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ORIGINAL CONTRIBUTIONS

Comprehensive Assessment of the Effects of Sleeve Gastrectomy on Glucose, Lipid, and Amino Acid Metabolism in Asian Individuals with Morbid Obesity

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Abstract

Background Obesity-induced insulin resistance leads to abnormalities in glucose, lipid, and amino acid metabolism. Our study examined the differences in insulin-mediated glucose, amino acid, and lipid metabolism between morbidly obese subjects with non-obese controls and the associated changes following sleeve gastrectomy (SG).

Methods Non-obese controls and individuals with morbid obesity and scheduled for SG were recruited. Metabolic assessments were performed for all subjects at baseline and at 6 months after SG for eight subjects. The hyperinsulinemic-euglycemic clamp technique together with comprehensive metabolomic profiling was used to quantify insulin-mediated glucose, amino acid, and lipid metabolism.

Results Eleven morbidly obese non-diabetic subjects scheduled for SG and nine non-obese controls were recruited. Compared to controls, obese subjects had significantly lower glucose uptake (4.4 ± 0.6 vs. 17.3 ± 2.4 mg/kg FFM/min per μU/mL·100) and higher concentration of branched-chain amino acids (BCAAs, 332.5 ± 26.8 vs. 235.3 ± 11.0 μM), non-esterified fatty acid (52.9 ± 9.9 vs. 25.6 ± 6.7 μM), and lipid-related acylcarnitines (intermediate chain 389.8 ± 32.5 vs. 285.9 ± 20.5; long chain 301.7 ± 22.1 vs. 236.0 ± 13.3 nM) during insulin clamp. Body weight significantly reduced at 6 months after bariatric surgery (92.5 ± 6.3 vs. 115.2 ± 6.9 kg), together with improvements in insulin-mediated glucose uptake, and suppression of BCAAs, non-esterified fatty acids, and lipid-related metabolites.

Conclusions Morbid obesity in Asian individuals was associated with impairment in the regulatory actions of insulin on glucose, amino acid, and lipid metabolism, and these obesity-induced regulatory dysfunctions improved significantly 6 months after SG.

Keywords Metabolomics · Sleeve gastrectomy · Hyperinsulinemic-euglycemic clamp · Branched-chain amino acids · Asians · Morbid obesity

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Background

Obesity is a public health concern that has reached epidemic proportions and weight loss is the cornerstone of obesity management. However, conventional weight management with lifestyle intervention and pharmacologic agents are often modest in efficacy and non-sustainable in the long term [1–3]. Further, weight loss drugs can be associated with intolerable side effects. Bariatric surgery, by contrast, provides a more effective and sustainable solution to weight management compared to conventional treatment [4]. Among the various bariatric surgery techniques, Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) are the two most commonly performed procedures. Both procedures are not only effective in

weight reduction but also improve various physiological parameters, including insulin resistance (IR) [5].

IR is closely associated with obesity and is commonly defined as the impaired ability of insulin to regulate glucose homeostasis. In addition, obesity-induced IR is also linked to abnormalities in amino acid and lipid metabolism [6]. In this regard, several amino acids such as the branched-chain amino acids (BCAAs) [7, 8] and intermediates of incomplete lipid oxidation [8, 9] are elevated in morbid obesity. These metabolites may participate directly in IR pathogenesis, and changes in their levels have been proposed to contribute towards the improvement of IR after bariatric surgery [10, 11]. Presently, our understanding regarding the relationship between amino acid and lipid homeostasis with IR improvement after bariatric surgery is derived mainly from patients undergoing RYGB [5, 12]. A better understanding of these changes after SG is needed, as SG has become one of the most commonly performed bariatric surgery worldwide [13, 14]. This clarification is especially relevant among Asian individuals, who are known to be more IR and develop type 2 diabetes mellitus at a lower body mass index (BMI) compared to other ethnicities [15]. The number of patients undergoing SG in Asia is also expected to increase with rising obesity and diabetes rates [16].

Aims

In this study, we used the hyperinsulinemic-euglycemic clamp technique together with comprehensive plasma metabolomic profiling to compare insulin-mediated glucose, amino acid, and lipid metabolism between morbidly obese scheduled for SG and non-obese Asian individuals at baseline. To further evaluate the metabolic effect of SG, similar evaluations were performed in morbidly obese individuals 6 months after surgery.

Materials and Methods

Subjects

Individuals with morbid obesity and scheduled for SG were recruited from the Singapore General Hospital's weight management clinic. Criteria for bariatric surgery include BMI ≥ 37.5 or ≥ 32.5 kg/m^2 in the presence of obesity-related complications and failure to achieve weight loss following conventional medical intervention. Non-obese subjects (BMI < 27.5 kg/m^2) were also recruited from our healthy volunteer database as controls. Subjects were excluded if they had diabetes, cardiovascular, kidney or liver disorders. None of the subjects were on any medication that could affect glucose, lipid, or amino acid metabolism. Participant recruitment flow is summarized in the consort diagram (Supplementary Fig. 1).

Study Protocol

All subjects underwent metabolic evaluations at baseline including standard biochemistry, anthropometric measurements, OGTT, and hyperinsulinemic-euglycemic clamp studies. The same study procedures were repeated for the morbidly obese subjects at 6 months following bariatric surgery.

Anthropometric Measurements and Body Composition

Height, weight, waist circumference (WC), hip circumference (HC), and blood pressure were taken three times and averaged. Body composition: total fat mass (FM), fat-free mass (FFM), and FM percentage were estimated using tetrapolar bioelectric impedance analysis (Tanita Body Composition scale, model TBF-300, Tanita Corporation, Tokyo, Japan). Lipid panel and liver and kidney function tests were performed with standard laboratory methods.

Oral Glucose Tolerance Test

OGTT was performed after overnight fasting, and venous blood was drawn at 0, 15, 30, 60, 90, and 120 min after 75 g dextrose administration for measurements of insulin and glucose concentration.

Hyperinsulinemic-Euglycemic Clamp

Hyperinsulinemic-euglycemic clamp was performed to assess the response of glucose, amino acid, and lipid metabolism to the direct effects of insulin stimulation. Insulin clamp was performed after overnight fasting of 8 h. Intravenous cannulas were inserted on opposite arms for intravenous infusion and collection of blood samples. A warming blanket was used on the blood-taking arm for the arterialization of venous blood. After collecting fasting blood samples for insulin, glucagon, glucose, non-esterified fatty acids (NEFAs), and metabolomics, insulin infusion was started at 40 mU/m^2/min for the next 180 min. Blood glucose was measured every 5 min using an on-site glucose analyzer (YSI 2300 STATPLUS, YSI Incorporated, Life Sciences, Yellow Springs, OH, USA), and dextrose 20% was infused at variable rates to maintain blood glucose at 100 mg/dL with a coefficient variation of $< 5\%$. Blood taken during the final 30 min of the insulin clamp was used for measurements of changes of hormones and metabolites in response to insulin stimulation. Indirect calorimetry (Quark RMR, Cosmed, Rome, Italy) was performed

30 min before and during the last 30 min of the clamp to measure the changes in respiratory quotient (RQ).

Laboratory Measurements

Glucose concentration was measured with glucose oxidase method (YSI Inc., Yellow Springs, OH, USA), and insulin measured with carbonylmetalloimmune assay (Abbott Diagnostics, Wiesbaden, Germany). Plasma NEFA concentration was measured with enzymatic essay (Wako Diagnostics, Richmond, VA, USA) and glucagon using ELISA method (EZGLU-30K, EMD Millipore Corporation, St. Charles, Missouri, USA). Metabolomic profiling was performed using liquid chromatography tandem mass spectrometry at the Duke-NUS metabolism core facility as previously described [17].

Calculations

During OGTT, homeostasis model assessment for insulin resistance (HOMA-IR), Matsuda index, and area under curve (AUC) for glucose and insulin concentration were calculated. HOMA-IR was calculated by the product of fasting glucose and insulin divided by 22.5 [18], and the whole-body insulin sensitivity index (Matsuda index) was calculated as 100,000 divided by the square root of the product of fasting glucose, fasting insulin, mean glucose, and mean insulin concentration [19]. Glucose and insulin AUC were calculated by trapezoidal law.

Insulin sensitivity was quantified using the insulin clamp and represented as the glucose infusion rate (GIR) and insulin sensitivity index (ISI). Subjects who were more IR would had lower GIR and ISI. GIR was calculated as the glucose infusion rate to maintain euglycemia during the final 30 min. ISI was calculated to account for the variation in the final plasma insulin reached during insulin clamp as the GIR divided by average insulin concentration in the last 30 min and multiplied by 100. Both GIR and ISI were expressed per kilogram FFM per minute. Insulin metabolic clearance rate (MCR) was calculated by insulin infusion rate divided by the delta increase between fasting state and steady-state insulin concentration [20].

Data Analysis

To examine the differences in various metabolic measurements between the obese subjects and healthy controls, independent sample *t* tests or Mann–Whitney *U* tests were used. To study the impact of insulin infusion of NEFAs, acylcarnitines (ACs), and amino acids, the percentage of change in metabolite concentration from baseline was calculated. Within-individual changes in metabolites after insulin infusion and following bariatric surgery were tested using paired *t* tests or Wilcoxon signed-rank tests. Data are presented as mean ± SEM (interquartile range) and a two-tailed p value ≤ 0.05 is considered statistically significant. Statistical testing was performed using the SPSS version 22 (SPSS Inc., Chicago, IL, USA) and Prism version 7 (GraphPad software Inc., USA).

Results

Body Composition and Metabolic Characteristics

Eleven morbidly obese non-diabetic subjects scheduled for SG and nine non-obese controls were recruited. Baseline characteristics of study subjects are summarized in Table 1. Compared to healthy non-obese controls, morbidly obese subjects before bariatric surgery had significantly higher body weight, BMI, total body FM, FM percentage, and waist–hip ratio. FFM, however, was not significantly different between the two groups. Triglyceride (TG), alanine transaminase (ALT), and asparagine transaminase (AST) were also higher in the morbidly obese subjects at baseline.

Three morbidly obese subjects were removed from the postoperative analysis: two chose not to go through bariatric surgery, and one decided for RYGB instead of SG due to personal preferences. The remaining subjects lost an average of 22% of their baseline weight at 6 months after bariatric surgery, mainly from FM loss (Table 1). Serum TG, ALT, and AST decreased significantly but there was no change in the HDL or LDL concentrations (Table 1).

Oral Glucose Tolerance Test

Before bariatric surgery, morbidly obese subjects had higher blood glucose and insulin after overnight fast and following OGTT compared to the non-obese controls (Table 2 and Supplementary Fig. 2). HOMA-IR was significantly greater, and the Matsuda index was significantly lower in the morbidly obese.

After surgery, fasting glucose, insulin, and measurement of IR significantly improved (HOMA-IR was reduced by 66% and Matsuda index increased by 84%). Post-OGTT glucose and insulin also tended to be lower but these values did not achieve statistical significance (Table 2 and Supplementary Fig. 2).

Hyperinsulinemic-Euglycemic Clamp Study

Before bariatric surgery, morbidly obese subjects were significantly more IR than non-obese controls with lower GIR and ISI. Blood glucose during insulin clamp steady state was comparable between the two groups, and insulin concentration was higher in the morbidly obese but this did not reach statistical significance. RQ increased as expected in response to

Table 1 Baseline characteristics

Parameters	Non-obese ($n = 9$)	Obese		p (preop vs. non-obese)	p (pre vs. postop)
		Preop ($n = 11$)	Postop ($n = 8$)		
Age (years)	30.2 ± 2.6 (24.5, 34.5)	36.8 ± 9.1 (26.0, 45.0)		0.103	
Male (%)	22.2	27.3			
Ethnicity					
Chinese	55.6%	45.5%			
Malay	11.1%	27.3%			
Indian	33.3%	27.3%			
Weight (kg)	69.8 ± 4.1 (61.6, 79.2)	114.8 ± 6.8 (91.6, 136.2)	92.5 ± 6.3 (75.7, 107.4)	< 0.001	< 0.001
BMI (kg/m^2)	23.3 ± 0.8 (21.7, 24.7)	39.1 ± 1.4 (34.5, 42.8)	31.5 ± 1.1 (28.8, 34.2)	< 0.001	< 0.001
Fat mass (kg)	18.5 ± 1.8 (13.1, 23.2)	53.5 ± 4.5 (37.3, 65.4)	34.4 ± 3.3 (25.8, 41.5)	< 0.001	< 0.001
FFM (kg)	51.3 ± 3.7 (42.0, 60.2)	61.4 ± 4.1 (51.0, 71.6)	58.1 ± 4.3 (46.6, 63.4)	0.089	0.003
FM (%)	26.8 ± 2.6 (20.8, 31.0)	46.3 ± 2.2 (41.5, 52.1)	37.1 ± 2.1 (30.6, 42.5)	< 0.001	< 0.001
SBP (mmHg)	114.0 ± 4.4 (103.5, 122.0)	127.0 ± 3.1 (121.0, 137.0)	115.8 ± 3.6 (110.5, 125.3)	0.023	0.001
DBP (mmHg)	66.7 ± 2.7 (61.0, 72.5)	77.8 ± 2.3 (70.0, 83.0)	74.0 ± 2.8 (68.8, 79.5)	0.006	0.401
HC (cm)	95.8 ± 2.2 (91.2, 101.3)	125.2 ± 3.4 (115.9, 136.7)	113.2 ± 2.9 (108.1, 120.5)	< 0.001	< 0.001
WC (cm)	83.2 ± 2.1 (77.3, 88.5)	123.1 ± 4.9 (111.2, 138.0)	107.9 ± 4.7 (98.7, 120.4)	< 0.001	< 0.001
Waist–hip ratio	0.87 ± 0.01 (0.85, 0.91)	0.98 ± 0.02 (0.94, 1.07)	0.95 ± 0.02 (0.90, 1.02)	0.001	0.007
TC (mmol/L)	4.9 ± 0.2 (4.4, 5.2)	4.9 ± 0.2 (5.0, 5.1)	5.3 ± 0.3 (4.6, 6.0)	0.944	0.853
Triglycerides (mmol/L)	0.9 ± 0.1 (0.7, 1.2)	1.3 ± 0.2 (0.9, 1.7)	1.0 ± 0.1 (0.8, 1.2)	0.048	0.025
HDL (mmol/L)	1.4 ± 0.1 (1.0, 1.8)	1.2 ± 0.1 (1.0, 1.4)	1.2 ± 0.1 (1.0, 1.6)	0.276	0.769
LDL (mmol/L)	3.2 ± 0.2 (2.7, 3.6)	3.1 ± 0.2 (2.5, 3.8)	3.7 ± 0.2 (3.3, 4.1)	0.988	0.495
ALT (U/L)	17.9 ± 2.4 (12.5, 21.5)	40.0 ± 7.0 (27.0, 42.0)	15.4 ± 1.8 (10.5, 20.5)	0.013	< 0.001
AST (U/L)	18.6 ± 1.2 (16, 20)	28.3 ± 3.3 (19.0, 33.0)	18.1 ± 2.3 (13.3, 21.5)	0.017	0.005

Data presented as mean ± SEM (interquartile range)

BMI body mass index, *FM* fat mass, *FFM* fat free mass, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HC* hip circumference, *WC* waist circumference, *TC* total cholesterol, *HDL* high-density cholesterol, *LDL* low-density cholesterol, *ALT* alanine transaminase, *AST* aspartate transaminase

insulin infusion but the magnitude of increase tended to be greater in the non-obese controls (Table 3). Plasma glucagon levels were also higher in the morbidly obese compared to those in the controls but the difference was only statistically significant during the insulin clamp. Similarly, plasma NEFAs were higher in the morbidly obese, and this difference was most apparent during the insulin clamp.

After bariatric surgery, both GIR and ISI in the morbidly obese increased indicating significant improvements in IR. Steady-state insulin concentration during the clamp was also

Table 2 Oral glucose tolerance test

Parameters	Non-obese ($n = 9$)	Obese		p (preop vs. non-obese)	p (pre vs. postop)
		Preop ($n = 11$)	Postop ($n = 8$)		
Fasting glucose (mg/dL)	89.0 ± 3.4 (84.2, 92.4)	106.1 ± 3.2 (98.4, 114.0)	91.5 ± 2.4 (84.8, 95.2)	0.002	0.005
Fasting insulin (μU/mL)	6.0 ± 0.9 (4.5, 8.0)	21.3 ± 2.7 (11.7, 25.5)	8.9 ± 1.4 (6.0, 12.2)	< 0.001	0.001
HOMA-IR	1.3 ± 0.2 (1.0, 1.8)	5.6 ± 0.7 (2.8, 6.7)	2.0 ± 0.3 (1.3, 2.8)	< 0.001	< 0.001
Matsuda Index	7.8 ± 1.3 (4.7, 9.7)	1.9 ± 0.3 (1.2, 2.4)	3.2 ± 0.4 (2.3, 4.1)	< 0.001	0.001
Glucose AUC (min·mg/dL)	12,829 ± 822 (10,970, 14,190)	20,672 ± 1226 (15,908, 23,723)	18,744 ± 672 (17,719, 20,281)	< 0.001	0.199
Insulin AUC (min·μU/mL)	7135 ± 2140 (3289, 9652)	17,742 ± 3047 (7751, 28,846)	16,146 ± 2953 (11,408, 20,848)	0.004	0.528

Data presented as mean ± SEM (interquartile range)

HOMA-IR homeostasis model assessment for insulin resistance, *AUC* area under curve

Table 3 Hyperinsulinemic-euglycemic clamp study

Parameters	Non-obese ($n = 9$)	Obese		p (preop vs. non-obese)	p (pre vs. postop)
		Preop ($n = 11$)	Postop ($n = 8$)		
Fasting glucose (mg/dL)	89.1 ± 2.8 (83.2, 96.5)	103.5 ± 3.2 (91.1, 110.5)	88.1 ± 2.6 (82.6, 95.4)	0.004	0.009
Fasting insulin (μU/mL)	5.7 ± 1.4 (3.2, 6.5)	21.3 ± 3.4 (13.1, 27.2)	7.7 ± 1.2 (5.1, 9.3)	< 0.001	0.005
Clamp glucose (mg/dL)	100.4 ± 2.1 (94.1, 105.5)	104.2 ± 1.3 (100.8, 108.3)	105.4 ± 6.9 (98.0, 111.6)	0.129	0.856
Clamp insulin (μU/mL)	70.3 ± 7.5 (50.3, 87.0)	88.7 ± 7.0 (75.6, 110.3)	71.1 ± 4.8 (57.5, 83.5)	0.091	0.007
GIR (mg/kg FFM/min)	11.6 ± 1.9 (7.5, 15.2)	3.7 ± 0.4 (2.8, 4.5)	8.3 ± 0.9 (6.8, 10.6)	0.003	0.002
ISI (mg/kg FFM/min per μU/mL·100)	17.3 ± 2.4 (11.1, 23.8)	4.4 ± 0.6 (3.4, 6.6)	12.7 ± 1.4 (8.5, 16.0)	0.001	< 0.001
Insulin MCR (mL/m^2/min)	680 ± 71 (530, 854)	622 ± 40 (565, 699)	651 ± 43 (527, 767)	0.473	0.232
Fasting RQ	0.81 ± 0.02 (0.76, 0.85)	0.78 ± 0.01 (0.75, 0.80)	0.80 ± 0.03 (0.74, 0.82)	0.230	0.808
Clamp RQ	0.87 ± 0.03 (0.79, 0.96)	0.82 ± 0.01 (0.79, 0.84)	0.87 ± 0.02 (0.85, 0.90)	0.143	0.152
Δ RQ	0.08 ± 0.02 (0.02, 0.14)	0.03 ± 0.01 (0.00, 0.06)	0.08 ± 0.01 (0.06, 0.11)	0.061	0.016
Fasting NEFA (μM)	177.5 ± 21.9 (184.7, 242.8)	256.7 ± 32.7 (200.3, 361.1)	232.7 ± 31.2 (148.2, 280.2)	0.061	0.664
Clamp NEFA (μM)	25.6 ± 6.7 (12.4, 37.4)	52.9 ± 9.9 (26.4, 77.3)	5.5 ± 1.9 (1.0, 9.1)	0.036	0.007
Fasting glucagon (ng/mL)	0.031 ± 0.002 (0.026, 0.034)	0.048 ± 0.009 (0.021, 0.074)	0.026 ± 0.002 (0.021, 0.032)	0.081	0.171
Clamp glucagon (ng/mL)	0.009 ± 0.001 (0.07, 0.011)	0.018 ± 0003 (0.012, 0.025)	0.008 ± 0.001 (0.006, 0.011)	0.005	0.022

Data presented as mean ± SEM (interquartile range). $n = 6$ for fasting glucagon for non-obese controls

GIR glucose infusion rate, *ISI* insulin sensitivity index, *MCR* metabolic clearance rate, *RQ* respiratory quotient

lower and delta RQ significantly higher. Postoperation plasma glucagon and NEFAs in the morbidly obese decreased slightly, but the differences were only statistically significant when measured during insulin clamp (Table 3).

Plasma Amino Acids

Compared to non-obese controls, fasting phenylalanine, glutamine/glutamate, and tyrosine concentrations were significantly higher in the morbidly obese before bariatric surgery, but the concentrations of BCAAs and the remaining amino acids were not. Plasma concentration of BCAAs and the other amino acids in both groups decreased in response to insulin infusion. However, compared to non-obese controls, the percentage of decrease in plasma BCAAs in the morbidly obese following insulin infusion was significantly smaller (Fig. 1a). Consequently, concentration of BCAAs and the majority of amino acids (except glycine and alanine) in the morbidly obese were significantly higher during insulin clamp. Glycine was the only amino acid that was lower in the obese than in the controls during fasting and following insulin infusion.

After surgery, blood concentration of the BCAAs, alanine, glutamate/glutamine, methionine, phenylalanine, and tyrosine decreased in the morbidly obese during fasting and insulin clamp. Fasting methionine, fasting glutamate/glutamine, and alanine concentrations during insulin clamp also decreased in the postoperation obese but failed to reach statistical significance. Both fasting and insulin clamp glycine concentrations by contrast increased significantly after surgery (Table 4).

Plasma Acylcarnitines

Before bariatric surgery, fasting plasma AC concentrations were similar in the obese and non-obese controls. Plasma AC levels decreased significantly for both groups during the insulin clamp. However, the magnitude of suppression of intermediate- and long-chain ACs was lower in the morbidly obese subjects. Thus, obese subjects had higher concentrations of intermediate- and long-chain ACs compared to controls during the insulin clamp (Table 5 and Fig. 1b).

Following bariatric surgery, plasma concentrations of AC during fasting or insulin clamp in the morbidly obese subjects were largely unchanged (Table 5), although there appeared to

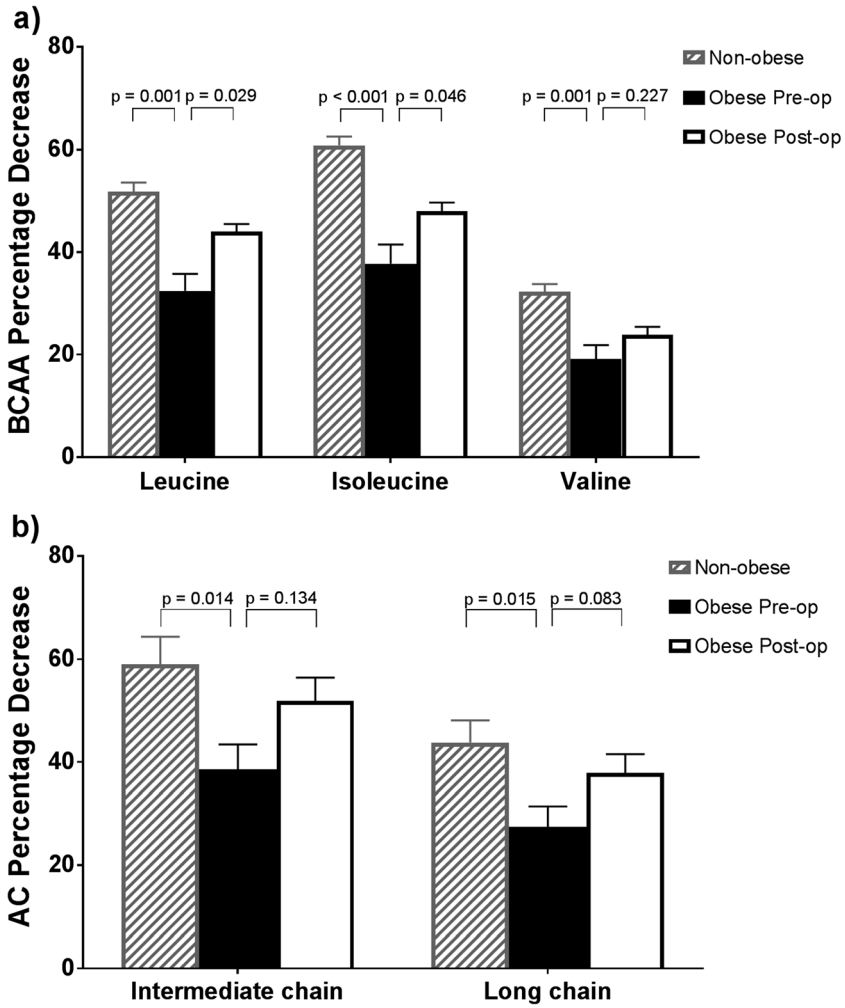

Fig. 1 Percentage reductions of branched-chain amino acids and acylcarnitines following insulin infusion. Percentage reductions of branched-chain amino acids (**a**) and intermediate- and long-chain acylcarnitines (**b**) were calculated for non-obese controls, pre- and postoperation obese subjects with concentrations during insulin infusion compared to fasting values. Data was presented as mean ± SEM

be a trend towards greater suppression of intermediate- and long-chain ACs during insulin clamp (Fig. 1b).

Discussion

In this study, the hyperinsulinemic-euglycemic clamp technique together with metabolomic profiling was used to comprehensively examine the changes in glucose, amino acid, and lipid metabolism in morbidly obese Asian individuals undergoing SG. The lower GIR and ISI together with the lack of insulin-mediated decrease in plasma amino acids, NEFAs, and lipid-associated AC in the morbidly obese individuals indicate that the regulatory actions of insulin on glucose, amino acid, and lipid metabolism were significantly impaired. These obesity-induced regulatory dysfunctions improved significantly after SG.

Excess adiposity, especially central adiposity, is a major determinant of IR and poor metabolic health. Morbidly obese subjects in this study demonstrated not only higher total and central adiposity but also other adverse metabolic parameters, such as higher blood pressures, serum triglycerides, and transaminases compared to controls. Further, although these subjects were non-diabetic by clinical criteria, fasting glucose and glucose excursion following OGTT were higher compared to controls despite higher insulin concentrations, indicating the presence of severe IR. Various markers have been developed to measure IR, but the hyperinsulinemic-euglycemic clamp is still considered the most accurate and the "gold-standard" method as it provides direct quantification of insulin-mediated glucose uptake [21]. Both direct measurements of IR using the hyperinsulinemic-euglycemic insulin clamp (GIR and ISI) and indirect measurements based on fasting (HOMA-IR) and OGTT (Matsuda Index) were used in this study, and the results demonstrated that the morbidly obese subjects were significantly more IR than non-obese controls. Following SG, all measurements of IR in the morbidly obese subjects improved significantly together with the decrease in adiposity (total body weight, fat mass, and waist-to-hip circumference). Limited studies have used the hyperinsulinemic-euglycemic clamp technique to measure changes in IR in patients undergoing SG [22–25], and this study adds to the literature by reporting

Table 4 Amino acid concentrations in fasting and clamp state

Amino acids (μM)	Non-obese ($n = 9$)	Obese		p (preop vs. non-obese)	p (pre vs. postop)
		Preop ($n = 11$)	Postop ($n = 8$)		
Isoleucine					
Fasting	78.7 ± 4.5 (67.6, 90.0)	83.4 ± 4.8 (68.5, 94.0)	61.7 ± 3.6 (55.2, 68.2)	0.484	< 0.001
Clamp	30.7 ± 1.9 (27.0, 34.6)	53.1 ± 5.7 (39.1, 64.5)	32.1 ± 2.2 (27.2, 33.3)	0.003	0.003
p*	< 0.001	< 0.001	< 0.001		
Leucine					
Fasting	111.2 ± 5.1 (100.7, 119.4)	119.0 ± 6.3 (104.1, 141.4)	85.5 ± 6.1 (68.8, 104.4)	0.363	< 0.001
Clamp	53.4 ± 2.5 (46.8, 61.2)	81.6 ± 7.3 (63.0, 100.9)	48.1 ± 4.2 (36.3, 59.5)	0.003	0.001
p*	< 0.001	< 0.001	< 0.001		
Valine					
Fasting	224.0 ± 11.6 (203.0, 258.3)	242.9 ± 12.1 (217.5, 284.8)	180.1 ± 11.8 (154.2, 212.3)	0.201	< 0.001
Clamp	151.2 ± 7.3 (132.9, 167.4)	197.7 ± 14.1 (150.4, 244.9)	137.0 ± 9.4 (115.8, 160.8)	0.013	< 0.001
p*	< 0.001	< 0.001	< 0.001		
Total BCAA					
Fasting	413.9 ± 20.5 (371.5, 463.6)	445.4 ± 22.7 (403.1, 520.2)	327.3 ± 20.7 (278.0, 391.7)	0.316	< 0.001
Clamp	235.3 ± 11.0 (204.7, 260.1)	332.5 ± 26.8 (244.5, 410.3)	217.2 ± 15.4 (178.4, 253.2)	0.005	< 0.001
p*	< 0.001	< 0.001	< 0.001		
Alanine					
Fasting	312.6 ± 14.4 (271.6, 353.7)	341.9 ± 15.5 (291.8, 369.8)	285.9 ± 24.4 (230.0, 349.7)	0.190	0.014
Clamp	265.29 ± 11.0 (241.4, 284.8)	285.3 ± 19.1 (238.3, 331.4)	261.8 ± 20.1 (210.6, 326.5)	0.404	0.129
p*	0.002	< 0.001	0.022		
Glutamate/glutamine					
Fasting	93.8 ± 6.7 (76.2, 117.0)	117.5 ± 8.0 (93.4, 140.9)	101.2 ± 6.3 (87.9, 121.6)	0.040	0.162
Clamp	72.4 ± 2.6 (67.0, 81.6)	103.5 ± 8.2 (75.4, 123.2)	77.7 ± 7.8 (55.2, 101.2)	0.007	0.032
p*	0.008	< 0.001	0.001		
Glycine					
Fasting	157.8 ± 6.9 (142.4, 180.1)	122.0 ± 5.7 (107.0, 135.2)	161.5 ± 10.6 (132.7, 187.3)	0.001	0.001
Clamp	138.5 ± 5.2 (124.5, 147.0)	116.9 ± 5.8 (103.8, 125.2)	146.0 ± 7.6 (130.6, 166.5)	0.014	< 0.001
p*	0.001	0.120	0.013		
Methionine					
Fasting	27.7 ± 1.2 (25.0, 29.9)	26.5 ± 1.3 (22.0, 31.0)	22.5 ± 1.1 (20.8, 24.1)	0.503	0.113
Clamp	15.5 ± 1.0 (13.9, 18.1)	20.2 ± 1.4 (17.5, 22.8)	14.4 ± 0.89 (12.7, 16.9)	0.018	0.008
p*	0.008	< 0.001	< 0.001		
Phenylalanine					
Fasting	51.5 ± 1.0 (47.7, 54.2)	57.7 ± 1.8 (54.6, 60.7)	45.1 ± 2.0 (40.0, 51.0)	0.013	0.001
Clamp	33.8 ± 1.2 (30.7, 36.8)	47.0 ± 3.1 (43.6, 50.5)	33.2 ± 1.7 (28.7, 38.7)	0.002	0.007
p*	< 0.001	< 0.001	< 0.001		
Tyrosine					
Fasting	55.4 ± 2.4 (49.9, 58.2)	72.4 ± 3.7 (65.9, 78.3)	52.3 ± 3.4 (43.0, 58.3)	0.002	< 0.001
Clamp	32.4 ± 1.4 (27.7, 36.2)	56.3 ± 4.4 (50.3, 62.4)	36.2 ± 3.2 (28.0, 38.4)	< 0.001	0.001
p*	< 0.001	< 0.001	< 0.001		

Data presented as mean ± SEM (interquartile range)

*Difference between fasting and clamp values

significant improvements of IR quantified using insulin clamp in morbidly obese Asian individuals after SG. IR involves dysregulated nutrient homeostasis of not only glucose but also amino acids.

Previous studies have demonstrated significantly higher plasma BCAAs in obese individuals, and these amino acids tracked closely with the progression and resolution of IR [8, 17, 26]. The reason for the higher BCAA in obesity is debatable

Table 5 Acylcarnitine concentrations in fasting and clamp state

Acylcarnitines (nM)	Non-obese ($n = 9$)	Obese		p (pre-op vs. non-obese)	p (pre vs. postop)
		Preop ($n = 11$)	Postop ($n = 8$)		
C2					
Fasting	7583 ± 990 (5674, 8901)	7678 ± 791 (6586, 8964)	8506 ± 1015 (6668, 10,558)	0.941	0.378
Clamp	4171 ± 384 (3374, 4971)	4222 ± 373 (2929, 5364)	4490 ± 335 (3563, 4935)	0.926	0.442
p*	0.003	< 0.001	0.001		
C3					
Fasting	478.0 ± 37.4 (370.1, 549.5)	393.7 ± 32.0 (274.6, 496.3)	391.3 ± 44.1 (294.2, 470.6)	0.102	0.829
Clamp	380.0 ± 34.4 (270.4, 474.2)	351.9 ± 30.6 (232.6, 427.4)	342.6 ± 32.8 (264.9, 405.9)	0.548	0.577
p*	0.001	0.002	0.019		
C5					
Fasting	129.1 ± 13.1 (97.5, 148.0)	114.6 ± 6.2 (97.0, 128.2)	95.6 ± 13.1 (69.9, 110.5)	0.302	0.141
Clamp	92.1 ± 11.0 (74.1, 105.4)	94.8 ± 11.2 (65.4, 135.9)	71.2 ± 7.9 (52.5, 92.2)	0.865	0.114
p*	< 0.001	0.033	0.015		
C4-OH					
Fasting	16.9 ± 3.3 (9.8, 21.9)	20.2 ± 2.9 (14.0, 27.0)	23.4 ± 4.2 (15.0, 29.6)	0.464	0.349
Clamp	11.6 ± 2.4 (7.5, 13.8)	13.6 ± 1.7 (9.0, 21.1)	14.7 ± 2.4 (7.4, 19.1)	0.503	0.242
p*	0.015	0.003	0.005		
C3 + C5					
Fasting	607.1 ± 43.4 (485.4, 703.5)	508.3 ± 37.2 (380.4, 636.3)	486.9 ± 52.9 (368.4, 618.7)	0.100	0.589
Clamp	472.0 ± 41.8 (350.3, 561.3)	446.7 ± 40.4 (306.5, 544.1)	413.8 ± 39.0 (315.3, 504.1)	0.67	0.357
p*	< 0.001	0.001	0.007		
Intermediate chain					
Fasting	757.7 ± 84.4 (574.5, 914.5)	658.9 ± 56.6 (537.7, 825.1)	761.8 ± 95.5 (594.3, 815.0)	0.329	0.340
Clamp	285.9 ± 20.5 (247.5, 330.2)	389.8 ± 32.5 (296.1, 521.6)	344.9 ± 28.0 (265.5, 406.2)	0.016	0.219
p*	< 0.001	< 0.001	0.002		
Long chain					
Fasting	438.4 ± 37.9 (344.4, 530.5)	422.6 ± 30.0 (338.2, 478.4)	460.4 ± 25.5 (413.4, 497.4)	0.744	0.328
Clamp	236.0 ± 13.3 (202.2, 275.6)	301.7 ± 22.1 (248.4, 352.1)	285.5 ± 23.9 (231.1, 357.9)	0.028	0.294
p*	< 0.001	< 0.001	< 0.001		

Data presented as mean ± SEM (interquartile range)

Intermediate chain, molar sum of C6-C12; long chain, molar sum of C14-C18

*Difference between fasting and clamp value

but could be due to (1) an increase in appearance of BCAA in the circulation secondary to enhanced proteolysis and/or (2) a decrease in clearance through impaired oxidation or other catabolic pathways [27]. Both of these conditions can be a consequence of IR: increased proteolysis may stem from an impairment of the anti-proteolytic action of insulin in the obese state; similarly, IR is known to be associated with impaired mitochondrial fuel oxidation, which could lead to reduced amino acid clearance. In this study, fasting plasma BCAA concentrations in the morbidly obese (pre-surgery) were not significantly higher than those in the non-obese controls. The reason for the lack of difference in fasting BCAA between the two groups may be due to the smaller sample size. It is also known that the magnitude of increase in plasma BCAA is proportionate to the severity of glucose intolerance [28], and none of the obese subjects in this study had diabetes. By pairing the insulin clamp technique with plasma metabolomics profiling, the anti-proteolytic action of insulin can be assessed by measuring the decrease of plasma amino acids during insulin infusion. A smaller decrease would indicate the lack of inhibition of proteolysis due to IR [29, 30]. Consistent with previous studies, the percentage suppression of plasma BCAA in response to insulin was significantly smaller among the morbidly obese subjects compared to controls. Consequently, the final concentrations of BCAAs in morbidly obese during the insulin clamp were significantly higher compared to the controls. Following SG, a greater suppression of plasma BCAAs during the clamp and lower plasma BCAAs was seen in the morbidly obese individuals compared to the pre-operation values, suggesting that the anti-proteolytic action of insulin was partially restored after

weight loss. Changes in amino acid catabolic pathways may also be responsible for changes in plasma amino acid in obesity and after bariatric surgery. C3 and C5 AC plasma concentrations reflect amino acid catabolism, including the breakdown of the BCAA. These ACs were elevated in patients with morbid obesity [8] and decreased after bariatric surgery [31], consistent with the idea that BCAA catabolism is higher in obesity. However, plasma concentration of these ACs in the morbidly obese subjects in this study was not significantly different compared to that in the controls before bariatric surgery and did not change after SG, suggesting that BCAA catabolism rates are unaffected by obesity. Collectively, these results indicate that elevated BCAA levels in obesity were due to the impairment in insulin-mediated suppression of proteolysis, rather than decreased catabolism. To the best of our knowledge, only one study had investigated changes in plasma BCAA using insulin clamp after RYGB and gastric banding [31], and this is the first study that has used this technique in patients undergoing SG.

Several other amino acids such as phenylalanine, tyrosine, glutamine/glutamate, and methionine followed the same pattern of changes as the BCAAs in the morbidly obese subjects. However, glycine was the only amino acid that was lower in the morbidly obese compared to controls before surgery and increased significantly after SG. Similar findings have been reported in other types of bariatric surgery, but the reason why glycine is low in morbid obesity and increases after weight loss remains an enigma. This might be related to the formation of urinary glycine adducts during periods of "hyperaminoacidemia" [32] or related to glycine's role in anti-oxidant defense [33], but these postulations will need to be clarified in future studies.

The relationship between lipid and obesity-induced IR is well established. NEFA concentrations were higher in morbidly obese subjects in this study compared to non-obese controls during fasting and even more so during insulin clamp. Consistent with this finding, the intermediate- (C6-12) and long-chain (C14-18) ACs, which reflect fatty acid oxidation, were higher in the morbidly obese subjects during insulin clamp. These results suggest that the accelerated rate of lipolysis in morbid obesity due to IR was also accompanied by increased mitochondrial fatty acid oxidation. After SG, the fasting plasma NEFAs as well as intermediate- and long-chain ACs did not decrease, despite a significant reduction in body weight and improvements in insulin-mediated glucose uptake. These results indicate a significant reliance of fatty acid as the metabolic fuel and is not unexpected as the morbidly obese subjects in this study were evaluated relatively early after SG when they remained at a state of significant negative energy balance. A state of negative energy balance drives the use of fatty acid as the metabolic fuel [34] and higher rates of lipolysis and blood concentrations of NEFAs [35] and intermediate- and long-chain ACs [11] have been reported during the early stages following RYGB. Interestingly, compared to preoperation values, plasma NEFA during insulin clamp was significantly lower post-SG. This was also accompanied by a trend towards greater reductions in intermediate- and long-chain ACs during insulin clamp. These results suggest (1) appropriate fuel selection in patients undergoing weight loss post-SG and (2) significant improvement in insulin's ability to inhibit lipolysis and FFA oxidation following SG. This interpretation is supported by findings of the increase in RQ (Δ RQ) with insulin clamp after bariatric surgery, which indicates significant improvement in skeletal muscle metabolism [36].

There are several limitations in this study. First, the sample size was small but was adequately powered to detect significant changes in insulin resistance and plasma BCAAs after bariatric surgery. The study population was also similar in numbers to another study involving subjects undergoing RYGB and gastric banding [31]. However, a larger sample size may be required to detect other subtle changes in physiologic parameters. Second, the measurements of static metabolites are indirect indicators of metabolic flux and findings from this study will need to be verified in future studies using stable isotope-labeled substrates. Finally, post-SG subjects were followed up over a relatively short duration and longer-term studies are necessary to ascertain the consistency of these observations.

Conclusion

In conclusion, morbid obesity in Asian individuals was associated with significant impairment in the regulatory actions of insulin on glucose, amino acid, and lipid metabolism and these obesity-induced regulatory dysfunctions improved significantly 6 months after SG.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Consent Statement Informed consent was obtained from all individual participants included in the study.

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