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Postprandial metabolite profiles reveal differential nutrient handling after bariatric surgery compared to matched caloric restriction

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Abstract

Background—Roux-en-Y gastric bypass (RYGB) surgery results in exaggerated postprandial insulin and incretin responses, and increased susceptibility to hypoglycemia. We examined whether these features are due to caloric restriction (CR) or altered nutrient handling.

Methods—We performed comprehensive analysis of postprandial metabolite responses during a 2-hour mixed-meal challenge test (MMT) in twenty morbidly obese subjects with type 2 diabetes who underwent RYGB surgery or matched CR. Acylcarnitines and amino acids was measured using targeted mass spectrometry. Linear mixed model was used to determine the main effect of interventions, and interaction term to assess the effect of interventions on postprandial kinetics.

Results—Two-weeks after these interventions, several gut hormones (insulin, GIP and GLP-1), glucose, and multiple amino acids, including branched-chain and aromatic species, exhibited a more rapid rate of appearance and clearance in RYGB subjects compared to CR during the MMT. In the RYGB group, changes in leucine/isoleucine, methionine, phenylalanine and GLP-1 responses were associated with changes in insulin response. Levels of alanine, pyruvate, and lactate decreased significantly at the later stages of meal challenge in RYGB subjects, but increased with CR.

Conclusions—RYGB surgery results in improved metabolic flexibility (i.e. greater disposal of glucose and amino acids, and more complete β -oxidation of fatty acids) compared to CR. The changes in the amino acid kinetics may augment the hormonal responses seen after RYGB

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Conflict-of-interest statement

The authors declare that there is no duality of interest associated with this manuscript.

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surgery. The reduction in key gluconeogenic substrates in the postprandial state may contribute to increased susceptibility to hypoglycemic symptoms in RYGB subjects.

Keywords

Roux-en-Y gastric bypass; caloric restriction; postprandial trajectories; amino acids; morbid obesity; type 2 diabetes

Roux-en-Y gastric bypass (RYGB) surgery results in improvement in glucose homeostasis before significant weight loss¹. Besides a smaller gastric pouch that enforces caloric restriction (CR), RYGB surgery rearranges the gut anatomy such that the shorter intestinal nutrient transit significantly alters several gut hormonal responses^{2, 3}. In particular, the insulin and incretin responses are unequivocally accentuated with meals after RYGB, but not after equivalent CR weight loss⁴. Importantly, a larger incretin response is observed when a meal is administered via the alimentary limb compared to administration into the bypassed gastric remnant, thus corroborating the “hindgut hypothesis”⁵. Increased incretin response is posited to contribute to hyperinsulinemia seen in RYGB subjects⁶, and has been proposed to mediate expansion of β -cell mass that contributes to post-surgical hyperinsulinemic hypoglycemia^{7, 8}.

In contrast to the widely described hormonal responses, much less is known about metabolite responses after different weight loss interventions. While fasting metabolite levels are informative, postprandial metabolite responses are related to insulin action along multiple physiologic axes⁹, and might provide an indication of changes in metabolic flexibility following an intervention¹⁰. Several studies have shown that the overall glucose response (measured as area-under-the-curve during meal challenge) is unchanged after RYGB^{11, 12}, but with a leftward shift of the trajectory of plasma glucose, indicating a more rapid absorption and clearance. A recent study shows strong correlation between fasting plasma branched-chain amino acids and insulin sensitivity after RYGB¹², suggesting that postprandial handling of nutrients other than glucose (i.e. amino acids and fatty acids) might be altered following RYGB.

The aim of this study was to compare metabolic responses to a meal challenge following RYGB surgery versus CR in morbidly obese diabetic patients. Our broader goal was to determine if metabolite profiles might help to explain the exaggerated hormonal responses seen after RYGB, and provide insights into mechanisms underlying increased susceptibility to hypoglycemia after RYGB.

METHODS

We recruited ten obese subjects from the Duke Metabolic and Weight Loss Surgery Program who underwent laparoscopic RYGB surgery. Ten control subjects matched for age, sex and body mass index (BMI) were recruited by advertisement and enrolled into the CR group. Exclusion criteria included diabetes duration >5 years, HbA1c >10%, use of insulin or incretin-based therapy, previous esophageal, gastric, pancreatic, or bowel surgery, tobacco use, or known substance abuse within six months of enrollment. All subjects provided informed consent, and the Duke University Institutional Review Board approved the protocol.

Mixed-meal challenge test (MMT) and feeding regimen

All subjects were examined on two occasions, pre- and 10-14 days post RYGB surgery or CR. Anti-diabetes medications were discontinued 3 days prior to MMT per study protocol. During each visit, subjects consumed 230 kcal liquid meal of 8 Oz Ensure High Protein. The

liquid supplement was provided to the subjects at 5-min intervals over a total of 20 min to accommodate for the reduced gastric capacity of subjects after RYGB. Blood samples were collected before meal ingestion and at 30, 60, 90 and 120 min after completion of the meal.

Upon hospital discharge, RYGB subjects consumed a standard liquid diet provided by the surgery program. The CR subjects were instructed to consume similar diet that matched RYGB subjects. Four 8-Oz cans of Ensure High Protein were given daily to each subject to provide 920kcal/day. Dietary intake was monitored in all subjects by fluid intake records from admission to the study through to the post-intervention visit. All anti-diabetes medications were stopped after RYGB, stopped for 5 CR subjects and reduced by 50% in the remainders.

Surgical procedure

Briefly, a small proximal gastric pouch (~30mL) was created. The jejunum was divided at 50 cm distal to the ligament of Treitz and a 2 cm end-to-side gastrojejunostomy was performed. A side-to-side jejunojejunostomy was then created 100 cm distal to the gastrojejunostomy. There were no post-operative complications and all subjects were discharged between post-operative day 1 and 3.

Analytical procedures

Blood glucose was measured using the hexokinase method (Beckman, Fullerton, CA, USA). Hormonal analytes were measured in duplicates using the Millipore MILLIPLEX Human Metabolic Hormone Panel based on the Luminex xMAP technology. The intra- and inter-assay coefficient of variation for the analytes were <8% and <16% respectively. Conventional metabolites, including non-esterified fatty acids (NEFA), total ketones, β -hydroxybutyrate and lactate were measured as previously described¹³. Plasma fibroblast growth factor-21 were measured using an ELISA (R&D Systems, Minneapolis, MN, USA), and glucagon using RIA (Millipore, Billerica, MA). Plasma acylcarnitines and amino acids (AA) were measured by tandem mass spectrometry¹⁴. Insulin sensitivity was calculated using the Matsuda equation¹⁵.

Statistical analysis

All values represent means $\pm$ standard error (SE) unless otherwise stated. Statistical analyses were carried out using SPSS ver.17 (SPSS Inc., Chicago, USA), with $p<0.05$ considered as significant. Linear mixed models with random effects were used to analyze postprandial metabolite trajectories in relation to intervention groups and follow-up period⁵. Linear-mixed effect estimation was carried out with the use of restricted maximum-likelihood methods. The postprandial time was treated as a categorical factor to represent longitudinal patterns observed in each metabolite measurement¹⁶. The main effect of the analysis modeled the mean metabolite response over time during MMT. Interaction terms were included to examine the impact of intervention (CR and RYGB) on the postprandial trajectories. Comparisons of mean metabolite concentrations were conducted using student t-tests. We performed cross-correlation analysis (described in the Supplementary Appendix) to assess the interrelationship between changes in metabolites and hormones of interest¹⁷.

RESULTS

Changes in BMI, insulin sensitivity, and hormone levels for the RYGB and CR groups are described in Supplementary Appendix. There was no difference in the percentage change in BMI, fasting glucose and insulin sensitivity between groups. The percentage weight loss was greater with RYGB than CR (-8.3 ± 0.5 kg vs. -4.3 ± 0.6 kg, $p<0.001$). Thus, body weight

was included as a covariate in the following analyses to account for differences between the two intervention groups.

Percentage change in metabolites from pre- to post-intervention

There were no significant differences in plasma AA between CR and RYGB groups pre-intervention. Significant reductions in fasting proline, histidine, valine, phenylalanine, molar sum of BCAA, aromatic AA and total AA were observed after RYGB (Table 1, left column), but not with CR. Fasting alanine was reduced significantly in both groups.

Consistent with the reduction in fasting total AAs and BCAA, there was a significant reduction in the molar sum of C3 and C5 acylcarnitines (byproducts of BCAA and other AA catabolism) in both intervention groups. Fasting C2, long-chain (C14-C22) and total acylcarnitines were significantly increased with both interventions. Fasting medium-chain (C6-C12) acylcarnitines were significantly increased in the CR group ($p=0.003$), but not with RYGB. Both interventions resulted in significant increases in fasting NEFA, total ketones and β -hydroxybutyrate, with a trend towards larger responses with RYGB.

Two hours after MMT, significant reductions of 20-30% were observed in multiple AA, molar sum of BCAA, aromatic and total AA in the RYGB but not the CR group (Table 1, right column). Plasma C2, medium-chain, long-chain and total acylcarnitines were significantly higher at 2-hour post-MMT after both interventions, with no between-group differences.

Changes in postprandial amino acid trajectories

Overall, the mean postprandial responses for valine, proline, histidine, serine, aspartate, ornithine, citrulline, and BCAA were highly significant after intervention in both groups (all p -values <0.005) [see Figure 1 and Supplemental Appendix Figure S1 (open vs. filled circles for CR; open vs. filled squares for RYGB)]. However, the mean postprandial responses for phenylalanine, glycine, glutamate/glutamine and aromatic AA were significant with RYGB (all p -values <0.05), but not with CR. The postprandial trajectories for valine, leucine/isoleucine, phenylalanine, tyrosine, proline, serine, methionine, and arginine were significantly altered after RYGB (all interaction term p -values <0.05), but not after CR.

Next, we examined the differences in the postprandial AA trajectories between CR and RYGB groups (compare filled circles vs. filled squares in Figure 1 and Supplemental Appendix Figure S1). In the post-intervention period, postprandial trajectories for proline, valine, leucine/isoleucine, phenylalanine, tyrosine, ornithine, methionine, and arginine were statistically different between the two experimental groups (all interaction term p -values <0.05). These AA trajectories were not different between groups in the pre-intervention period, demonstrating a unique effect of RYGB on the trajectories of these amino acids with meal challenge. The post-RYGB trajectories exhibited a steep increase in the first phase (0-60 min) with rapid decline in the second phase (60-120 min) of MMT.

Changes in postprandial lipid trajectories

The postprandial trajectories for NEFA tracked higher after CR ($p<0.001$), but not with RYGB (Figure 2). The postprandial trajectories for C2, long-chain acylcarnitines, total ketones and β -hydroxybutyrate were significantly increased with both interventions (all p -values <0.05). Changes in the postprandial trajectories for medium-chain acylcarnitines were only significant with CR ($p<0.001$).

Changes in postprandial glucose and glucoregulatory hormones trajectories

The postprandial trajectories for glucose, insulin, C-peptide, GLP-1, GIP and glucagon showed a steeper rise after RYGB (all p -values <0.05), but not after CR (Supplementary Appendix Figure S2 and S3). The increment in insulin, GIP and GLP-1 responses from baseline to peak were larger and occurred more rapidly after MMT in RYGB compared to CR.

Changes in postprandial gluconeogenic substrate trajectories

The postprandial trajectories for alanine, lactate, and pyruvate tracked significantly lower from pre- to post-intervention in both groups (Figure 3). However, at the later time points of MMT, marked divergence in trajectories were observed post-RYGB vs. post-CR for alanine ($p<0.001$), lactate ($p<0.001$) and pyruvate ($p=0.013$), such that levels of these metabolites were much lower in the RYGB group.

Interrelationship between the temporal changes in metabolites and gut hormones

Since temporal changes in metabolites (in particular glucose and amino acids) and gut hormones (in particular insulin, GIP and GLP-1) were distinctly different between CR and RYGB, we performed cross-correlation analysis to assess the interrelationship between their postprandial responses. In the RYGB group, leucine/isoleucine and methionine exhibited significant positive cross-correlation ranging from 0.624 to 0.679 at lag no=-1, indicating that the changes in these AA led the changes in insulin response (Table 2). Similarly, changes in GLP-1 led changes in insulin response (lag no=-1, CCF =0.564) in the RYGB group. A positive cross-correlation at negative lag numbers for tyrosine and glutamate/glutamine and GLP-1 suggests that changes in these AA led changes in GLP-1 response after RYGB.

DISCUSSION

Although RYGB is known to cause early improvements in glucose homeostasis, this is the first study that reports on a broad spectrum of metabolic changes occurring early after RYGB surgery. The key strength of the study is that subjects were well-matched for gender, race, age and body weight. CR subjects received controlled amounts of a liquid diet matched to post-surgical subjects in order to achieve similar negative energy balance. We thereby establish that the preferential lowering of BCAA and aromatic AA occurs early (2 weeks) after RYGB surgery, and is not due to differences in food intake, because unlike previous studies^{12, 18}, food intake was controlled in both intervention groups in this study. The changes in metabolites are more pronounced in the postprandial than in the fasted period after RYGB. In addition, the rate of changes in the appearance and clearance of multiple amino acids mirror the rate of changes in glucose, insulin, and incretins after RYGB surgery. The rate of change for several of these metabolites correlated with and predicted changes in insulin and incretin responses, suggesting a biological link between the rate of changes of these metabolites and the augmented gut hormonal responses. Finally, we observe distinct effects of RYGB on clearance of gluconeogenic substrates following meal challenge. The rapid peak in plasma glucose is consistent with more efficient entry of nutrients into the Roux-limb as postulated¹¹, but we demonstrate for the first time that this applies to multiple amino acids.

Almost all amino acid levels were significantly lower after RYGB than CR at the end of meal challenge, implying improved peripheral uptake and utilization of these metabolites. Since the postprandial trajectories of amino acids track closely to that of insulin, the enhanced nutrient clearance could be due to either increased insulin secretion or improved insulin action, or both. Amino acids are known as potent insulin secretagogues, and

metabolic clearance of amino acids is related to their effectiveness as insulin secretagogues¹⁹. In this study, changes in leucine/isoleucine, methionine and phenylalanine levels were correlated with changes in insulin levels after RYGB. Early changes in GLP-1 are also correlated with insulin after RYGB, a relationship that was not seen after CR⁶. Our findings suggest a link between the rapid appearance of amino acids, incretins and insulin secretion, agreeing with previous studies that show a synergistic relationship between incretins and amino acid levels in stimulation of insulin secretion²⁰. The exaggerated postprandial incretin response after RYGB may also be due to early and rapid exposure of distal gut to luminal glucose²¹ and partial protein hydrolysates²². While alanine, serine and glutamine have been shown to stimulate GLP-1 release from the Glutag cell line²³, we found that tyrosine and glutamate/glutamine appear to lead the changes in GLP-1 response to a meal after RYGB surgery. The exact physiological role of these amino acids in the regulation of incretin responses is a subject worthy of further investigation. We also observed that RYGB has a larger impact on lipid metabolism than CR. Multiple intermediates and endpoints of fatty acid oxidation tended to be higher in the fasted state in RYGB subjects. This includes acetylcarnitine (C2), total ketones, β -hydroxybutyrate, and long-chain acylcarnitines. Interestingly, with RYGB surgery, postprandial NEFA levels decline at a rate similar to CR, but levels of endproducts of fatty acid oxidation, including ketones, decline more slowly. The medium-chain (incompletely oxidized) acylcarnitines levels are higher after CR than after RYGB. We interpret these results to indicate a higher rate and efficiency of fatty acid oxidation after RYGB. Increased catabolism of ketogenic amino acids such as leucine may also contribute to the sustained elevation of ketones post-RYGB surgery.

Finally, the postprandial trajectories for alanine, pyruvate and lactate differ remarkably between CR and RYGB which could possibly explain the greater susceptibility to hypoglycemia reported for the latter group⁸. While these key gluconeogenic substrates levels generally increase with CR, they decline after RYGB, and most strikingly towards the conclusion of meal challenge. Prior studies have suggested that β -cell hyperplasia with resultant hyperinsulinemia is the primary cause of hypoglycemia after RYGB²⁴. However, plasma glucose and insulin responses to oral glucose in patients with symptomatic hypoglycemia after RYGB are rather similar to individuals who remain asymptomatic⁷. Thus, our finding of exaggerated postprandial insulin responses in RYGB subjects followed by similarly exaggerated clearance for glucose, amino acids, lactate, and pyruvate may suggest that the subjects become more reliant on gluconeogenesis to maintain glucose levels after RYGB, but now in the face of reduced availability of key gluconeogenic precursors.

The study has several limitations. Static metabolite profiles can identify perturbations in fuel metabolism, but they do not provide definitive information about metabolic flux. Although cross-correlation analysis suggests that amino acids could modulate gut hormone responses, no direct evidence for a cause-effect relationship was provided. The magnitude of weight loss was slightly greater with RYGB than CR. Although most of the weight loss during this period is likely due to reduction in total body water²⁵, we included body weight as a covariate in our analyses to account for the group differences. Thus, the early and specific adaptive metabolic responses in RYGB compared to CR subjects described herein are independent of the amount of weight lost. Although post-surgery period might influence the metabolic responses, it is more likely to interfere with rather than enhance improvement in glycemic control. Finally, further studies are needed to ascertain if the findings of postprandial metabolite profiles observed in patients after RYGB bypass are similar to those induced by other bariatric procedures, e.g. sleeve gastrectomy. In conclusion, greater disposal of glucose and amino acids, and more complete β -oxidation of fatty acids are early and positive changes in metabolic flexibility post-RYGB might contribute to the continuous and sustained improvement in insulin sensitivity in these subjects seen with longer follow-

up. The changes in the trajectories of these metabolites is distinct after RYGB surgery and is likely due to enhanced luminal nutrient transit and absorption as a result of structural alterations in the gut anatomy. Findings of a sharp reduction in key gluconeogenic substrates may also help to explain the greater susceptibility of RYGB subjects to hypoglycemic symptoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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CMK wrote the manuscript and performed statistical analysis of the data. MJM and RDS performed the metabolite and hormonal profiling assays, and ZP and JC helped in hormonal profiling. CBN and AT designed the study and reviewed and edited the manuscript.

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Figure 1. Postprandial trajectories for selected amino acids in response to mixed meal challenge
 The overall postprandial mean responses for the amino acids shown here were significantly different from pre- to post-intervention for both groups (all p -values < 0.005), except for phenylalanine ($p < 0.001$) which was significant with RYGB surgery, but not with CR. The postprandial trajectories for valine, leucine/isoleucine, phenylalanine, tyrosine, proline, methionine, serine, and arginine were significantly altered with between post-CR and post-RYGB surgery (all interaction terms p -values < 0.001), but not with CR. * $p < 0.05$ for differences between post-CR and post-RYGB surgery in plasma levels at the indicated meal challenge time-point.

Figure 2. Postprandial trajectories for C2, molar sum of C3 + C5, medium-chain and long-chain acylcarnitines (AC), non-esterified fatty acids and total ketones in response to mixed meal challenge

No statistical difference between post-CR and post-RYGB surgery were observed for these postprandial metabolite trajectories. * $p < 0.05$ for differences in plasma levels between post-CR and post-RYGB surgery at the indicated meal challenge time-point.

Figure 3. Postprandial trajectories for gluconeogenic substrates (alanine, pyruvate and lactate) in response to mixed meal challenge

The overall postprandial mean responses for the metabolites shown here were significantly different from pre- to post-intervention for both groups (all p-values < 0.05). The postprandial trajectories between post-CR and post-RYGB surgery are significantly different for alanine (p < 0.001), pyruvate (p = 0.013), and lactate (p < 0.001). *p < 0.05 for differences in plasma levels at the indicated meal challenge time-point.

Table 1
Plasma metabolites in overnight fasted subjects, and two hours after a mixed meal tolerance test

Data are expressed as percentage change (%) post-intervention relative to pre-intervention for the caloric restriction (CR) or Roux-en-Y gastric bypass (RYGB) surgery subjects. Data are presented as mean $\pm$ SE. ¹P and ²P are p-values for comparison between changes occurring as a result of RYGB or CR, adjusted for differences in body weight. The symbol * indicates p-value <0.05 for differences between pre-and post-CR, or pre- and post-RYGB surgery. Total AA, molar sum of all amino acids measured; BCAA, molar sum of branched-chain amino acids (Val + Leu/Ile); Aromatic AA, molar sum of Phe + Tyr.

	Fasting (Percentage change, %)			2-hour Post-meal Challenge (Percentage change, %)		
	CR	RYGB	¹ P	CR	RYGB	² P
Amino acids						
Glycine	3.25 $\pm$ 4.41	14.37 $\pm$ 7.59	0.222	9.45 $\pm$ 5.40	6.84 $\pm$ 4.5	0.724
Alanine	-11.65 $\pm$ 5.87*	-18.39 $\pm$ 5.48*	0.412	1.09 $\pm$ 8.62	-23.64 $\pm$ 7.15*	0.040
Serine	15.27 $\pm$ 6.57	22.53 $\pm$ 7.00*	0.459	13.94 $\pm$ 7.56	2.51 $\pm$ 4.91	0.221
Proline	-7.58 $\pm$ 6.30	-16.34 $\pm$ 6.06*	0.330	-1.57 $\pm$ 6.47	-20.10 $\pm$ 4.86*	0.034
Methionine	-1.88 $\pm$ 5.66	-3.32 $\pm$ 5.99	0.863	3.31 $\pm$ 7.46	-19.98 $\pm$ 4.65*	0.016
Histidine	-3.66 $\pm$ 4.97	-17.57 $\pm$ 2.97*	0.027	-4.32 $\pm$ 3.27	-27.70 $\pm$ 4.91*	0.001
Valine	-2.07 $\pm$ 6.98	-15.40 $\pm$ 5.26*	0.145	-1.68 $\pm$ 4.73	-18.76 $\pm$ 4.99*	0.023
Leucine/Isoleucine	1.52 $\pm$ 8.03	-8.75 $\pm$ 5.57	0.309	-1.71 $\pm$ 5.73	-20.80 $\pm$ 6.76*	0.045
Phenylalanine	1.93 $\pm$ 3.08	-16.62 $\pm$ 2.58*	<0.001	2.50 $\pm$ 4.02	-26.82 $\pm$ 2.38*	<0.001
Tyrosine	1.37 $\pm$ 7.59	-9.12 $\pm$ 5.96	0.293	3.48 $\pm$ 5.85	-16.04 $\pm$ 3.77*	0.012
Aspartate/Asparagine	8.50 $\pm$ 6.81	10.59 $\pm$ 8.35	0.848	17.69 $\pm$ 4.67*	34.46 $\pm$ 12.67*	0.239
Glutamate/glutamine	-4.79 $\pm$ 3.96	9.64 $\pm$ 7.48	0.105	1.06 $\pm$ 6.74	9.14 $\pm$ 13.32	0.595
Arginine	-0.13 $\pm$ 4.18	-1.15 $\pm$ 5.18	0.880	2.45 $\pm$ 5.94	-17.87 $\pm$ 5.56*	0.022
BCAA	-0.86 $\pm$ 7.16	-12.87 $\pm$ 5.13*	0.190	-1.85 $\pm$ 4.83	-19.73 $\pm$ 5.62*	0.027
Aromatic AA	0.99 $\pm$ 5.06	-13.52 $\pm$ 3.43*	0.029	2.88 $\pm$ 4.81	-21.30 $\pm$ 2.56*	<0.001
Total AA	-3.36 $\pm$ 3.79	-6.75 $\pm$ 2.76*	0.480	1.39 $\pm$ 4.47	-13.83 $\pm$ 2.22*	0.007
Acylcarnitines						
C2	49.79 $\pm$ 10.55*	75.39 $\pm$ 17.15*	0.220	26.30 $\pm$ 10.16*	50.82 $\pm$ 18.92*	0.273
C3 + C5	-17.48 $\pm$ 5.77*	-20.96 $\pm$ 7.17*	0.710	-7.09 $\pm$ 5.14	-10.47 $\pm$ 9.70	0.762
Medium-chain	45.17 $\pm$ 10.31*	7.23 $\pm$ 8.61	0.011	39.74 $\pm$ 14.11*	9.15 $\pm$ 7.66*	0.073
Long-chain	51.73 $\pm$ 4.14*	50.41 $\pm$ 10.58*	0.909	29.30 $\pm$ 5.47*	33.17 $\pm$ 11.68*	0.768

	Fasting (Percentage change, %)			2-hour Post-meal Challenge (Percentage change, %)			p
	CR	RYGB	IP	CR	RYGB		
Total acylcarnitines	41.70 ± 8.29*	58.58 ± 13.09*	0.290	22.48 ± 7.69*	38.52 ± 14.14*		0.336
Urea cycle products							
Ornithine	-5.04 ± 7.11	-7.36 ± 5.12	0.794	-7.32 ± 4.44	-13.60 ± 5.35*		0.378
Citrulline	8.43 ± 9.57	-1.87 ± 9.13	0.446	-9.27 ± 9.81	-10.91 ± 10.14		0.909
Free fatty acid and products							
Non-esterified fatty acids	45.9 ± 10.8*	27.8 ± 11.6*	0.271	35.2 ± 18.1	29.0 ± 22.5		0.883
Total ketones	312.9 ± 79.5*	584.2 ± 229.8*	0.279	41.4 ± 15.8	162.4 ± 92.8		0.229
3β-hydroxybutyrate	349.7 ± 96.3*	710.9 ± 280.4*	0.239	34.3 ± 14.5	184.4 ± 102.4		0.180
Glucose	-10.1 ± 5.4	-9.4 ± 11.2	0.956	-9.1 ± 7.6	-24.1 ± 8.0*		0.191

Table 2

Maximum cross-correlation function (CCF) value with its corresponding lag no for the relationship between the changes in the metabolites and gut hormones (insulin, GIP and GLP-1). Lag no<0 indicates that metabolite of interest leads the corresponding hormone, lag no>0 indicates that metabolite of interest lags the corresponding hormone and lag no=0 means that the changes in metabolite and hormone occurs simultaneously. CCF value>0.500 are bold for easy reading. All the values are statistically significant at p<0.05. We omitted CCF values with p>0.05. CR, caloric restriction; RYGB, Roux-en-Y bypass; glucose-dependent insulinotropic polypeptide, GIP; glucagon-like peptide-1, GLP1

	Percent Change in Insulin				Percent Change in GIP				Percent Change in GLP-1			
	CR		RYGB		CR		RYGB		CR		RYGB	
	CCF	Lag no	CCF	Lag no	CCF	Lag no	CCF	Lag no	CCF	Lag no	CCF	Lag no
Glycine	-	-	-	-	0.303	0	-	-	0.391	2	-	-
Alanine	-0.358	3	0.528	1	0.483	0	0.365	0	0.382	1	0.317	2
Serine	0.387	-1	0.318	1	0.313	1	-	-	-0.404	3	-	-
Proline	0.521	0	0.598	0	0.626	0	0.642	0	0.363	1	0.424	-3
Valine	0.534	0	0.479	-1	0.518	0	0.404	0	0.328	0	0.611	0
Leucine/isoleucine	0.587	0	0.679	-1	0.546	0	0.566	0	-0.379	-2	0.636	0
Methionine	0.401	0	0.624	-1	0.667	0	0.579	0	0.366	-4	0.524	0
Histidine	0.394	-1	0.339	1	-	-	-	-	-	-	0.473	2
Phenylalanine	-0.324	-3	0.646	-1	0.463	0	0.507	0	-	-	0.597	0
Tyrosine	0.483	0	0.616	0	0.605	0	0.555	0	0.381	0	0.506	-3
Aspartate/Asparagine	0.307	2	-	-	0.652	2	-	-	0.336	2	0.355	-2
Glutamate/glutamine	-	-	-	-	-	-	-	-	-	-	0.535	-2
Arginine	0.395	0	0.431	1	0.370	0	0.493	-1	-0.385	-2	0.420	-3
Ornithine	-0.389	-2	0.486	-1	-	-	-	-	-0.335	-2	0.439	0
Citrulline	0.500	-1	0.542	-4	0.405	-1	-	-	-	-	-	-
Glucose	0.580	0	0.593	0	-0.351	3	0.404	0	-	-	0.414	0
NEFA	-	-	-	-	0.334	2	-	-	-0.356	0	-	-
GIP	0.465	0	0.725	0	-	-	-	-	-	-	-	-
GLP-1	-	-	0.564	-1	-	-	-	-	-	-	-	-