

# Glucose-Dependent Insulinotropic Polypeptide May Enhance Fatty Acid Re-esterification in Subcutaneous Abdominal Adipose Tissue in Lean Humans

Meena Asmar,<sup>1,2</sup> Lene Simonsen,<sup>1</sup> Sten Madsbad,<sup>3</sup> Bente Stallknecht,<sup>2</sup> Jens Juul Holst,<sup>2</sup> and Jens Bülow<sup>1</sup>

**OBJECTIVE**—Glucose-dependent insulinotropic polypeptide (GIP) has been implicated in lipid metabolism in animals. In humans, however, there is no clear evidence of GIP effecting lipid metabolism. The present experiments were performed in order to elucidate the effects of GIP on regional adipose tissue metabolism.

**RESEARCH DESIGN AND METHODS**—Eight healthy subjects were studied on four different occasions. Abdominal subcutaneous adipose tissue metabolism was assessed by measuring arterio-venous concentration differences and regional adipose tissue blood flow during GIP (1.5 pmol/kg/min) or saline infused intravenously alone or in combination with a hyperinsulinemic-hyperglycemic (HI-HG) clamp.

**RESULTS**—During GIP and HI-HG clamp, abdominal subcutaneous adipose tissue blood flow, hydrolysis of circulating triacylglycerol (TAG) ( $P = 0.009$ ), and glucose uptake ( $P = 0.03$ ) increased significantly while free fatty acid (FFA) output ( $P = 0.04$ ) and FFA/glycerol release ratio ( $P = 0.02$ ) decreased compared with saline and HI-HG clamp.

**CONCLUSIONS**—In conclusion, GIP in combination with hyperinsulinemia and slight hyperglycemia increased adipose tissue blood flow, glucose uptake, and FFA re-esterification, thus resulting in increased TAG deposition in abdominal subcutaneous adipose tissue. *Diabetes* 59:2160–2163, 2010

Several animal studies support the idea that glucose-dependent insulinotropic polypeptide (GIP) may play a direct role in lipid metabolism, which could be to ensure efficient deposition of dietary fat in body stores in times of plenty (1,2). GIP enhances insulin release during a meal and because insulin is a major hormonal regulator of lipogenesis, a component of GIP's action on fat metabolism is probably indirect (3–5). However, there is no clear evidence of a GIP effect on lipid metabolism in humans. We recently studied the effect of GIP on the removal rates of plasma triacylglycerol (TAG) and on free fatty acid (FFA) concentrations, which were increased after either a mixed meal or infusion of Intralipid and insulin (6). Under these experimental condi-

tions, we were not able to demonstrate any effects of GIP on the removal rates of either chylomicron-TAG or Intralipid TAG concentrations. However, we found evidence for enhanced FFA re-esterification under conditions with combined high plasma GIP and insulin concentrations. Based on these findings, we hypothesized that GIP per se plays a role in the regulation of adipose tissue re-esterification of FFA, a process that is of central importance in adipose tissue handling of fatty acids (7,8). Therefore, the aim of the present study was to elucidate the effects of GIP alone or in combination with hyperinsulinemia and hyperglycemia on regional adipose tissue metabolism.

## RESEARCH DESIGN AND METHODS

Eight healthy males with a mean age of 30 years  $\pm$  6 (means  $\pm$  SD) and BMI of 23.2  $\pm$  1.4 were studied. The ethics committee in Copenhagen Municipality approved the protocol (H-D-2008-043).

**Experimental design.** Each subject participated in randomized order in four different experiments separated by approximately 3 weeks. On two of the occasions, the subjects underwent a hyperinsulinemic (150–200 pmol/l)–hyperglycemic (6.5–7 mmol/l) (HI-HG) clamp with continuous infusion of either GIP (1.5 pmol<sup>-1</sup> · kg<sup>-1</sup> · min<sup>-1</sup>) or saline during 300 min. On two other occasions, GIP or saline was infused alone during the study.

**Protocol and methods.** The subjects' habitual dietary intakes were recorded for 1 day before the first experiment and were replicated before subsequent experiments. The subjects arrived at 8:00 a.m. after having fasted for at least 12 h. The investigations were performed with the subjects in a supine position in a room kept at 24°C. A catheter was inserted into an antecubital vein for the infusion of GIP, glucose, insulin, or saline. The subjects were further catheterized in a subcutaneous vein on the anterior abdominal wall (9) and in a radial artery. After three baseline measurements, a continuous infusion of either GIP or saline alone or in combination with an HI-HG clamp was initiated at time = 0.

**GIP infusion and HI-HG clamp.** Synthetic human GIP (1–42) (Polypeptide Laboratories, Wolfenbüttel, Germany) was dissolved in sterilized water containing 2% human serum albumin (Human Albumin; CSL Behring, Germany) and sterile filtrated. Vial content was tested for sterility and bacterial endotoxins (European pharmacopoeia 2.6.14, Method C, turbidimetric kinetic method). The peptide was demonstrated to be >97% pure and identical to the natural human peptide by high-performance liquid chromatography, mass, and sequence analysis.

Insulin (Actrapid Human; Novo Nordisk, Denmark) was infused at a continuous rate of 10  $\mu\text{U} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ . During concomitant GIP infusion, the insulin infusion rate was adjusted according to the expected endogenous insulin secretion in response to GIP aiming toward plasma insulin levels comparable with those obtained during insulin infusion alone. Therefore, insulin was infused at a lower rate (7  $\mu\text{U} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ) under these conditions.

**Adipose tissue metabolism.** Adipose tissue metabolism was measured by Ficks principle (10). Adipose tissue blood flow (ATBF) was measured continuously by the <sup>133</sup>Xenon washout technique (11).

**Blood samples and analysis.** Arterial and venous blood samples were drawn simultaneously at time –30, –15, 0, and hereafter every 30 min until discontinuation of the infusion. Glucose concentrations were measured every 10 min for clamp adjustments.

Blood samples were analyzed for TAG, glycerol, FFA, and glucose by enzymatic methods modified to run on a Hitachi 912 automatic analyzer (Boehringer Mannheim). In addition, arterial blood was analyzed for GIP (12)

From the <sup>1</sup>Department of Clinical Physiology/Nuclear Medicine, Bispebjerg Hospital, Copenhagen, Denmark; the <sup>2</sup>Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; and the <sup>3</sup>Department of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark.

Corresponding author: Jens Bülow, jlb04@bbh.regionh.dk.

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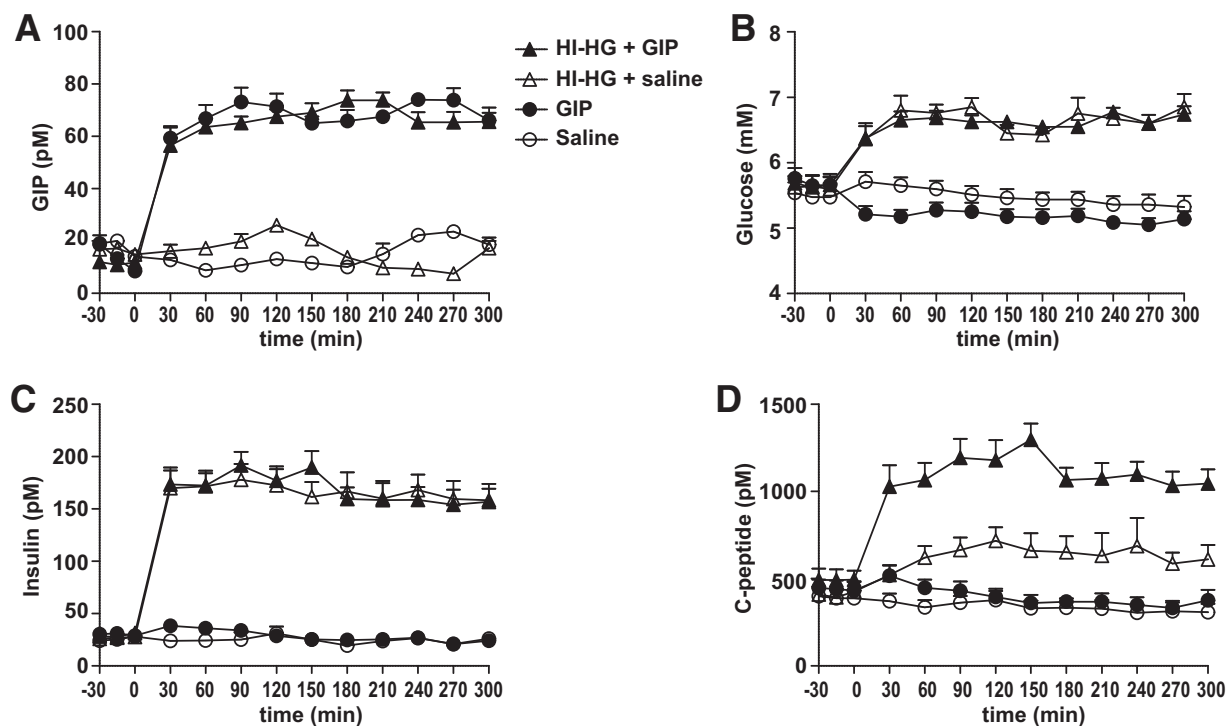


FIG. 1. Data are means  $\pm$  SEM. Plasma arterial GIP (A), glucose (B), insulin (C), and C-peptide (D) concentrations during HI-HG clamp experiment with GIP ( $\blacktriangle$ ) and without GIP ( $\triangle$ ), GIP alone ( $\bullet$ ), or saline alone ( $\circ$ ).

and insulin and C-peptide by autoDELPHIA automatic fluoroimmunoassays (Wallac, Turku, Finland).

**Statistical analysis.** All results are presented as means  $\pm$  SEM. Changes in concentrations with time and between the four experiments were compared using mixed linear models with repeated measures where time, treatment, and interaction time  $\times$  treatment were included in the model as fixed factors, and the subjects were included as random effects. The *t* test for paired data was used for comparing GIP and saline experiments. A significance level of 0.05 was chosen. The Statistical Analysis Software, version 9.1, was used for statistical calculations.

## RESULTS

**Arterial hormone and glucose concentrations.** During GIP alone and GIP in combination with the HI-HG clamp experiment, intact GIP concentrations reached physiological postprandial levels after  $\sim$ 60 min at  $68.3 \pm 1.5$  and  $66.5 \pm 1.6$  pmol/l, respectively (Fig. 1A). During infusion of saline alone and saline in combination with the HI-HG clamp experiment, GIP concentrations remained at fasting levels ( $14.7 \pm 1.6$  and  $15.8 \pm 1.8$  pmol/l, respectively; *P* = not significant).

During the HI-HG clamp experiment with and without GIP, insulin concentrations increased rapidly during the first 30 min to a mean plateau of  $169 \pm 4$  and  $167 \pm 2$  pmol/l, respectively (*P* < 0.0001) (Fig. 1C). C-peptide concentrations increased as well during the HI-HG clamp experiment with and without GIP from fasting levels at  $493 \pm 7$  and  $409 \pm 9$  pmol/l, respectively (*P* = not significant) (Fig. 1D) to a mean plateau of  $1,108 \pm 27$  and  $635 \pm 17$  pmol/l, respectively (*P* < 0.0001). During GIP alone, insulin and C-peptide concentrations increased slightly but significantly during the first 60 min compared with saline (*P* = 0.02) (Fig. 1C and D), while no difference occurred during 60–300 min.

During the HI-HG clamp experiments with and without GIP, glucose levels increased toward target levels during the first 60 min ( $6.7 \pm 0.1$  and  $6.8 \pm 0.2$  mmol/l, respectively) (Fig. 1B) and remained constant and similar during

60–300 min ( $6.6 \pm 0.1$  and  $6.7 \pm 0.1$  mmol/l). There was no significant difference between the amounts of glucose infused during these two experiments ( $118 \pm 11$  and  $110 \pm 8$  g). During GIP infusion alone, a decrease in glucose concentrations occurred during the first 60 min compared with saline infusion (*P* = 0.01) as a result of stimulation of endogenous insulin secretion during GIP infusion (Fig. 1B). No significant differences in plasma glucose concentrations were seen during 60–300 min.

**Arterial metabolite concentrations.** The arterial concentrations of the metabolites during the four experiments are given in Fig. 2. During the HI-HG clamp experiments with and without GIP, arterial TAG, FFA, and glycerol concentrations decreased significantly with no differences seen between the two experiments. During GIP or saline alone, arterial TAG concentrations remained constant and no differences were seen between the two experiments. Arterial FFA and glycerol concentrations did not differ significantly during saline and GIP infusions alone and increased steadily during the 300 min.

**Adipose tissue metabolism.** Figure 3 shows the subcutaneous abdominal adipose tissue blood flow during the four experiments. During the GIP and HI-HG clamp experiment, ATBF increased significantly during the first 30 min, reached a plateau of  $3.9 \pm 0.4$  versus  $1.4 \pm 0.1$  ml  $\cdot$  min $^{-1}$   $\cdot$  (100 g tissue) $^{-1}$  after 90 min, and differed significantly from the saline and HI-HG clamp experiment ( $1.4 \pm 0.1$  ml  $\cdot$  min $^{-1}$   $\cdot$  [100 g tissue] $^{-1}$ , *P* < 0.0001). During the saline and HI-HG clamp experiment, ATBF remained virtually constant with a tendency toward a slight decrease at the end of the experiment. During GIP alone, a slight but significant ATBF increase was seen during the first 60 min when compared with saline ( $1.6 \pm 0.02$  vs.  $1.1 \pm 0.01$  ml  $\cdot$  [100 g tissue] $^{-1}$   $\cdot$  [60 min] $^{-1}$ , respectively; *P* = 0.04) concomitant with elevated insulin levels. After 60 min, ATBF was similar during the two

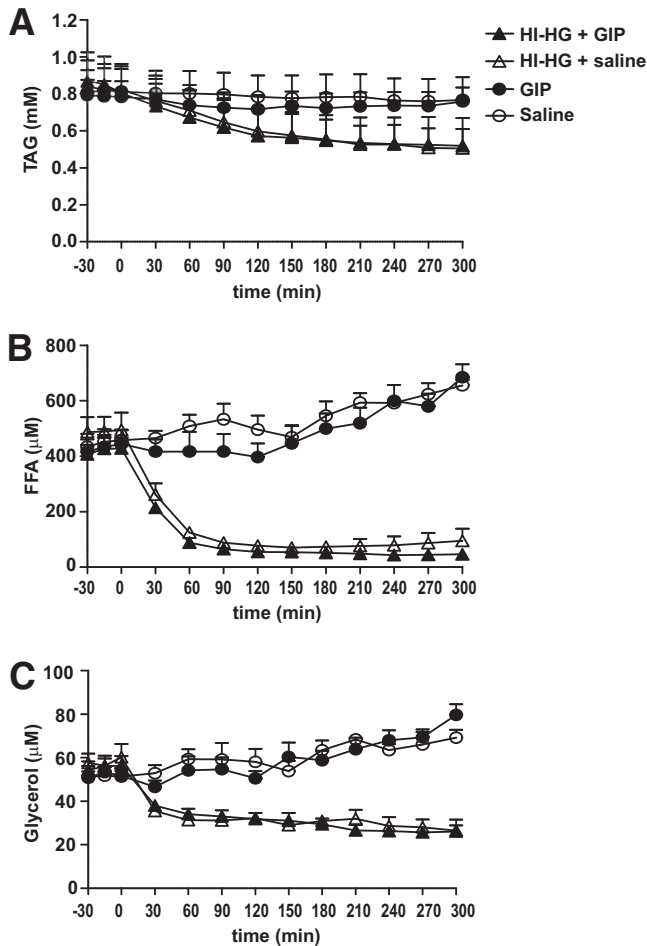


FIG. 2. Plasma arterial TAG (A), FFA (B), and glycerol (C) concentrations during HI-HG clamp experiment with GIP ( $\blacktriangle$ ) and without GIP ( $\triangle$ ), GIP alone ( $\bullet$ ), or saline alone ( $\circ$ ).

experiments and increased steadily and differed significantly from ATBF during the HI-HG clamp experiment without GIP during the last hour ( $P < 0.05$ ).

Figure 4 shows net fluxes of TAG, FFA, glycerol, and glucose in adipose tissue. Baseline TAG, FFA, glycerol, and glucose fluxes were similar in the four experiments (Fig. 4A, C, E, and G). TAG hydrolysis ( $P = 0.009$ ) (Fig. 4B) and glucose uptake ( $P < 0.03$ ) (Fig. 4H) increased and FFA output ( $P = 0.04$ ) (Fig. 4D) decreased significantly

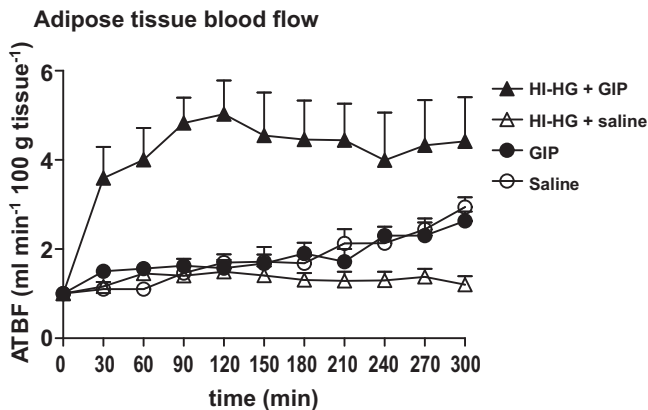


FIG. 3. Data are means  $\pm$  SEM. Adipose tissue blood flow during HI-HG clamp experiment with GIP ( $\blacktriangle$ ) and without GIP ( $\triangle$ ), GIP alone ( $\bullet$ ), or saline alone ( $\circ$ ).

during the GIP and HI-HG clamp experiment compared with the HI-HG experiment without GIP. In addition, a decreased FFA-to-glycerol output ratio was seen during the GIP and HI-HG clamp experiment ( $P = 0.02$ ) (Fig. 4J) compared with the HI-HG clamp alone. No differences were seen in TAG, FFA, glycerol, and glucose fluxes between the GIP and saline experiments.

## DISCUSSION

The major finding in the present study is that GIP in combination with hyperinsulinemia and hyperglycemia increased blood flow, glucose uptake, TAG hydrolysis, and FFA re-esterification, resulting in increased TAG deposition in the anterior, abdominal, subcutaneous adipose tissue. Under fasting conditions, GIP did not affect adipose tissue lipid metabolism.

Since the possible metabolic effects elicited via GIP in adipose tissue has not been described previously, we used a prolonged HI-HG clamp technique with plasma glucose and insulin concentrations similar to those found after ingestion of a carbohydrate-rich meal. It was found that during the HI-HG clamp experiment in combination with GIP, TAG hydrolysis increased significantly compared with the HI-HG clamp experiment without GIP. This effect was brought about concomitantly with a significant increase in the subcutaneous ATBF. ATBF increases significantly postprandially, and this increase appears to be of particular importance in the regulation of lipid metabolism by facilitating transport and deposition of lipids in adipose tissue (7). Insulin per se does not seem to affect ATBF, however insulin may indirectly stimulate ATBF. Recently, a study reported that ATBF was markedly higher after oral glucose than during the intravenous insulin-glucose infusions (13). The present findings suggest that GIP has vasoactive effects in adipose tissue, although the design of the study cannot rule out that other substances may play a role. C-peptide has been found to have dose-dependent vascular effects in skeletal muscle in the concentration range between 0–1 nmol/l (14). In the present experiments, the C-peptide concentration increased from about 0.4 to 0.7 nmol/l in the HI-HG clamp experiments without a concomitant increase in adipose tissue blood flow. In the HI-HG clamp experiment with GIP, the C-peptide concentration increased to about 1 nmol/l. However, in light of the missing flow increase in GIP HI-HG clamp experiment, it seems unlikely that the flow increase found in clamp experiments with GIP primarily is elicited via C-peptide.

Simultaneously with the increase in adipose tissue blood flow, an increase in adipose tissue TAG hydrolysis took place, probably reflecting an increased substrate supply to lipoprotein lipase (LPL). Samra et al. (15) have demonstrated that when adipose tissue blood flow increased by infusion of adrenaline, TAG hydrolysis increased exactly in parallel with increased blood flow, implying that TAG hydrolysis is normally limited by substrate delivery, which is consistent with our present study.

While TAG hydrolysis was significantly higher during the HI-HG clamp with GIP compared with the clamp without GIP (Fig. 4), the FFA release was lower in the clamp experiment with GIP. This suggests that FFA derived from LPL-mediated hydrolysis of the circulating TAG was directed into the adipose tissue probably to be esterified and stored, similar to what has been shown previously in subjects examined in the fed state (8). Concomitantly with the FFA uptake, there was an increase

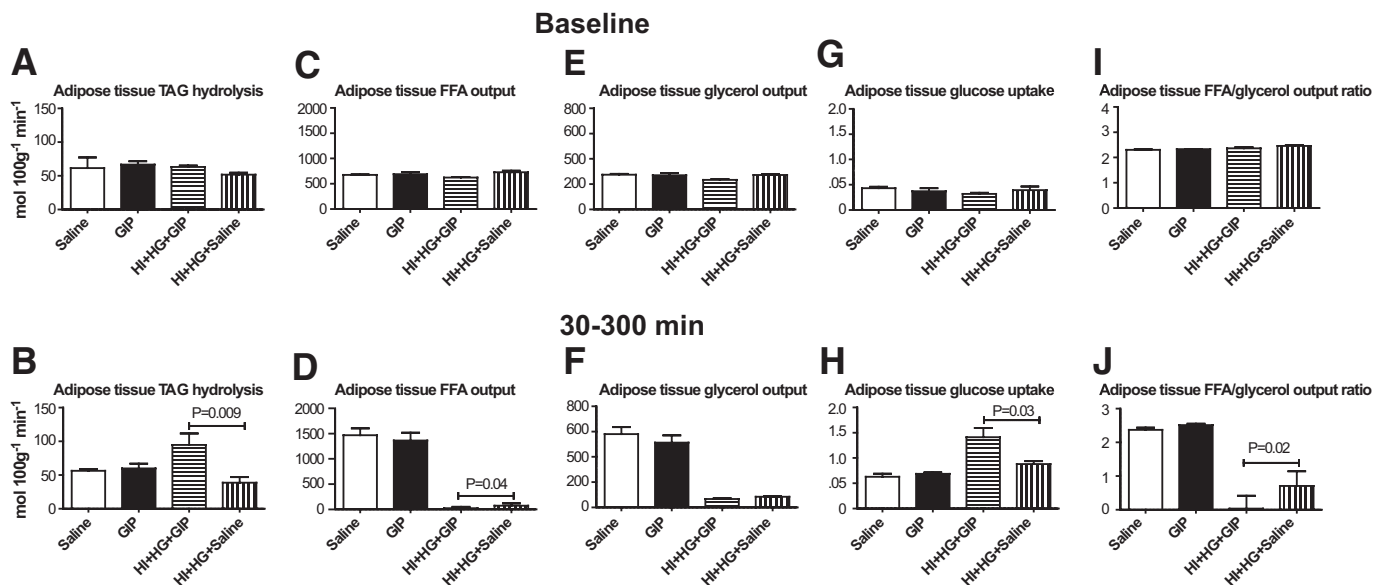


FIG. 4. Data are means  $\pm$  SEM. Average net fluxes of TAG (A and B), FFA (C and D), glycerol (E and F), and glucose (G and H) in abdominal subcutaneous adipose tissue during the preinfusion baseline period and in the period from 30–300 min after commencement of infusions. Average adipose tissue FFA-to-glycerol output ratio (I and J) during the preinfusion baseline period and in the period from 30–300 min after commencement of infusions.

in glucose uptake during GIP in combination with insulin. Taken together, these results indicate that GIP directs FFA released by LPL-mediated TAG hydrolysis toward tissue uptake instead of release to the circulation.

In vitro studies have shown that GIP stimulates lipolysis (16,17). However, McIntosh et al. (16) found that GIP-stimulated lipolysis in 3T3 cells was inhibited by insulin, suggesting that the lipolytic effect of GIP is weaker than the antilipolytic effect of insulin. In the present study, we could not demonstrate any lipolytic effect of GIP under any of the experimental circumstances.

In conclusion, GIP in combination with hyperinsulinemia and light hyperglycemia increased adipose tissue blood flow, increased adipose tissue glucose uptake, increased FFA re-esterification, and thus resulted in increased adipose tissue TAG deposition.

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No potential conflicts of interest relevant to this article were reported.

M.A. researched the data, contributed to the discussion, and wrote the manuscript. L.S. researched the data, contributed to the discussion, and reviewed the manuscript. S.M. and B.S. reviewed the manuscript. J.J.H. contributed to the discussion and reviewed the manuscript. J.B. researched the data, contributed to the discussion, and wrote the manuscript.

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#### REFERENCES

- Wasada T, McCorkle K, Harris V, Kawai K, Howard B, Unger RH. Effect of gastric inhibitory polypeptide on plasma levels of chylomicron triglycerides in dogs. *J Clin Invest* 1981;68:1106–1107
- Ebert R, Nauck M, Creutzfeldt W. Effect of exogenous or endogenous gastric inhibitory polypeptide (GIP) on plasma triglyceride responses in rats. *Horm Metab Res* 1991;23:517–521
- Beck B, Max JP. Gastric inhibitory polypeptide enhancement of the insulin effect on fatty acid incorporation into adipose tissue in the rat. *Regul Pept* 1983;7:3–8
- 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. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* 2002;8:738–742
- Starich GH, Bar RS, Mazzaferrri EL. GIP increases insulin receptor affinity and cellular sensitivity in adipocytes. *Am J Physiol* 1985;249:E603–E607
- Asmar M, Tangaa W, Madsbad S, Hare K, Astrup A, Flint A, Bülow J, Holst JJ. On the role of glucose-dependent insulintropic polypeptide in postprandial metabolism in humans. *Am J Physiol Endocrinol Metab* 2010;298:E614–E621
- Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW. Integrative physiology of human adipose tissue. *Int J Obes Relat Metab Disord* 2003;27:875–888
- Ruge T, Hodson L, Cheeseman J, Dennis AL, Fielding BA, Humphreys SM, Frayn KN, Karpe F. Fasted to fed trafficking of fatty acids in human adipose tissue reveals a novel regulatory step for enhanced fat storage. *J Clin Endocrinol Metab* 2009;94:1781–1788
- Simonsen L, Bülow J, Madsen J. Adipose tissue metabolism in humans determined by vein catheterization and microdialysis techniques. *Am J Physiol* 1994;266:E357–E365
- Bülow J. Adipose tissue blood flow during exercise. *Dan Med Bull* 1983;30:85–100
- Simonsen L, Enevoldsen LH, Bülow J. Determination of adipose tissue blood flow with local  $^{133}\text{Xe}$  clearance: evaluation of a new labelling technique. *Clin Physiol Funct Imaging* 2003;23:320–323
- Deacon CF, Nauck MA, Meier J, Hücking K, Holst JJ. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J Clin Endocrinol Metab* 2000;85:3575–3581
- Karpe F, Fielding BA, Ardilouze JL, Ilic V, Macdonald IA, Frayn KN. Effects of insulin on adipose tissue blood flow in man. *J Physiol* 2002;540:1087–1093
- Ekberg K, Johansson BL, Wahren J. Stimulation of blood flow by C-peptide in patients with type 1 diabetes. *Diabetologia* 2001;44:A323
- Samra JS, Simpson EJ, Clark ML, Forster CD, Humphreys SM, Macdonald IA, Frayn KN. Effects of epinephrine infusion on adipose tissue: interactions between blood flow and lipid metabolism. *Am J Physiol* 1996;271:E834–E839
- McIntosh CH, Bremsak I, Lynn FC, Gill R, Hinke SA, Gelling R, Nian C, McKnight G, Jaspers S, Pederson RA. Glucose-dependent insulintropic polypeptide stimulation of lipolysis in differentiated 3T3-L1 cells: wortmannin-sensitive inhibition by insulin. *Endocrinology* 1999;140:398–404
- Yip RG, Boylan MO, Kieffer TJ, Wolfe MM. Functional GIP receptors are present on adipocytes. *Endocrinology* 1998;139:4004–4007