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Differential Metabolic Impact of Gastric Bypass Surgery Versus Dietary Intervention in Obese Diabetic Subjects Despite Identical Weight Loss

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Abstract

Glycemic control is improved more after gastric bypass surgery (GBP) than after equivalent diet-induced weight loss in patients with morbid obesity and type 2 diabetes mellitus. We applied metabolomic profiling to understand the mechanisms of this better metabolic response after GBP. Circulating amino acids (AAs) and acylcarnitines (ACs) were measured in plasma from fasted subjects by targeted tandem mass spectrometry before and after a matched 10-kilogram weight loss induced by GBP or diet. Total AAs and branched-chain AAs (BCAAs) decreased after GBP, but not after dietary intervention. Metabolites derived from BCAA oxidation also decreased only after GBP. Principal components (PC) analysis identified two major PCs, one composed almost exclusively of ACs (PC1) and another with BCAAs and their metabolites as major contributors (PC2). PC1 and PC2 were inversely correlated with pro-insulin concentrations, the C-peptide response to oral glucose, and the insulin sensitivity index after weight loss, whereas PC2 was

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uniquely correlated with levels of insulin resistance (HOMA-IR). These data suggest that the enhanced decrease in circulating AAs after GBP occurs by mechanisms other than weight loss and may contribute to the better improvement in glucose homeostasis observed with the surgical intervention.

INTRODUCTION

Gastric bypass surgery (GBP) results in significant weight loss and remission of type 2 diabetes mellitus (T2DM) in 50 to 80% of cases (1). After GBP, diabetes remission occurs rapidly (within days), well before a large amount of weight loss has occurred, or even without weight loss in patients with lower starting body mass index (BMI; the ratio of weight in kilograms to height in meters squared). Insulin release in response to an oral glucose load is delayed in T2DM because of pancreatic β cell dysfunction, which results in elevated postprandial blood glucose concentrations. In obese subjects with T2DM, weight loss induced by GBP, but not by diet intervention, results in recovery of early-phase insulin release after an oral glucose load and a greater reduction of postprandial plasma glucose concentrations (2). Thus, factors other than weight loss play a pivotal role in the improved glucose tolerance observed in diabetic subjects in response to GBP.

Incretins—the gut hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are secreted during meals that stimulate postprandial insulin release—have emerged as potential mediators of the remarkable improvements in glucose homeostasis after GBP. Not only do nutrient-stimulated plasma GLP-1 and GIP concentrations increase significantly shortly after GBP (3, 4), but their incretin effect on insulin secretion, blunted in diabetes, is restored to the level of a nondiabetic individual (4). These changes occur 1 month after GBP, persist for years (5, 6), and are not seen after diet-induced weight loss (2).

Factors other than incretins may also contribute to improvements in metabolic control in response to GBP. Recent studies demonstrate a strong correlation between the concentrations of plasma branched-chain amino acids (BCAAs) and related metabolites with insulin resistance in multiple human cohorts (7–9), and animal studies suggest that elevations in BCAAs can contribute to loss of insulin sensitivity (7). These findings raise the possibility that the rapid remission of diabetes in GBP subjects may be related to more pronounced changes in BCAAs or other metabolites compared to other weight loss interventions. Therefore, here, we have profiled a spectrum of amino acids (AAs) and acylcarnitines (ACs) to gain an understanding of the differential metabolic responses to weight loss induced by diet intervention compared to GBP surgery in morbidly obese patients with T2DM.

RESULTS

Subject cohorts

Two independent cohorts of obese subjects were studied at the New York Obesity Nutrition Research Center (NYONRC) and Duke University, and protocols for each study group are detailed individually in Materials and Methods. Data described in Results refer to the NYONRC cohort unless otherwise specified.

Choice of metabolic assays

Here, we have profiled 45 different species of ACs and 15 AAs by flow injection–tandem mass spectrometry. As highlighted in other work from our group (7–9) and summarized in Fig. 1, this set of analytes provides important information about substrate selection and

pathways of energy metabolism. ACs are generated during mitochondrial metabolism of fatty acids, AAs, and glucose, with some species being particularly reflective of one or the other of these pathways (Fig. 1). For example, even-chained, medium-to-long–chained AC species are generated almost exclusively from fatty acid oxidation, whereas C3 and C5 ACs come primarily from AA oxidation pathways, particularly BCAA oxidation (7, 10). AA and AC profiles can be correlated with an important set of physiological variables previously measured in the current study subjects, including levels of key glucoregulatory hormones. All of the subjects in the NYONRC cohort underwent an oral glucose tolerance test (OGTT; see Materials and Methods), which included measurements of blood glucose, insulin, and incretin hormone levels during the test (4). Insulin secretion is normally biphasic during OGTT, with an early (first 30 min) and a late phase. In T2DM, glucose-stimulated insulin secretion is impaired, with the early response being essentially absent, and this alteration contributes to deterioration of glucose tolerance during OGTT in T2DM. In our studies, early-phase insulin secretion was measured as the difference (Δ) in plasma concentrations of C-peptide and/or insulin 30 min after oral glucose administration compared to baseline (before oral glucose administration).

Baseline metabolic measures

As described previously (2), there were no differences between the GBP and diet intervention subject groups at baseline, before the weight loss intervention, according to these parameters: mean age, BMI, duration of diabetes since diagnosis, and fasting hemoglobin A1c (HbA1c; a measure of glycated Hb); fasting plasma concentrations of glucose, pro-insulin, insulin, C-peptide (a product of pro-insulin processing to insulin), and glucagon (a hormone that opposes the anabolic actions of insulin); and plasma glucose, insulin, GLP-1, and GIP concentrations during an OGTT. Further measurements conducted as part of this study and summarized in Table 1 reveal that subjects in the GBP and diet intervention groups did not differ in terms of total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, uric acid (a product of purine metabolism), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (liver enzymes), C-reactive protein (CRP; a marker of inflammation), leptin (an adipocyte hormone indicative of fat stores), ghrelin (a gastric peptide implicated in food intake regulation), ketones (total and β-hydroxybutyrate), lactate (product of anaerobic glucose metabolism), and, finally, a wide array of AAs and ACs measured by tandem mass spectrometry (Table 1).

Effects of GBP and diet intervention

Similar metabolic effects of GBP and diet intervention—The decreases in body weight and BMI were similar in the GBP and diet intervention groups as per study design (Table 2). Although the rate of weight loss was faster after GBP (2.7 kg/week) than after diet intervention (1.3 kg/week, $P = 0.003$), the concentrations of plasma ketones and β-hydroxybutyrate increased significantly and similarly in both groups at the end of the weight loss interventions. Because ketones rise as a consequence of increased fatty acid oxidation during fasting or caloric restriction, this finding serves as further evidence that the extent of calorie restriction and weight loss resulting from the GBP and diet interventions was similar. The following parameters were shown previously to change significantly and with similar magnitude after diet intervention and GBP (2): fasting plasma concentrations of glucose, insulin, C-peptide, and pro-insulin; the fasting insulin-to-glucose ratio; and the composite index of insulin sensitivity derived from plasma glucose and insulin concentrations during an OGTT [insulin sensitivity index (ISI)] (Table 2) (see Materials and Methods). Weight loss by either intervention caused no significant changes in the subjects' serum lipid profiles or plasma concentrations of non-esterified fatty acids (NEFAs), uric acid, or lactate over the time periods studied.

Differential metabolic effects of GBP and diet intervention—A major difference between the GBP and diet groups as reported previously is the significant lowering of glucose concentrations during an OGTT in the GBP group (2). Also, plasma concentrations of ghrelin increased as expected after diet intervention but did not change significantly after GBP (11) (Table 2), and plasma concentrations of glucagon decreased as expected after diet intervention, but exhibited a surprising increase after GBP, suggesting that mechanisms other than weight loss were implicated in this change (2). Here, we found that plasma concentrations of leptin decreased more after GBP than after diet intervention (Table 2).

Metabolic changes observed after GBP but not after diet intervention—Plasma incretin concentration and the incretin effect on insulin secretion, as well as the early-phase insulin and C-peptide response to OGTT increased significantly, and glucose concentrations at 120 and 180 min during OGTT decreased after GBP but not after diet intervention, as shown previously (2). The concentrations of several individual plasma AAs decreased significantly after GBP only (Ala, $P = 0.03$; Val, Leu/Ile, Phe, Tyr, and Cit, $P < 0.001$; Met, $P = 0.014$; His, $P = 0.001$; Orn, $P = 0.006$). These same AAs exhibited a trend to decrease in response to diet intervention, but these trends did not achieve statistical significance (Table 2). Because plasma levels of a larger group of AAs have been described to increase in insulin-resistant states, and a particular correlation of BCAA concentrations with insulin resistance has been reported (7–9), we also investigated the changes in the molar sum of total AAs (TAAs; sum of Ala, Val, Leu/Ile, Phe, Tyr, Cit, Met, His, Orn, Asx, Glx, Arg, Ser, and Pro) and of all the BCAAs (sum of Val and Leu/Ile) after the two weight loss interventions. Plasma concentrations of TAAs and BCAAs decreased by 19.7% ($P = 0.008$) and 38.3% ($P < 0.001$), respectively, after GBP, but only by 12.8% ($P = 0.025$) and 12.6% ($P = 0.083$) after diet intervention, respectively. Consistent with these findings, BCAAs were lower after GBP compared to diet ($P < 0.001$) (Fig. 2A).

Also consistent with the data for TAAs and BCAAs, serum concentrations of propionyl carnitine (C3) ($P = 0.004$), methylmalonyl/succinyl carnitine (C4-DC) ($P = 0.019$), and 2-methylbutyryl and isovaleryl carnitines (C5) ($P = 0.027$), which are all by-products of oxidation of BCAAs and other AAs, decreased after GBP but not after the dietary intervention (Table 2, Fig. 1, and table S1). Although the sum of all ACs increased after both types of weight loss intervention (Fig. 3A), the sum of C3 and C5 ACs decreased significantly after GBP ($P = 0.001$) but not after diet intervention ($P = 0.956$) (Fig. 3B).

Similar analyses in an independent group of subjects studied at Duke confirmed the findings in the NYONRC cohort. In the Duke subjects, matched at baseline for BMIs and metabolic measures (table S2) and for amount of weight lost (table S3), serum BCAA concentrations were reduced significantly after GBP by 35.8% and after diet intervention by 11.9%, with a more marked reduction after GBP ($P = 0.02$ between groups) (Fig. 2B and table S3).

Principal components analysis

Of the six principal components (PCs) identified (fig. S1 and table S4), the two most significant contributors to overall variance were PC1, composed mainly of AC metabolites, and PC2, which contained BCAAs and related metabolites (fig. S1 and table S5). The PC1 scores increased significantly after GBP and after diet intervention ($P = 0.005$), whereas PC2 scores decreased significantly after GBP only ($P < 0.001$) (table S6). In addition, the changes in PCs 2, 3, 4, and 5 after weight loss were significantly greater in the GBP group compared to the diet intervention group (table S6).

Before weight loss, in both groups, PC1 scores (mainly ACs) were negatively correlated with BMI ($r = -0.521$, $P = 0.015$), fasting plasma glucagon concentrations ($r = -0.563$, $P = 0.007$), and HOMA-IR ($r = -0.66$, $P = 0.0009$) and positively correlated with ISI ($r = 0.437$,

$P = 0.040$). PC2 scores (BCAAs and related metabolites) were positively correlated with the C-peptide and pro-insulin response to oral glucose challenge (respectively: $r = 0.614$, $P = 0.003$ and $r = 0.577$, $P = 0.006$) and HOMA-IR ($r = 0.626$, $P = 0.002$). After weight loss, controlling for group, PC1 correlated positively with ISI and negatively with fasting and post–oral glucose concentrations of insulin, C-peptide, and pro-insulin (Table 3). PC2 correlated positively with HOMA-IR and with fasting and post–oral glucose concentrations of insulin, C-peptide, and pro-insulin, and correlated negatively with ISI (Table 3).

In a regression model including PC1, group (GBP or diet intervention), and weight loss as predictors, PC1 and group, but not weight loss, predicted 61.2% of total variance in plasma C-peptide concentrations during early-phase secretion [area under the curve (AUC), 30 min] ($P = 0.001$) and 42.2% of variance in total plasma pro-insulin concentrations (AUC, 180 min) ($P = 0.023$) in response to an oral glucose challenge. Similarly, PC1 and group, but not weight loss, predicted 44.9% of total variance in ISI ($P = 0.015$). In a separate regression analysis including PC2, group (GBP or diet intervention), and weight loss as predictors, PC2 and group, but not weight loss, predicted 53.7% of total variance in plasma C-peptide concentrations during early-phase secretion (AUC, 30 min) ($P = 0.004$) and 51.5% of variance in pro-insulin (AUC, 180 min) ($P = 0.006$) in response to an oral glucose challenge. Finally, PC2 and group, but not weight loss, predicted 49.4% of total variance in ISI ($P = 0.008$) and 55.9% of total variance in HOMA-IR ($P = 0.003$) (Table 4).

DISCUSSION

We compared the metabolic effects of two methods of weight loss, diet intervention and GBP, under conditions in which the amount of weight loss was identical between the two groups. To provide a comprehensive understanding of metabolic changes that accompany these two kinds of interventions, we used a targeted mass spectrometry metabolomics approach supplemented with measurement of other key hormones and metabolites by colorimetric and antibody-based assays. We hoped to identify metabolic factors that change more markedly in response to GBP compared to diet at equivalent weight loss, because such factors might help to explain previous reports of superior diabetes remission and overall metabolic improvement in response to GBP versus diet intervention (1, 2). Diabetes remission, defined as normalization of HbA1c concentrations in the absence of medication, is observed in up to 80% of patients after GBP (1), but only rarely after diet intervention. In the NYONRC cohort, all patients were off their diabetes medications 1 month after GBP, whereas half of the patients in the diet intervention group still required antidiabetes medications, even if at lower dosages, after a similar amount of weight loss (2). Although fasting blood glucose and insulin (that is, HOMA-IR) concentrations decreased similarly after matched weight loss by GBP and diet, glucose tolerance, measured during an OGTT, improved more after GBP than after diet intervention (2). Here, at baseline, the GBP and diet intervention groups displayed similar conventional metabolite values and hormonal profiles, confirming previous reports (2, 6, 11), and also had matched serum concentrations of a wide array of AAs and ACs as measured by targeted mass spectrometry (Table 1). Although our cohort of obese diabetic individuals was small, the metabolomic profile before weight loss was similar to data reported previously from 73 obese nondiabetic individuals (7).

Our most intriguing finding is that AAs, particularly the BCAAs and related metabolites, decreased more significantly after GBP than after an equivalent 10-kg weight loss induced by diet intervention. In addition to the BCAAs leucine, isoleucine, and valine, the aromatic AAs phenylalanine and tyrosine, as well as ornithine, citrulline, and histidine all decreased after GBP. Similar trends were noted for all of these AAs in response to the dietary intervention, but the magnitude of change was clearly larger in the GBP than in the diet

group, despite the equivalent decrease in body weight. These data were confirmed with individual variable analysis, composite variable analysis (studies of the molar sums of all AAs and all BCAAs), and PC analysis (PCA). A similar preferential decline in the molar sum of BCAAs was observed in a comparison of a completely different set of GBP and dietary intervention subjects at Duke, after an average 27-kg weight loss over 6 months in both intervention groups. Thus, the main finding of this study was consistent in an index (NYONRC) and a validation (Duke) cohort, and the Duke study demonstrates that the effect is robust for many months after intervention.

Elevation of circulating AA concentrations, and particularly BCAAs, has been shown in obese compared to lean (7, 12–15) and in diabetic compared to nondiabetic individuals (16). Recent work using the same targeted metabolomics platform as that used in the current investigation has demonstrated that BCAA and metabolites derived from their catabolism form a PC that associates more strongly with insulin resistance than any other PC, including lipid-related factors, in three separate studies conducted on several ethnic groups (7–9). Our data are also consistent with the report of a 35% decrease in circulating BCAA concentrations in obese individuals after an ~56-kg weight loss induced by GBP surgery (17) and with another study conducted at 3 and 6 months after GBP surgery (18). The current work demonstrates that the changes in serum AA concentrations that occurred after GBP surgery are significantly greater than those after diet-induced weight loss. This suggests a role for alterations in circulating AAs in mediating enhanced glycemic control in GBP compared to diet-induced weight loss.

PCA of the metabolomics data identified two major components—PC1, which contained mainly ACs, and PC2, which was composed of BCAAs and related metabolites—that together accounted for 43% of the variance in the metabolite data after weight loss. Only PC2 was significantly (and positively) correlated with HOMA-IR and the variables that determine HOMA-IR, fasting glucose, and fasting insulin (Table 3). In addition, regression analysis showed that both PC1 and PC2 predicted changes in ISI, pro-insulin, and C-peptide, but only PC2 predicted changes in HOMA (Table 4). The amount of weight lost failed to predict any of these outcomes.

AA signaling is integrated by the mammalian target of rapamycin (mTOR), and mTOR is involved in the sensing of nutrient availability and modulation of insulin action in vivo via its further effects to activate S6 kinase 1 (S6K1), which phosphorylates the S6 ribosomal protein to enhance protein synthesis (19). Supplementation of a high-fat diet with BCAA causes chronic activation of the mTOR/S6K1 pathway in rodents, as does infusion of a cocktail of multiple AAs in humans (7, 20); this activation may contribute to the diet-induced insulin resistance that results from increasing serine phosphorylation of insulin receptor substrate-1 (IRS-1). IRS-1 transmits signals from the insulin and insulin-like growth factor-1 (IGF-1) receptors to stimulate cellular metabolic functions such as glucose transport and glycogen synthesis, and mTOR/S6K1-mediated phosphorylation of IRS-1 on serine appears to impede these functions (7, 14, 20). Thus, the marked decrease in BCAAs and related metabolites in GBP patients may reverse chronic mTOR activation and lead to improved insulin action. AAs, including leucine, also stimulate insulin secretion from pancreatic β cells, but prolonged exposure to elevated amounts of these metabolites could conceivably cause chronic activation of mTOR or other signaling molecules and lead to impaired β cell function. Further studies will be required to determine whether the marked fall in BCAAs observed in GBP has a mechanistic linkage to the improvement in insulin secretion in response to a glucose challenge, either via direct actions on the β cell or via effects on incretin concentrations or activity.

The decrease in circulating plasma AAs after GBP could result from a decrease in protein intake (and in parallel, essential AAs such as BCAAs) (21, 22), an increase in AA catabolism, a decrease in protein degradation, or a combination of these. Although the two subject groups were not pair-fed and not matched for the macronutrient composition of their diets, and it took longer for the diet intervention group to achieve the same weight loss as the GBP group (~2 months versus 1 month, respectively), similar increases in ketones and β-hydroxybutyric acid (markers of fatty acid oxidation during caloric restriction) were found in the diet and surgical groups at the end of the intervention. The calorie content of the meal supplements in the diet intervention groups was controlled at 1000 to 1200 kcal/day, with ~100 g of protein per day. In the GBP group, the diet was not controlled in a similar fashion, but based on food records, the mean calorie intake after GBP ranged from 600 to 800 kcal, with 70 g of protein per day intake. On the basis of these estimates, it is possible that the different calorie and protein contents of the diets accounted for the difference in circulating AAs. However, all of our analyses were performed on samples taken from overnight-fasted subjects, minimizing the potential impact of recent ingestion of a protein-containing meal on the AA profile.

A reported 35% decrease in fasting plasma BCAA concentrations after GBP was associated with an increase in two key BCAA catabolic enzymes, the branched-chain amino acid aminotransferase (BCATm) and the branched-chain α-keto acid dehydrogenase E1 (BCKD E1 α), in both subcutaneous and visceral fat depots, compared to before surgery (17). To the extent that these changes in expression of BCAA catabolic enzymes actually enhance metabolic flux, increased catabolism of BCAAs after GBP in adipose tissue may contribute to the decreased plasma BCAA concentrations after this surgery (17). Such a conclusion would also be consistent with recent observations in animal models that demonstrate that metabolism of BCAAs in adipose tissue exerts a significant impact on circulating BCAA concentrations (23). Less is known about changes in BCAA catabolism in muscle and liver after GBP, but such changes could also contribute to our findings.

Concentrations of ACs reflect the pool of mitochondrial acyl co-enzyme A (CoA) species and therefore report on mitochondrial oxidation of fatty acids, AAs, and glucose (Fig. 1). Although the sum of all ACs increased significantly after GBP and diet intervention, C3 and C5 ACs decreased only after weight loss by GBP, reflecting the changes in BCAA concentrations (Figs. 1 to 3). The close correlation between BCAAs and C3 and C5 ACs is expected on the basis of our previous work and by the generation of three- and five-carbon AC species during oxidation of BCAAs (7). We also observed a significant decrease in dicarboxylated C4 AC (C4-DC AC) concentrations in plasma only after GBP. The C4-DC AC analyte as measured by flow injection–tandem mass spectrometry represents the sum of methylmalonyl- and succinylcarnitine concentrations, because these analytes are isomers of the same molecular mass that are not resolved by our method. Recently, several studies have reported that elevations in ACs may be an indicator of dysregulation of mitochondrial fatty acid oxidation that may contribute to the development of insulin resistance and T2DM (10, 24–26). One of these studies showed a positive correlation between C4-DC AC concentrations and fasting HbA1c and glucose, and a negative correlation between plasma C4-DC AC concentrations and glucose disposal under hyperinsulinemic and euglycemic conditions, suggesting that elevated C4-DC AC concentrations may be a predictor for poor glycemic control (25). Both C4-DC AC isomers are generated downstream of C3 ACs (propionylcarnitine) in the valine catabolic pathway. Thus, the preferential decrease in C3s, C4-DCs, and C5 ACs can all be interpreted to indicate that GBP results in enhanced oxidation of BCAAs. Whether the decrease in these metabolites and the implied activation of fuel oxidation is a cause or consequence of the diabetes remission after GBP remains to be determined.

The complexity of GBP surgery results in the alteration of many physiological pathways. We and others have shown marked increases in postprandial concentrations of the incretins GLP-1 and GIP. In addition, plasma concentrations of the gut hormones peptide YY3-36 (PYY3-36) and oxyntomodulin (OXM), which are co-secreted with GLP-1 by intestinal endocrine L cells and regulate food intake, were increased after GBP but not after equivalent diet-induced weight loss (2, 27, 28). Data suggest that gut peptides implicated in meal-to-meal satiety, regulation of insulin secretion, or both can also modulate liver and adipose tissue metabolic pathways (29). Similar to what was described with the diet-induced obesity mouse model (29), the marked increase of GLP-1 (3, 4), OXM (28), and glucagon (2, 6) after GBP could modify intermediary metabolism in liver or adipose tissue, contributing to the unique metabolic signature of GBP-induced weight loss described herein. Future studies will further characterize the pathways involved in these metabolic alterations and will seek to understand whether the specific metabolic signature of GBP is related to changes in gut peptides after surgery.

MATERIALS AND METHODS

NYONRC cohort

Details of the NYONRC subjects and the methods used for the study are described elsewhere (2).

Subjects—Invited to participate in this study were obese patients with BMIs >35 kg/m^2 (eligible candidates for GBP surgery) who were younger than 60 years, of both genders and all ethnic groups, diagnosed with T2DM for less than 5 years, and not on insulin, thiazolidinedione, exenatide, or dipeptidyl peptidase-4 (DPP-4) inhibitors, with plasma HbA1c values of less than 8%. Before enrolling in the study, all participants signed an informed consent approved by our institution. One group of patients was studied before and 1 month after GBP (surgical group, $n = 10$). A second group of patients, fulfilling the same recruitment criteria, was studied before and after a 10-kg diet-induced weight loss (diet group, $n = 11$). In addition, patients in the diet group were matched for age, weight, BMI, T2DM duration, and glycemic control (HbA1c) to patients from the surgical group.

Roux-en-Y GBP and diet intervention protocols—All patients in the surgical group underwent a laparoscopic GBP, as described previously (4). The food intake after GBP was monitored by food records but not directly supervised. In the diet group, weight loss was achieved by a meal replacement diet (1000 kcal/day, Robard Corp.) distributed to the subjects during weekly visits (2). The diet in the few days preceding the initial testing before weight loss was not controlled for either group (see Supplementary Materials and Methods).

Metabolic measurements—Plasma concentrations of incretins, pro-insulin, insulin, C-peptide, and glucose were measured during a 50-g, 3-hour OGTT. Parameters of insulin secretion and insulin resistance were derived from the OGTT. In addition, the incretin effect on insulin secretion was assessed by comparing insulin response to oral glucose and to an isoglycemic IV glucose infusion as described previously (4) (see Supplementary Materials and Methods). Plasma concentrations of conventional metabolites and hormones were measured at the NYONRC by radioimmunoassay (RIA) for plasma leptin, insulin, C-peptide, glucagon, total ghrelin, PYY3-36, and total GLP-1 and total GIP (Millipore) as described (2, 4). Plasma concentrations of total and low- and high-density lipoprotein cholesterol, as well as of triglycerides, nonesterified fatty acids, ketones, CRP, liver enzymes, and uric acid were measured as described (2, 4, 7). Fifteen AAs and 45 ACs were measured by tandem mass spectrometry using stable isotope dilution for quantification, as previously described (7).

Duke cohort

An independent cohort of obese individuals without diabetes was studied at Duke University before and 6 months after GBP ($n = 6$) or after a matched ~27-kg weight loss resulting from diet intervention ($n = 6$). The GBP subjects were a subset of the STEDMAN (Study of the Effects of Diet on Metabolism and Nutrition) Project cohort (30). The diet intervention group was a subset of the Weight Loss Maintenance (WLM) clinical trial of behavioral therapies for weight loss (31, 32). Details of the GBP and dietary/behavioral intervention, using a low-fat diet (the DASH diet), were described previously (31, 32). Hormone and metabolite measurements were performed on blood samples from the Duke subjects as described (7, 30).

Statistical methods used to analyze metabolomic data

Paired comparisons—Paired comparisons were made for each individual variable within each group and between groups to assess the effects of weight loss as well as to compare the effects of GBP and dietary intervention. Although actual *P* values are reported in Results and in the tables, a nominal *P* value of 0.01 was considered statistically significant, as a reasonable compromise between type I (false-positive) and type II (false-negative) error, with no adjustment for multiple comparisons.

Composite variables—We also calculated the molar sum of TAAs (sum of Ala, Val, Leu/Ile, Phe, Tyr, Cit, Met, His, Orn, Asx, Glx, Arg, Ser, and Pro) and of all the BCAAs (sum of Val and Leu/Ile), of total ACs, and of C3 and C5 ACs (C3-C5), which are derived from BCAA oxidation. Because glycine is unique among AAs in being lower rather than elevated in obese compared to lean subjects (7) and exhibits a trend opposite to the rest of the AAs after GBP, it was excluded from the composite TAA variable. Also, AC variables with greater than 25% of samples having a 0-value output (likely the result of plasma concentrations below the threshold of detection) were removed from the data set for all subsequent analyses.

Principal components analysis—Because of the large number of AA and AC variables, PCA was performed to extract those components that explained a significantly large proportion of the total variation in the data set. PCA was run on all AA and AC variables before intervention, and the factor scores so derived were then applied through a generated formula to the data set after weight loss. Six PCs with eigenvalues >1 accounted for 73.2% of the total variation, with the first two PCs selected for analysis, representing the most cumulative variance (table S4 and fig. S1). Important variables that composed a PC were determined by the variable-component correlation within the component correlation matrix (significance: variable-component correlation >0.400 or <−0.400). The results of the PCA and the composition of the two major PCs, accounting for 42.85% of the variance, are described in fig. S1 and table S5.

Paired comparisons of the PC scores after and before weight loss were performed for each group (GBP and diet intervention), and the changes were compared between groups with independent Student's *t* tests. Partial correlations were performed for each main PC against outcome variables, adjusting for group. Separately, regression analyses were run with PC1 and PC2, weight loss, and group (GBP or diet intervention) as predictors of key outcome variables.

Data from the NYONRC and Duke cohorts were analyzed separately. All data presented in Results are from the NYONRC cohort, unless otherwise specified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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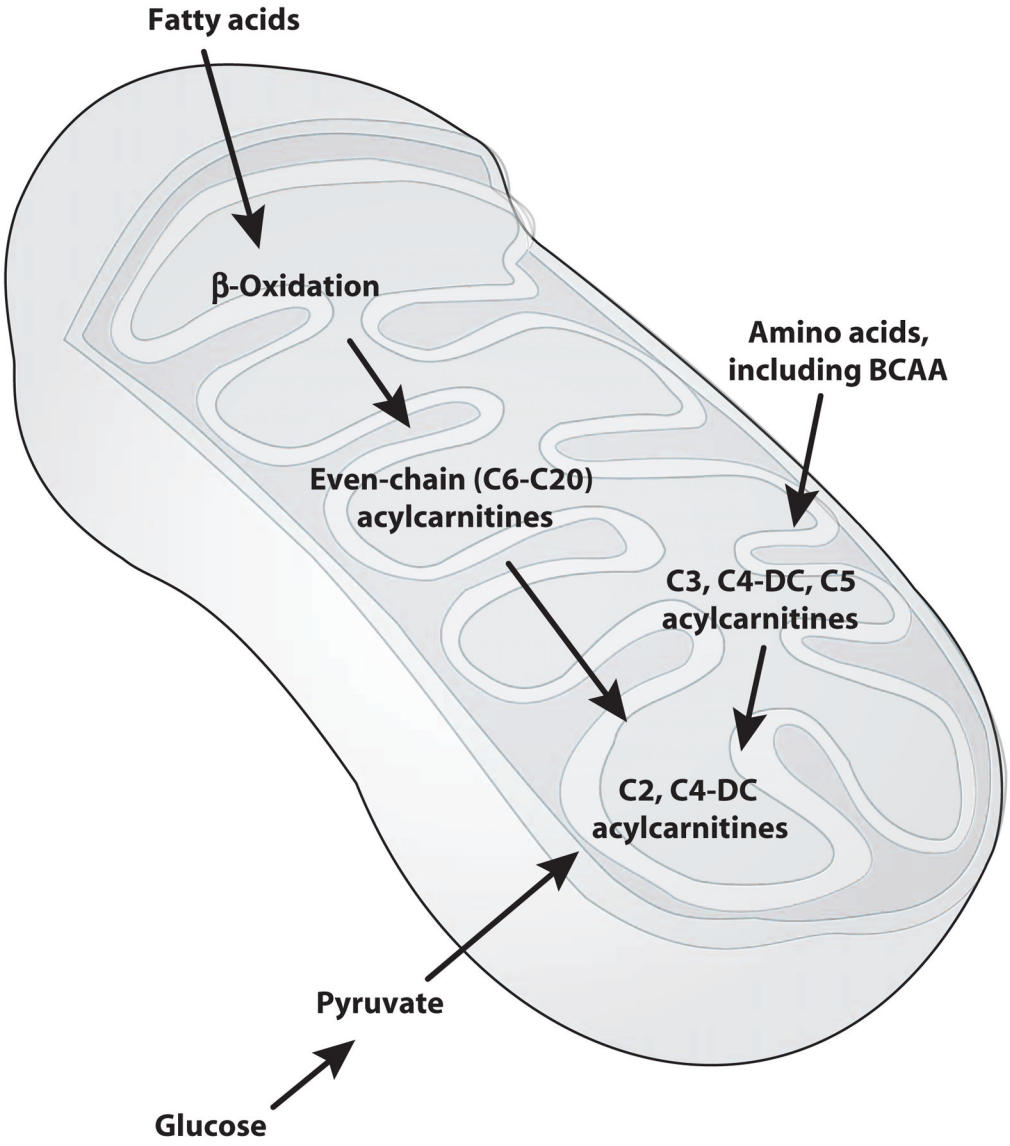

Fig. 1.
Metabolic pathways. Schematic summary of metabolic pathways that generate metabolites correlated with outcome variables in this study.

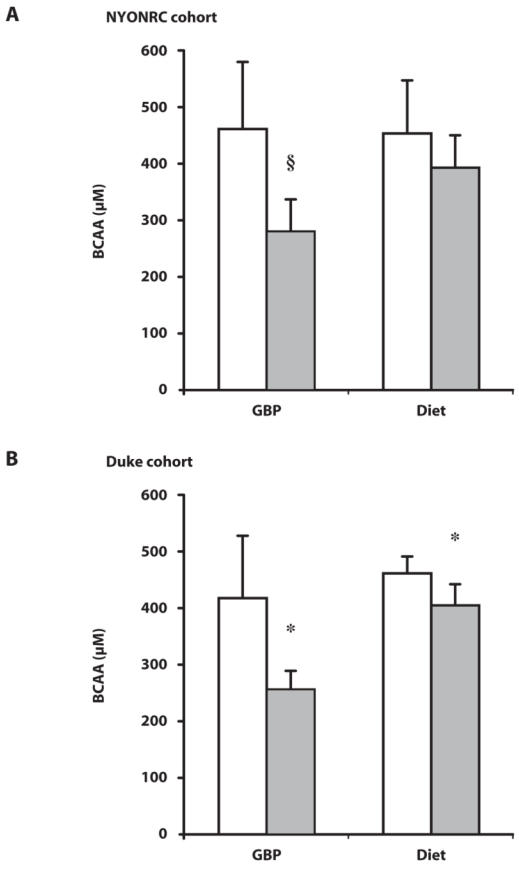

Fig. 2.

Changes in plasma BCAA concentrations in response to surgical or dietary interventions. (**A** and **B**) Total circulating (plasma) BCAA concentrations in the NYONRC (A) and Duke (B) cohorts before (white bars) and after (shaded bars) weight loss interventions. Data are means ± SD. $^{*}P < 0.05$; $^{\S}P < 0.01$, by Student's paired *t* test. GBP, gastric bypass surgery.

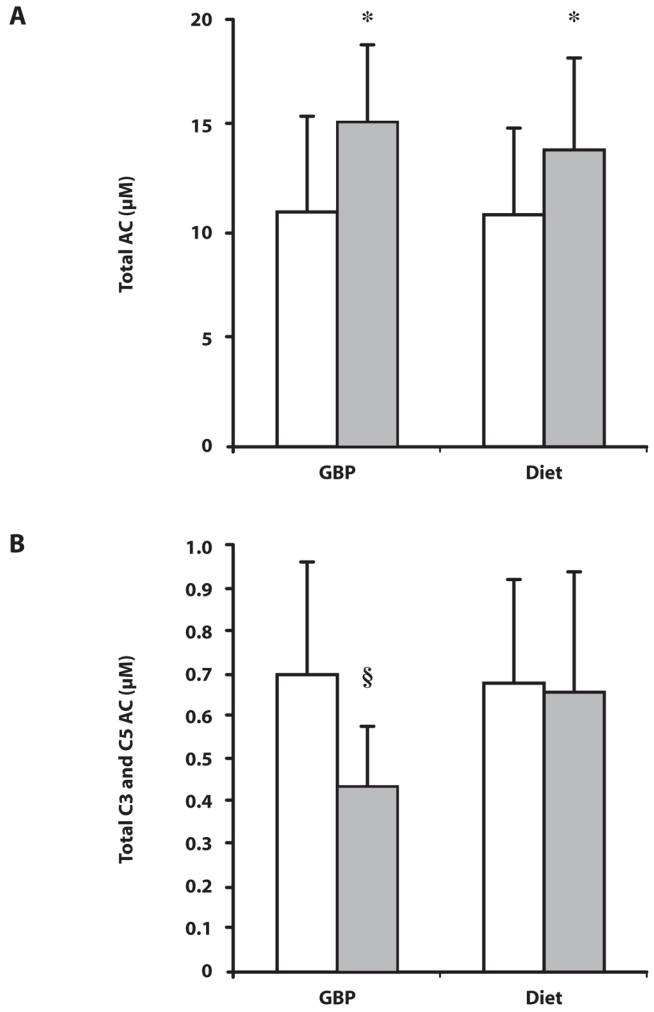

Fig. 3.

Changes in plasma AC concentrations in response to surgical or dietary interventions. (**A** and **B**) Molar sum of all circulating (plasma) ACs (total ACs) (A) and C3 and C5 ACs (B) before (white bars) and after (shaded bars) weight loss interventions in the NYONRC cohort. Data are means ± SD. $^{*}P < 0.05$; $^{\S}P < 0.001$, by Student's paired *t* test.

Table 1

Baseline characteristics before intervention. Baseline characteristics (mean ± SD) in patients before GBP or dietary intervention in the NYONRC cohort. $P < 0.05$ was considered significant, and P values are for differences between groups, before intervention, by independent t test.

Parameter	Pre-GBP	Pre–diet intervention	*P*
Age (years)	43.3 ± 10.0	47.9 ± 7.8	0.251
Weight (kg)	115.7 ± 19.0	110.6 ± 9.7	0.438
BMI (kg/m^2)	44.9 ± 8.7	42.8 ± 3.8	0.469
T2DM duration (months)	22.3 ± 18.0	18.7 ± 14.0	0.697
HbA1c (%)	6.87 ± 0.57	6.45 ± 0.61	0.117
Insulin sensitivity index (ISI composite)	2.27 ± 1.37	2.39 ± 1.12	0.826
HOMA-IR	9.34 ± 5.38	8.37 ± 4.19	0.653
Fasting leptin (ng/liter)	35.9 ± 14.9	31.2 ± 10.6	0.411
Fasting ghrelin (pg/ml)	499.9 ± 125.2	508.3 ± 319.2	0.938
CRP (mg/liter)	8.68 ± 5.13	6.39 ± 7.55	0.433
Lactate (mM)	1.55 ± 0.66	1.32 ± 0.56	0.392
Cholesterol (mg/dl)	127.5 ± 31.3	141.3 ± 25.7	0.282
Triglycerides (mg/dl)	132.8 ± 86.7	91.7 ± 62.1	0.224
NEFA (mM)	0.653 ± 0.246	0.643 ± 0.189	0.915
Total ketones (μM)	78.7 ± 48.4	123.2 ± 92.4	0.190
3-OH butyrate (μM)	61.0 ± 44.7	94.4 ± 84.0	0.277
Uric acid (mg/dl)	5.51 ± 1.76	5.06 ± 1.12	0.484
Glycine (μM)	266.1 ± 104.8	263.5 ± 37.9	0.939
Alanine (μM)	400.5 ± 91.5	372.9 ± 90.0	0.494
Serine (μM)	77.50 ± 15.91	76.87 ± 16.91	0.931
Proline (μM)	182.7 ± 68.3	199.1 ± 53.4	0.545
Valine (μM)	282.8 ± 79.5	281.1 ± 64.5	0.958
Leucine/isoleucine (μM)	177.8 ± 43.6	173.3 ± 34.7	0.796
Methionine (μM)	26.63 ± 4.38	26.59 ± 2.86	0.979
Histidine (μM)	55.08 ± 7.65	55.90 ± 7.83	0.811
Phenylalanine (μM)	61.84 ± 8.60	61.54 ± 6.45	0.927
Tyrosine (μM)	82.14 ± 16.31	75.42 ± 18.57	0.391
Asparagine/aspartic acid (μM)	67.47 ± 39.81	140.63 ± 125.67	0.095
Glutamine/glutamic acid (μM)	72.62 ± 31.30	75.83 ± 16.90	0.770
Ornithine (μM)	54.46 ± 19.27	53.15 ± 12.45	0.853
Citrulline (μM)	29.12 ± 7.88	30.61 ± 12.27	0.748
Arginine (μM)	68.36 ± 8.42	78.25 ± 21.49	0.181
C5s isovaleryl carnitine, 2-methylbutyryl carnitine (μM)	0.226 ± 0.123	0.192 ± 0.093	0.483

Table 2

Changes in weight and metabolic variables after GBP and diet intervention. Weight loss–induced changes (mean ± SD) in patients after GBP and after diet in the NYONRC cohort. The reported *P* values by independent *t* test are for differences between the changes occurring as a result of GBP and the changes occurring as a result of diet intervention. Significant differences between groups are bolded.

Parameter	ΔPre-Post-GBP	ΔPre-Post-Diet	*P*
Weight (kg)	−11.8 ± 5.3 *	−9.9 ± 2.3 *	0.303
BMI (kg/m^2)	−4.60 ± 2.13 *	−3.81 ± 0.87 *	0.296
Fasting glucose (mM)	−1.39 ± 1.34 *	−1.46 ± 0.69 *	0.873
Fasting pro-insulin (pM)	−23.7 ± 17.8 *	−17.0 ± 17.7 *	0.399
HOMA-IR	−3.98 ± 4.57 *	−4.39 ± 3.12 *	0.809
ISI composite	0.688 ± 0.822 *	1.53 ± 1.18 *	0.076
Fasting leptin (ng/liter)	**−16.8 ± 4.8** *	**−9.92 ± 7.71** *	**0.026**
Fasting ghrelin (pg/ml)	**−32.4 ± 156.4**	**124.3 ± 149.1** *	**0.030**
CRP (mg/liter)	−5.26 ± 5.25 *	−1.46 ± 3.56	0.065
Lactate (mM)	−0.300 ± 0.625	−0.227 ± 0.659	0.799
Cholesterol (mg/dl)	−20.50 ± 38.88	−18.6 ± 44.7	0.920
Triglycerides (mg/dl)	−33.2 ± 80.9	−19.8 ± 42.8	0.636
NEFA (mM)	0.051 ± 0.254	0.197 ± 0.386	0.323
Total ketones (μM)	273.3 ± 195.8 *	277.3 ± 363.3 *	0.975
3-OH butyrate (μM)	233.1 ± 177.8 *	239.7 ± 309.4 *	0.953
Uric acid (mg/dl)	−0.070 ± 1.84	0.300 ± 1.401	0.608
Creatinine (mg/dl)	−0.017 ± 0.150	−0.013 ± 0.145	0.948
ALT (IU/liter)	**12.40 ± 14.22** *	**−6.18 ± 9.91**	**0.003**
AST (IU/liter)	3.40 ± 16.99	7.36 ± 23.41	0.665
Glycine (μM)	53.5 ± 79.8	33.7 ± 57.7	0.521
Alanine (μM)	−74.1 ± 91.0 *	−30.1 ± 82.4	0.259
Serine (μM)	10.3 ± 21.5	9.41 ± 19.77	0.919
Proline (μM)	−37.4 ± 55.0	−24.4 ± 44.8	0.559
Valine (μM)	**−113.1 ± 64.1** *	**−33.2 ± 68.9**	**0.013**
Leucine/isoleucine (μM)	**−63.3 ± 34.4** *	**−24.4 ± 36.8**	**0.022**
Methionine (μM)	−5.00 ± 5.22 *	−2.46 ± 3.65	0.208
Histidine (μM)	**−9.11 ± 6.14** *	**−0.09 ± 9.85**	**0.022**
Phenylalanine (μM)	**−20.62 ± 7.74** *	**−6.11 ± 10.29**	**0.002**
Tyrosine (μM)	**−29.12 ± 16.57** *	**−10.5 ± 18.2**	**0.025**
Asparagine/aspartic acid (μM)	**59.0 ± 172.5**	**−85.4 ± 139.5**	**0.047**
Glutamine/glutamic acid (μM)	−11.9 ± 28.6	−0.46 ± 19.18	0.290
Ornithine (μM)	**−17.3 ± 15.5** *	**−0.84 ± 15.71**	**0.026**
Citrulline (μM)	−7.12 ± 3.80 *	−3.92 ± 11.78	0.423

Parameter	ΔPre-Post-GBP	ΔPre-Post-Diet	*P*
Arginine (μM)	−4.57 ± 15.09	−6.77 ± 27.03	0.823
C5s isovaleryl carnitine, 2-methylbutyryl carnitine (μM)	−0.122 ± 0.147*	−0.007 ± 0.156	0.099

*$P < 0.05$, significant change in either group (GBP or diet) by Student's paired *t* test.

Table 3

Partial correlations of PCs with major outcome variables. Partial correlations for each main PC against outcome variables, after weight loss, adjusting for group (GBP or diet intervention) in the NYONRC cohort. $P < 0.05$ was considered significant (bolded). ISI, insulin sensitivity index; AUC, area under the curve during the OGTT.

Outcome variables		PC1	PC2
Fasting glucose	*r*	−0.277	**0.584**
	P	0.238	**0.006**
Fasting insulin	*r*	−0.406	**0.584**
	P	0.075	**0.006**
Fasting C-peptide	*r*	**−0.665**	**0.694**
	P	**0.001**	**0.001**
Fasting pro-insulin	*r*	**−0.604**	**0.737**
	P	**0.004**	**<0.001**
Fasting glucagon	*r*	−0.423	**0.470**
	P	0.063	**0.036**
HOMA-IR	*r*	−0.393	**0.674**
	P	0.086	**0.001**
ISI (composite)	*r*	**0.597**	**−0.650**
	P	**0.005**	**0.001**
3-OH butyrate	*r*	**0.504**	**−0.605**
	P	**0.023**	**0.005**
Ketones	*r*	**0.492**	**−0.631**
	P	**0.028**	**0.003**
Triglycerides	*r*	**−0.521**	0.441
	P	**0.018**	0.052
C-peptide AUC (30 min)	*r*	**−0.716**	**0.596**
	P	**<0.001**	**0.005**
Insulin AUC (180 min)	*r*	**−0.476**	0.397
	P	**0.033**	0.083
Pro-insulin AUC (180 min)	*r*	**−0.553**	**0.705**
	P	0.011	<0.001

Table 4

Regression models. Regression models with group (GBP or diet intervention), weight change, and PC1 or PC2 as predictors of key outcome variables in the NYONRC cohort. $P < 0.05$ was considered significant. r^2 is the squared correlation coefficient between the outcome and the predictor and is also the proportion of the total variation in the outcome explained by the predictor. The standardized β coefficient is the usual (unstandardized) regression coefficient multiplied by the ratio of the SDs of the predictor and the outcome. AUC, area under the curve during the OGTT. ISI, insulin sensitivity index derived from plasma glucose and insulin levels during the OGTT.

Dependent variables	Predictors	Model r^2 and P	Standardized β coefficient	P
C-peptide AUC (30 min)	PC1	$r^2 = 0.612$ $P = 0.001$	−0.772	0.001
	Group		0.790	<0.001
	Weight change		−0.012	0.939
ISI	PC1	$r^2 = 0.449$ $P = 0.015$	0.668	0.005
	Group		−0.689	0.005
	Weight change		−0.068	0.461
Pro-insulin AUC (180 min)	PC1	$r^2 = 0.422$ $P = 0.023$	−0.695	0.005
	Group		0.535	0.026
	Weight change		0.342	0.096
C-peptide AUC (30 min)	PC2	$r^2 = 0.537$ $P = 0.004$	0.733	0.003
	Group		0.855	0.001
	Weight change		−0.234	0.193
ISI	PC2	$r^2 = 0.494$ $P = 0.008$	−0.803	0.002
	Group		−0.825	0.002
	Weight change		0.065	0.669
HOMA-IR	PC2	$r^2 = 0.559$ $P = 0.003$	0.880	0.001
	Group		0.791	0.001
	Weight change		−0.248	0.159
Pro-insulin AUC (180 min)	PC2	$r^2 = 0.537$ $P = 0.004$	0.880	0.001
	Group		0.702	0.005
	Weight change		0.106	0.558