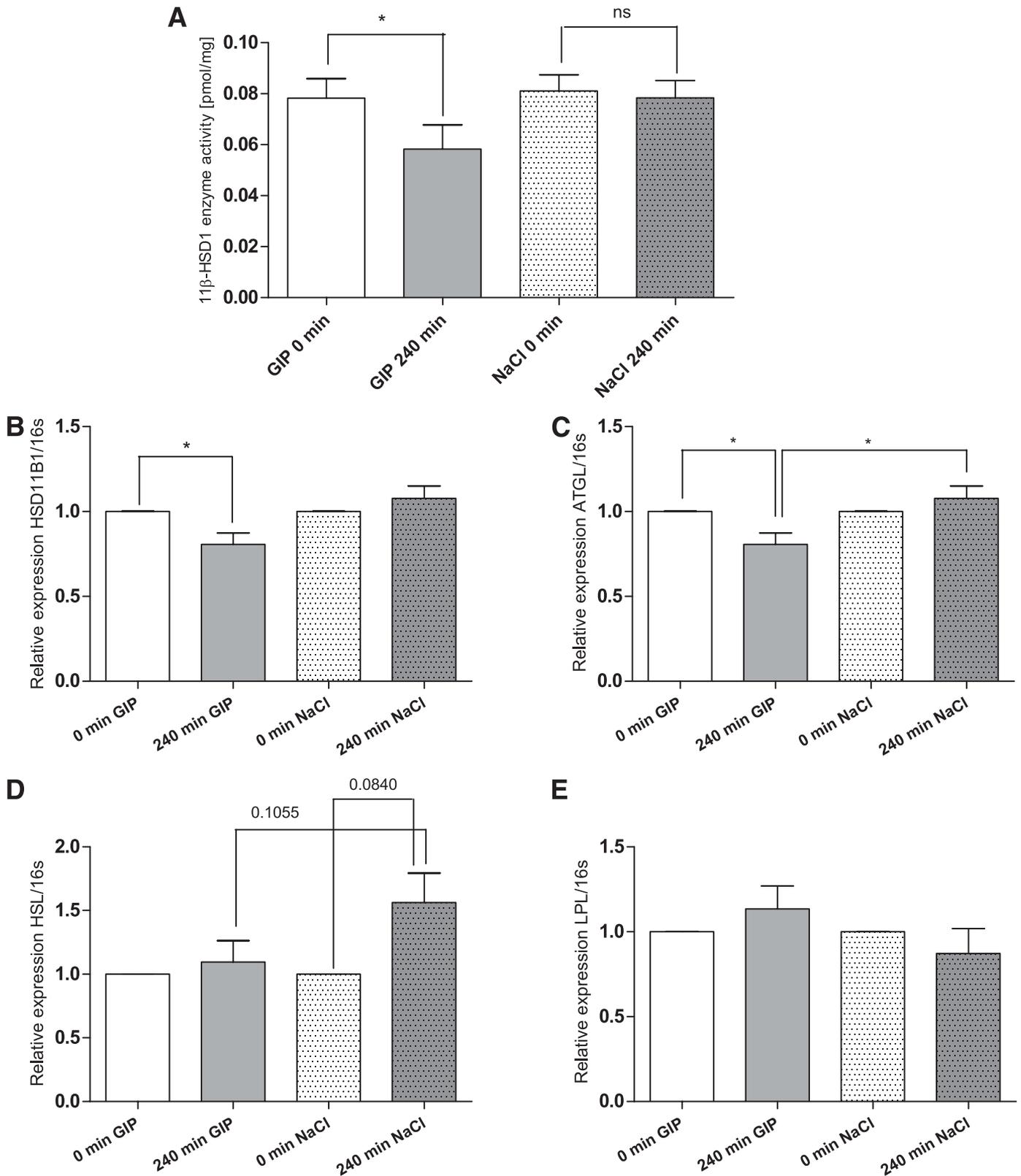
FIG. 6. GIP- and NaCl-induced effects on ex vivo 11β-HSD1 activity (*A*), HSD11B1 (*B*), ATGL (*C*), HSL (*D*) and LPL mRNA expression (*E*) in human subcutaneous fat biopsies (*n* = 11) relative to baseline (0 min). Data are mean ± SEM; *P* < 0.05.

Mutation of these binding sites abolished GIP effects, suggesting that these transcription factors may be involved in GIP-induced suppression of 11β-HSD1 expression. It is notable that the promoter constructs used here contain the human promoter, whereas the experiments were performed in a murine cell line, which may have influenced results.

We next found a GIP-induced decrease of 11β-HSD1 gene expression and enzyme activity in differentiated

3T3-L1 cells. GIP and both a pharmacological and an siRNA-induced inhibition of 11β-HSD1 reduced FFA release in vitro in 3T3-L1 cells, whereas the intake of FFAs was not affected. These findings would be consistent with an inhibition of lipolysis or an increased reesterification rate of intracellular FFAs (7,8). Our data cannot conclusively differentiate between those mechanisms. Although the changes of enzymes involved in lipolysis (ATGL and HSL) in 3T3-L1 and subcutaneous fat biopsies may support a changed rate of lipolysis (but not directly demonstrate it), the unchanged circulating glycerol levels may argue against this and support more pronounced effects on reesterification. Of note, two previous studies demonstrate an increased glycerol release under GIP exposure, which, however, was found only at supraphysiological GIP concentrations, whereas no effect on glycerol release was described at concentrations comparable to those used in our study (7,26). ATGL and HSL, two central enzymes regulating lipolytic pathways (27), were reduced by both GIP and inhibition of 11β-HSD1, supporting that GIP and 11β-HSD1 directly interfere with lipolytic pathways. We next analyzed whether those two pathways are functionally linked. Pharmacological preinhibition and siRNA-based knockdown of 11β-HSD1 abolished further effects of GIP on lipolysis. These data provide evidence that the GIP-induced inhibition of 11β-HSD1 has physiological relevance by mediating the inhibitory effects of GIP on FFA release in adipose tissue. 11β-HSD1 has been shown to affect the conversion of cortisone to cortisol, and intracellular cortisol availability is therefore likely to contribute to the described effects. However, we did not directly measure the intracellular cortisol concentration and, therefore, cannot completely exclude that other 11β-HSD1–associated effects may contribute to our observations. Although a widely used model of adipocyte biology, 3T3-L1 cells are functionally not directly comparable to the functionality of human adipocytes, which may also limit interpretation of our results. It is notable that all observed findings occurred relatively quickly. In fact, we believe that our results do not demonstrate that the downregulation of 11β-HSD1 mRNA is mandatory for the fast effects on FFA release, whereas reduced HSD1 activity appears to be crucial. The rather fast changes may be induced by protein modification or by regulation of cofactor availability and potentially prolonged changes based on the observed mRNA changes. A second model might explain the fast response by fast mRNA changes and a high protein turnover, although this was not investigated in our study. Finally, early changes of other hormones (i.e., insulin secretion) might also contribute to the post-GIP changes of lipolysis within the clinical trial. Although we found no formally significant early insulin changes, we cannot exclude such a mechanism, considering that we did not measure insulin at very early time points. Although not depicted in our study, such changes may influence subsequent FFA release.

To support the existence of the effects in humans presented herein, a controlled crossover trial investigating the effects of an acute elevation of GIP on fatty acid metabolism and adipose tissue gene expression was used. This trial was performed under euglycemic and normoinsulinemic conditions. Thus, this study did not aim to mirror the postprandial situation, which is characterized by changes of various hormones, making the identification of GIP-specific effects difficult. Nevertheless, the circulating GIP concentrations induced by GIP infusion and the time period of infusion (4 h) were comparable to conditions found in a postprandial situation (28,29). Indeed, FFAs were time-dependently reduced during GIP infusion compared with saline. This clinical finding directly supports the in vitro data from our and other groups and recent results from other human trials. Specifically, the elegant studies by Asmar et al. (8) are supported by the findings presented here, although the studies differ to some extent; Asmar et al. did not describe differences in fatty acid metabolism under euglycemia, which may be partially explained by different patient's characteristics. Obese or older individuals may show different responses to circulating GIP-respective adipose tissue metabolism, and we recently described that metabolic actions of GIP in mice vary significantly with age (30). The data of Asmar et al. (8) support that reesterification may be a relevant mechanism linking GIP and fatty acid metabolism. Accordingly, no significant differences of glycerol were found in our human trial. We nevertheless show that ATGL and HSL expression is modified by GIP. Although changes at the protein level may not be realistic within the time frame analyzed here, we also found a GIP-induced reduction of a Ser565 phosphorylation of HSL under rather high GIP concentrations in 3T3-L1 cells. Therefore, a differential phosphorylation mechanism may potentially link GIP and regulation of lipolysis. These data suggest that both mechanisms, reesterification and lipolytic pathways, may be relevant in the GIP-dependent regulation of fatty acid metabolism.

Given the in vitro data presented herein, which suggests a functional link between GIP and 11β-HSD1, we next analyzed potential effects on 11β-HSD1 in subcutaneous fat tissue biopsies. GIP induced a reduction of 11β-HSD1 expression and enzyme activity ex vivo, which supports that the mechanism demonstrated in vitro may be comparably relevant in humans in vivo. These findings add novel perspectives to the potential value of 11β-HSD1 inhibition because this is, to our knowledge, the first set of data directly linking the incretin-based regulation of peripheral tissue metabolism and 11β-HSD1 under basal conditions.

Although the comparable data of in vitro studies and a human randomized controlled trial strengthen the presented results, some limitations need to be considered. The physiological relevance of our data in the postprandial situation was not analyzed in our study. A previous report demonstrates that obese individuals have elevated GIP levels (9), which supports that our findings are likely to have physiological relevance in the fasting situation. Only obese males were investigated, and it is unclear whether the findings can be transferred to women or lean individuals. Other hormonal systems (e.g., estrogens) may have considerable effects on the regulation of lipolysis, making a generalization of the presented results difficult. A reduced 11β-HSD1 expression and activity was found ex vivo in subcutaneous adipose tissue biopsies of the clinical trial. However, the biopsies were not further separated into adipocytes and a stromal vascular fraction. Although the finding of comparable modifications in 3T3-L1 adipocytes supports that the demonstrated effects in human biopsies are likely to result from changes in adipocytes, effects on other cell types, such as vasculature or macrophages, cannot be excluded. Some of the mentioned limitations may be sufficiently addressed in rodent experiments (e.g., with mice having a fat-specific 11β-HSD1 knockout), which therefore would be highly desirable to further improve our understanding of GIP-dependent peripheral effects. Finally, all of our data are based on short-term exposure to GIP. There is considerable evidence that hormonal

short-term effects may affect physiological end points, which are opposing those later observed in long-term trials. Therefore, a definitive answer to the question of whether GIP should be enhanced or blocked to prevent or treat metabolic syndrome remains elusive and cannot be answered by the data presented.

In summary, we demonstrate within a randomized controlled human crossover trial that the incretin hormone GIP lowers circulating FFAs in obese men and has direct effects on 11β-HSD1 and ATGL expression and 11β-HSD1 enzyme activity in human adipose tissue. Considering the provided in vitro data, the observed effects most likely reflect a GIP-induced modification of fatty acid metabolism in an 11β-HSD1–dependent fashion.

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