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*Published in:*  
Journal of Clinical Endocrinology and Metabolism

*DOI:*  
[10.1210/jc.2012-3996](https://doi.org/10.1210/jc.2012-3996)

2013

[Link to publication](#)

*Citation for published version (APA):*  
Lindqvist, A., Ekelund, M., Mulder, H., Spéjel, P., Groop, L., Hedenbro, J., & Wierup, N. (2013). Effects of ingestion routes on hormonal and metabolic profiles in gastric-bypassed humans. *Journal of Clinical Endocrinology and Metabolism*, 98(5), E856-E861. <https://doi.org/10.1210/jc.2012-3996>

*Total number of authors:*  
7

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## Effects of Ingestion Routes on Hormonal and Metabolic Profiles in Gastric-Bypassed Humans

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**Context:** Gastric bypass surgery (GBP) results in the rapid resolution of type 2 diabetes. Most studies aiming to explain the underlying mechanisms are limited to data obtained after a postsurgical recovery period, making assessment of confounding influences from, for example, weight loss and altered nutrient intake difficult.

**Objective:** To examine the impact of GBP on hormonal and metabolite profiles under conditions of identical nutrient intake independent of weight loss, we studied GBP patients fitted with a gastrostomy tube to enable the administration of nutrients to bypassed segments of the gut. Thus, this model allowed us to simulate partially the preoperative condition and compare this with the postoperative situation in the same patient.

**Design:** Patients ( $n = 4$ ) were first given a mixed meal test (MMT) orally and then via the gastrostomy tube, preceded by overnight and 2-hour fasting, respectively. Blood samples were assessed for hormones and metabolites.

**Results:** The oral MMT yielded 4.6-fold increase in plasma insulin ( $P < .05$ ), 2-fold in glucagon-like peptide-1 ( $P < .05$ ), and 2.5-fold in glucose-dependent insulintropic peptide ( $P < .05$ ) plasma levels, compared with the gastrostomy MMT. The changes in hormone levels were accompanied by elevated branched-chain amino acid levels (1.4–2-fold,  $P < .05$ ) and suppressed fatty acid levels ( $\sim 50\%$ ,  $P < .05$ ).

**Conclusions:** These data, comparing identical nutrient delivery, demonstrate markedly higher incretin and insulin responses after oral MMT than after gastric MMT, thereby providing a potential explanation for the rapid remission of type 2 diabetes observed after GBP. The simultaneous increase in branched-chain amino acid questions its role as a marker for insulin resistance. (*J Clin Endocrinol Metab* 98: E856–E861, 2013)

Obesity and type 2 diabetes (T2D) are effectively treated by gastric bypass surgery (GBP) (1). Remarkably, GBP improves glycemia prior to weight loss (2) and reduces the risk of developing T2D in obese patients (3). The effect on improved glycemia has been associated with altered levels of the incretin hormones glucagon-like peptide 1 (GLP-1) (4) and glucose-dependent insulintropic peptide (GIP), but the exact mechanism remains

unknown. Enhanced GLP-1 secretion has been established after GBP (2), whereas the effect on GIP is less clear (5). Two hypotheses for the beneficial effects of GBP prevail: 1) excluding the foregut from nutrient exposure eliminates secretion of potential foregut-derived antiincretins (foregut theory); and 2) accelerated delivery of nutrients to the hindgut increases the secretion of incretins (hindgut theory) (6, 7). Most studies on the effects of GBP on T2D rely on samples

ISSN Print 0021-972X ISSN Online 1945-7197  
Printed in U.S.A.

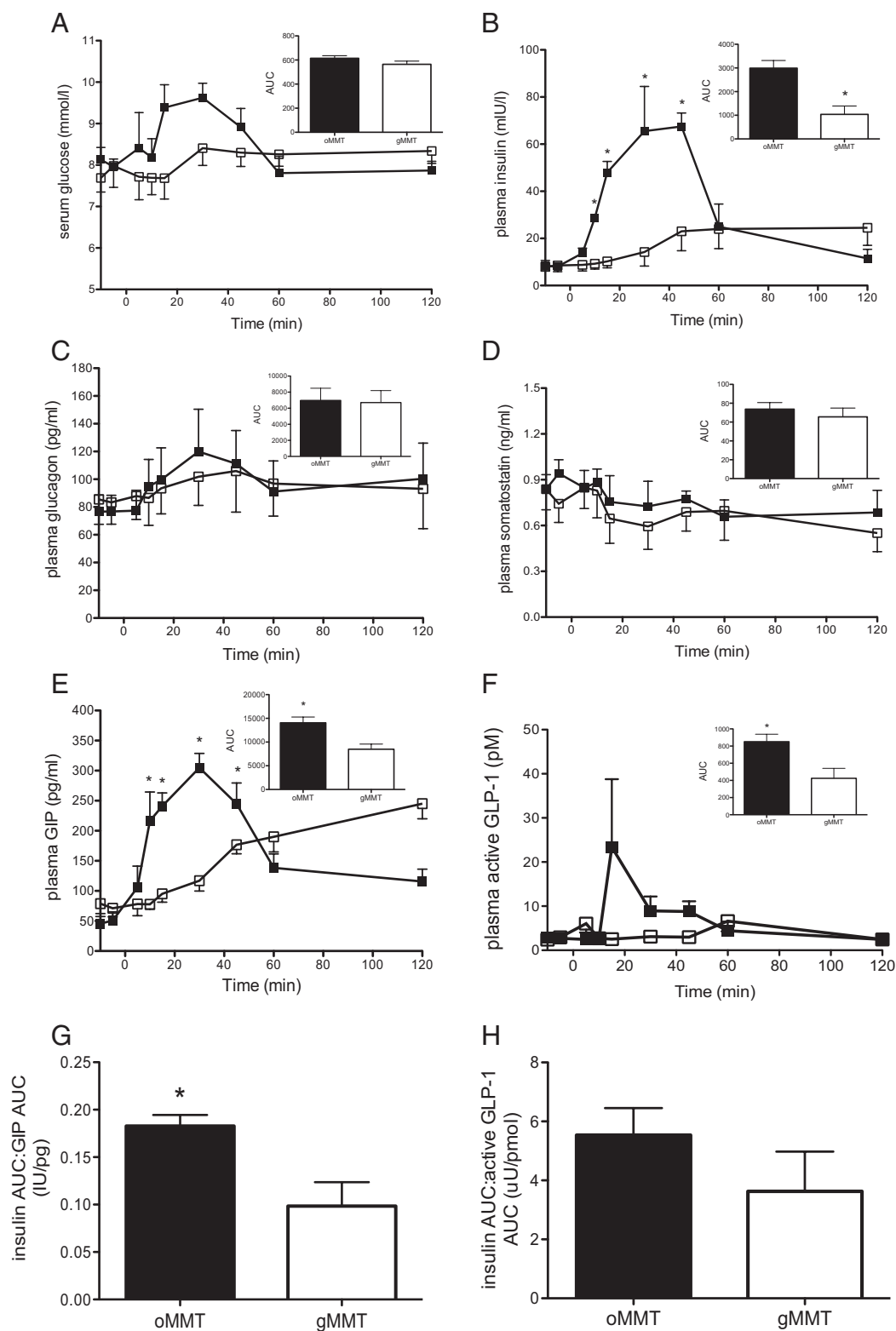
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Received November 26, 2012. Accepted February 22, 2013.

First Published Online April 30, 2013

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Abbreviations: AUC, area under the curve; BCAA, branched-chain amino acid; FA, fatty acid; GBP, gastric bypass surgery; GIP, glucose-dependent insulintropic peptide; GLP-1, glucagon-like peptide 1; gMMT, MMT with a gastrostomy tube; MMT, mixed-meal test; oMMT, oral MMT; T2D, type 2 diabetes.



**Figure 1.** Administration of a MMT orally (closed squares) or via the gastrostomy tube (open squares) elicits differential hormonal response. A, Response in serum glucose to oral ingestion or administration via a gastrostomy tube. In the oMMT, serum glucose responded with a 1.4-fold increase compared with baseline levels, whereas the response to the gMMT was much less pronounced. B, Plasma insulin was greatly increased (6.5-fold) compared to basal levels after the oMMT. The gMMT did not elicit such a response. From 10 minutes until 45 minutes, the oMMT resulted in significantly higher levels of insulin than did the gMMT. C, Plasma glucagon did not differ between the different administration routes of the MMT. D, Somatostatin was unresponsive to both routes of administering the MMT. E, The oMMT resulted in higher GIP secretion than did the gMMT (from 10 until 45 minutes). F, AUC was significantly higher for GLP-1 after the oMMT than after the gMMT. G, Insulin AUC/GIP AUC was significantly higher after the oMMT than after the gMMT. H, Insulin AUC/GLP-1 AUC did not differ between the 2 routes. Inserts in panels A–F show AUC calculations. Data are expressed as means  $\pm$  SEM. Statistical significance was assessed using the paired Student's *t* test. \**P* < .05.

obtained after a postsurgery recovery period, making it difficult to assess potential influence of weight loss, altered nutrient intake, and inflammation.

In the present study, we evaluated the influence of GBP on hormones and metabolites in GBP patients fitted with a temporary gastrostomy tube. This enabled the administration of identical amounts of nutrients to the bypassed segments of the gut (gastroduodenal route) and the new route created by GBP (orojejunal route) (Supplemental Figure 1, published on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>). Thus, we could mimic the preoperative condition and compare to the postoperative condition.

## Subjects and Methods

The study was approved by the Human Ethical Committee in Lund, Sweden, and adhered to the standards of the Declaration of Helsinki. All patients gave informed consent. Patients were recruited from 2 units specializing in bariatric surgery (Departments of Surgery, Scania University Hospital, and Aleris Obesity, Lund, Sweden). The patients were all women; average age  $37.5 \pm 4$  years, weight  $92.8 \pm 12.3$  kg, height  $164 \pm 2.2$  cm, and body mass index  $34.6 \pm 4.6$  kg/m<sup>2</sup>. The decision to insert a gastrostomy tube was taken by the surgeon as a precaution for problems in the immediate postoperative period. One patient was diagnosed with T2D 6 months prior to GBP and was treated with metformin preoperatively but not postoperatively.

Subjects underwent Roux-en-Y gastric bypass. A small (15–20 mL) gastric pouch was created and gastrointestinal continuity established via Roux-en-Y-gastrojejunostomy. The gastrostomy tube was placed in the excluded stomach fundus with access through the abdominal wall.

Mixed-meal tests (MMTs) were performed  $30 \pm 3$  days after surgery. After an overnight fast, a catheter was placed in the cubital vein. Blood samples were collected  $-10$  and  $-5$  minutes and 5, 10, 15, 30, 45, 60, and 120 minutes after MMT initiation for a maximum of 20 minutes (maximum 200 mL) [oral MMT (oMMT)] of a low-calorie drink (220 kcal, 14 g protein, 28 g carbohydrates, 5 g fat; Modifast, Stocksund, Sweden). After a 2-hour fasting period, the same procedure was repeated with the exception that Modifast was given via the gastrostomy tube (gMMT) at the same amount and rate as in the oMMT. Water recovery tests (8) verified an empty stomach before and after the gMMT. Blood was collected in chilled EDTA tubes (0.1 mmol/L diprotin A, 500 KIU/mL aprotinin) and serum tubes and centrifuged ( $1500 \times g$ , 15 minutes, 4°C). Samples were stored at  $-80^\circ\text{C}$ .

Glucose was measured using Infinity glucose oxidase kit (ThermoScientific, Middleton, Virginia). An ELISA was used to measure insulin (developed in-house, Department of Clinical Chemistry, Scania University Hospital, Malmö, Sweden), plasma glucagon, GIP and active GLP-1 (Millipore, Billerica, Massachusetts), and plasma somatostatin (Phoenix Pharmaceuticals, Burlingame, California).

Metabolites were profiled by gas chromatography/mass spectrometry as detailed in the Supplemental Material and previously described in detail (9).

## Statistical analyses

Multivariate analysis of metabolite data is described in the Supplemental Material. A paired Student's *t* test and area under the curve (AUC) calculations were performed in GraphPad Prism 5 (GraphPad Software, San Diego, California).

## Results

The oMMT resulted in a 1.4-fold increase in serum glucose ( $P < .05$ ; Figure 1A, Supplemental Table 2) and a 6.5-fold increase in plasma insulin ( $P < .05$ ; Figure 1B) compared with preprandial levels. Levels of glucose and insulin remained elevated for 60 minutes and then returned to baseline. The gMMT provoked a less pronounced response in glucose and insulin. The oMMT yielded a more robust peak in insulin secretion than the gMMT (10 minutes to 45 minutes;  $P < .05$ ). The administration routes did not affect glucose.

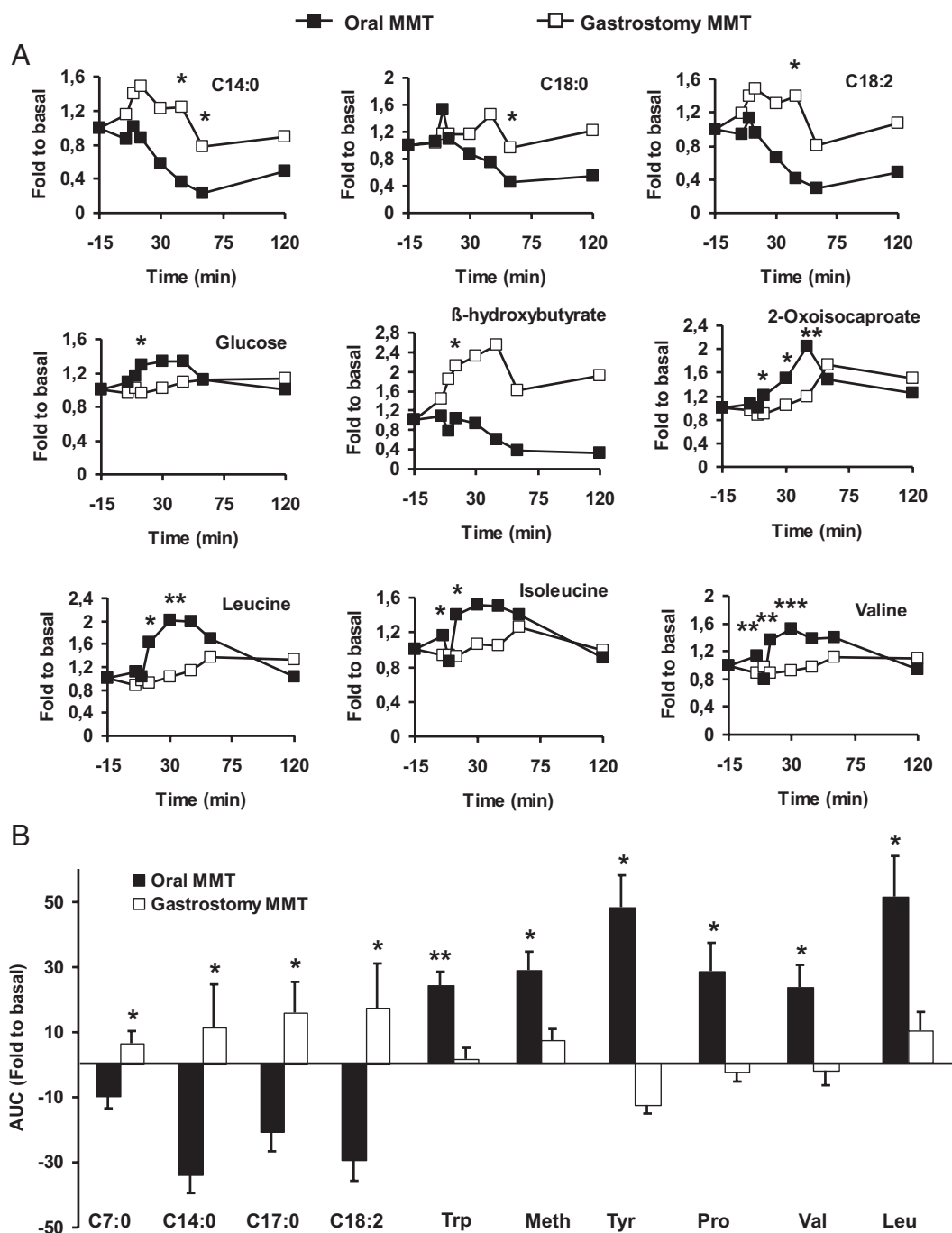
Plasma glucagon and somatostatin levels were similar in the oMMT and gMMT (Figure 1, C and D, respectively). The oMMT yielded a 6-fold increase in GIP ( $P < .05$ ; Figure 1E) compared with the preprandial levels, the response being less pronounced after the gMMT (Figure 1E). The oMMT produced more robust increases in GIP (10 minutes to 45 minutes;  $P < .05$ ) and GLP-1 (Figure 1F) compared with the gMMT. Accordingly, the GIP-AUC and GLP-1-AUC were 2-fold higher during the oMMT compared with the gMMT ( $P < .05$ ; Figure 1, E and F, respectively). The insulin to GIP-AUC-, and insulin to GLP-1-AUC ratios were calculated to investigate the influence of the administration route on insulin secretion in relation to incretins. The insulin to GIP-AUC ratio was 1.8-fold higher ( $P < .05$ ; Figure 1G) during the oMMT compared with the gMMT. No difference was observed in the insulin to GLP-1-AUC ratio (Figure 1H).

Multivariate statistical analysis of the metabolite data revealed a clear impact of administration route on metabolite profiles (Supplemental Figure 2A and Supplemental Table 1). A massive difference in levels of branched-chain amino acids (BCAAs), fatty acids (FAs), and the ketone body  $\beta$ -hydroxybutyrate was observed 30 minutes after

**Table 1.** Metabolic Parameters of the Subjects Participating in the Study

Variable	Value
Age, y	$37.5 \pm 4$
Weight, kg	$92.8 \pm 12.3$
Length, cm	$164 \pm 2.2$
BMI, kg/m <sup>2</sup>	$34.6 \pm 4.6$
HOMA-IR, AU	$2.94 \pm 0.78$
HOMA- $\beta$ , %	$36.8 \pm 12.5$

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA- $\beta$ , homeostasis model assessment of  $\beta$ -cell capacity. Data are presented as mean  $\pm$  SEM for 4 patients.



**Figure 2.** Levels of BCAA and glucose are elevated and levels of ketone bodies and FFA decreased after the oMMT compared with the gMMT. A, Raw data trajectories of metabolite levels expressed as mean normalized to basal levels. B, The AUC of metabolite levels expressed as mean  $\pm$  SEM. Statistical significance was assessed using the paired Student's *t* test. \**P* < .05; \*\**P* < .01; \*\*\**P* < .001.

nutrient administration between the oMMT and the gMMT (Supplemental Figure 2, B–D). Data analysis (Figure 2A) revealed that levels of BCAAs (leucine, valine) and 2-oxoisocaproate were increased 2- (*P* < .01), 1.4- (*P* < .05), and 1.5-fold (*P* < .05), respectively, 30 minutes after the oMMT; after 120 minutes, levels returned to baseline. Glucose reached maximal level after 30 minutes (*P* = .07). Differences in levels of FAs became apparent after 15 minutes. At 45 minutes after the oMMT, levels of C14:0,

C16:0, C17:0, C18:1, and C18:2 were decreased by 65% (*P* < .001), 49% (*P* < .01), 48% (*P* < .05), 58% (*P* < .01), and 59% (*P* < .001), respectively, and at 60 minutes by 76% (*P* < .001), 62% (*P* < .001), 59% (*P* < .001), 75% (*P* < .001), and 71% (*P* < .001), respectively.  $\beta$ -Hydroxybutyrate levels increased by 85% 10 minutes after the gMMT (*P* < .05), whereas there was a continuous decrease after the oMMT; the level of  $\beta$ -hydroxybutyrate was decreased by 52% at 60 minutes (*P* < .01). To de-

termine the overall load of metabolites after the meal, AUCs were calculated (Figure 2B and Supplemental Table 3). The FA AUC increased after the oMMT and decreased after the gMMT. Conversely, AUCs for valine, proline, and tyrosine were increased after the oMMT and decreased after the gMMT. Leucine, tryptophane, and methionine levels increased after both MMTs, although these levels were more pronounced after the oMMT.

## Discussion

The mechanism underlying improved glycemia after GBP remain to be elucidated (10). Hitherto, confounding influence from weight loss, altered nutrient intake, and inflammation (11) have obscured most studies. To circumvent these influences, we investigated GBP-patients fitted with a temporary gastrostomy tube, allowing for a simulated comparison of the pre- and postoperative conditions.

We found that the oMMT generated pronounced elevation of plasma insulin, GIP, and GLP-1 compared with the gMMT. Clearly these results may be affected by oral hydrolysis of carbohydrates by amylase, differences in cephalic phase response, the orosensory effect on hormones and enzymes involved in digestion and secretion, and epithelial changes in the biliopancreatic limb. Water recovery tests (8) ascertained the efficient emptying of the stomach. Thus, faster onset of GIP secretion after the oMMT may suggest a more rapid gastric emptying and a shorter transit time from pouch to intestine in the bypassed route. Plasma levels of GIP and GLP-1 were higher in the oMMT compared with the gMMT, supporting previous observations (12). Hence, distal GIP production may be induced to compensate for the loss of proximal GIP production in the intestine. Contrary to Hansen et al (12), the insulin to GIP-AUC and insulin to GLP-1-AUC ratios presented here suggest that the GIP-mediated incretin effect is altered, whereas the GLP-1-mediated incretin effect is not.

Previous studies have compared the metabolome of GBP patients before surgery with 1 (10% weight loss) (13) or 3 months (17% weight loss) (14) after surgery. Here we observed clear differences in metabolite levels in the absence of weight loss. Suppression of FA release reflected elevated insulin levels and was efficient only in the oMMT. This alteration is likely to contribute to the beneficial effects of GBP on glycemia (15). Interestingly, aromatic amino acids and BCAAs were elevated after the oMMT. Aromatic amino acids have previously been associated with BCAA levels and insulin resistance (16). It has previously been shown that expression of enzymes involved in BCAA metabolism are decreased in obesity and increased after GBP; these changes are negatively correlated with

plasma BCAA levels (17). The mitochondrial branched-chain amino transferase is highly expressed in secretory epithelia throughout the digestive tract, expression being highest in the stomach (18). Additionally, circulating BCAAs may originate from food, proteolysis, and the gut microbiota, all possibly influenced by GBP.

Recently it was shown that BCAAs required elevated FA levels to cause insulin resistance and T2D (16). Thus, our observation of increased BCAAs and decreased FA levels do not necessarily imply detrimental effects on glycemia. A larger decrease in BCAA levels has been observed after GBP than after diet intervention (13), although this may reflect lower nutrient intake after GBP (19). In that study baseline levels of BCAAs were monitored (13). Here we show that BCAA levels are acutely elevated after a meal in GBP patients. One of these BCAAs is the insulin secretagogue leucine (20).

Notably, by using GBP patients fitted with a temporary gastrostomy tube, we were able to mimic the preoperative condition and compare it with the postoperative condition on the same day and in the same patient. Our results revealed complex hormonal and metabolic regulation, partially reflecting the complex composition of the meal. One caveat of the study was that the order of the meals was not randomized, nor did we include a preoperative meal test. Therefore, we cannot exclude a second meal effect. This study design was determined by the limited number of patients requiring gastrostomy tubes. Also, because both intake rate and volume of oMMT differed between patients, it was necessary to perform the oMTT prior to the gMTT to match nutrient intake. To conclude, by performing a mixed-meal test using a gastrostomy tube and comparing it with an oral mixed-meal test, we were able to demonstrate differences in hormones and metabolites between administration routes in gastric-bypassed patients.

## Acknowledgments

Rebecca Johnson, Kristin Gustafsson, Sofia Polits, Ilona Sidlovskaja, and Lena Wierup are gratefully acknowledged for technical assistance.

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This work was supported by grants from the Swedish Research Council including Project Grant Dnr. 521-2007-4037 (to L.G.); Grants Dnr. 2008-4216 and 2013-22243 (to N.W.); Strategic Research Area Grant (EXODIAB: Dnr. 2009-1039); Lin-



naeus Grant (LUDC): Dnr. 349-2008-6589); and an Advanced Research Grant from the European Research Council (Grant GA 269045, to L.G.) as well as equipment grants from Wallenberg (Grant KAW 2009-0243) and Lundberg Foundation (Grant 359). In addition, the project was funded by the European Union Grant ENGAGE, The Albert Pahlsson Foundation, The Swedish Diabetes Foundation, ALF, and The Medical Faculty at Lund University.

Disclosure Summary: The authors have nothing to disclose.

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