

## Original

# Euglycemia and normolipidemia after anti-obesity gastric bypass

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### Abstract

Weight loss and resolution of comorbidities is well established after modern bariatric procedures, however chronology of glyco-lipidic biochemical response is still debated.

**Objective:** Aiming to analyze this variable as well as its correlation with food amount and composition, a prospective study was designed.

**Methodology:** Eighty consecutive patients undergoing Roux-en-Y gastric bypass were investigated every three months until one year after surgery. Females only were accepted and variables included general and nutritional course as well as glucose and lipid measurements. Energy intake was documented including percentage of macronutrients in the diet.

**Results:** Surgery was successful with about 71% excess body weight loss at the end of the first year. Mean energy intake on the 4 postoperative quarters was respectively  $519.6 \pm 306.6$ ,  $836.0 \pm 407.9$ ,  $702.1 \pm 313.1$  and  $868.8 \pm 342.8$  kcal/day (mean  $\pm$  SD). Fat intake was initially low but reached  $34.1 \pm 7.9\%$  of total calories at final measurement. Blood glucose and lipid fractions tended to be borderline or abnormal preoperatively, and favorably changed by 12 months. Consumption of glucose-and lipid-lowering medication significantly diminished, but each of these was still necessary in 6.3% of the group. Correlation between body mass index and also calorie intake versus glucose and lipid measurements was highly significant ( $P = 0.000$ ).

**Conclusions:** 1) Energy intake after operation was very low; 2) Weight loss proceeded rapidly and correlated with meal pattern; 3) Improvement of glucose and lipid tests was adequate but took several quarters to normalize; 4) Decreased requirements for glucose- and lipid-lowering medication was significant but not absolute; 4) Fat percentage of total calories exceeded 30% at the end of the observation period, despite recommendations to the contrary.

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Key words: *Morbid obesity. Bariatric surgery. Diabetes mellitus. Hyperlipidemia. Dietary intake. Macronutrients.*

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### EUGLUCEMIA Y NORMOLIPIDEMIA DESPUÉS DE DERIVACIÓN GÁSTRICA ANTI-OBESIDAD

### Resumen

Pérdida de peso y resolución de las comorbidades están bien establecidas luego de los modernos procedimientos bariátricos, pero la cronología de la respuesta bioquímica glico-lipídica es aun debatida.

**Objetivo:** Con el interés de analizar esta variable y sus correlaciones con la cantidad y composición de la dieta, un estudio prospectivo fue preparado.

**Metodología:** Ochenta pacientes consecutivas sometidas a bypass gástrico en Y de Roux fueron investigadas a cada tres meses hasta el fin del primero año. Aceptaronse solamente mujeres y las variables involucraban evolución general y nutricional asimismo medidas de glucemia y lípidos. La ingestión energética fue documentada con su porcentual de macronutrientes.

**Resultados:** La operación fue exitosa con pérdida de un promedio de 71% de exceso de peso al término de un año. Los ingresos calóricos en los 4 trimestres fueron respectivamente  $519,6 \pm 306,6$ ,  $836,0 \pm 407,9$ ,  $702,1 \pm 313,1$  y  $868,8 \pm 342,8$  kcal/día. El consumo inicial de grasas fue bajo pero alcanzó progresivamente el  $34,1 \pm 7,9\%$  del total calórico. La glucemia y fracciones lipídicas quedaban en el límite superior del normal en el preoperatorio, con mejora significativa a los 12 meses. La utilización de medicación hipoglucemiante y hipolipemiante disminuyó, pero al término era aún necesaria para 6,3% de la población. Las correlaciones entre índice de masa corporal y también ingestión calórica versus valores lipídicos y glucídicos fueron muy significativas ( $P = 0,000$ ).

**Conclusiones:** 1) La ingestión calórica postoperatoria fue muy baja; 2) La pérdida de peso avanzó rápidamente y fue proporcional a los parámetros dietéticos; 3) La respuesta de la glucemia y hiperlipidemia fue adecuada pero solo se completó al cabo de varios trimestres; 4) El porcentaje final de grasas en la dieta fue superior al 30% pese a recomendaciones contrarias.

(Nutr Hosp. 2009;24:32-39)

Palabras clave: *Obesidad mórbida. Cirugía bariátrica. Diabetes mellitus. Hiperlipidemia. Ingestión dietética. Macronutrientes.*

## Introduction

Substantial weight loss and resolution of major comorbidities is unquestionable after Roux-en-Y gastric bypass (RYGB) for morbid obesity.<sup>1</sup> Nevertheless, conflicting results regarding longitudinal course of glucose and lipid measurements during the first 12 months can be found. Normalization of cholesterol levels is reported as early as within three months,<sup>2,3</sup> whereas in the experience of others some indices take up to six or nine months to significantly change.<sup>4,5</sup> In superobese adolescents (age 13-21) Lawson et al documented decrease of triglycerides and total cholesterol after one year, but HDL and LDL, which were in the acceptable range preoperatively, did not further improve.<sup>6</sup>

By the same token, incretin expression is increased one month after operation<sup>7</sup> and glucose and insulin normalization reportedly occurs within days, before any weight loss is apparent.<sup>8,9</sup> Clinical benefit is believed to materialize by the first quarter,<sup>2,9,10</sup> but subjects may require a longer time to react, and 22% of diabetic candidates are still drug-dependent by one year.<sup>11</sup> Also Perugini et al., observed improved fasting glucose and insulin levels as early as after 12 days, but significant additional improvement up to one year was not found.<sup>12</sup>

In the experience of Alexandrides et al with type-2 diabetics, even after two years blood glucose was above 110 mg/dL in 34%, and total cholesterol and triglycerides failed to normalize in respectively 67% and 22% of the population.<sup>13</sup>

Food intake and macronutrient composition is likely to influence these findings, but again incomplete and uneven numbers can be found after operation. Carrasco indicates ingestion of approximately 800 kcal/day (21.3% protein) at six months,<sup>4</sup> similarly to Anderson et al with figures at three months of about 700 kcal/day (37% protein) and 1100 kcal/day after one year (23% protein).<sup>14</sup> In contrast the values of Sjostrom et al., at six months were in the range of 1,500 kcal/day and at 12 months of 1,700 kcal/day.<sup>15</sup> In between, Bobbioni et al., registered nearly 1,000 kcal/day (17.9% protein) at three months, 1,140 (17.5% protein) at six months, and 1,420 kcal (16.2% protein) by one year.<sup>16</sup>

Given the importance of euglycemia and normolipidemia for the bariatric population and the highlighted controversies, a prospective study was designed in a homogeneous group of females, which traditionally represent the majority of surgical candidates. Aims were to analyse postoperative pattern of the diet, along with glucose and lipid measurements, during the first 12 months. To the best of our knowledge, this is the first protocol in which macronutrients were registered and correlated with other findings.

## Materials and Methods

### Population

Female subjects admitted to operation for morbid obesity by RYGB during a 24-month period (n = 80)

were prospectively submitted to nutritional and dietetic investigation, during the first year following that procedure. Criteria for inclusion were similar to those for bariatric surgery in general, namely preoperative body mass index (BMI) > 40 kg/m<sup>2</sup>, or > 35 kg/m<sup>2</sup> with major comorbidities, failure of conservative weight-loss treatment, absence of alcohol or drug addiction, and adequate psychologic profile.

Criteria for exclusion were postoperative hospitalization, bariatric reintervention, critical illness, shock, coma, use of nutritional supplements except for a daily multivitamin preparation, and refusal to participate in this protocol.

### Surgical Technique

Open RYGBP involved the creation of a vertical gastric pouch of approximately 30 ml, a Roux-en-Y jejunal limb of 100 cm, and a biliopancreatic limb of 60-80 cm. The gastric reservoir had a length of 8-10 cm and a volume of 30-50 ml, and a silastic ring (6.3 cm in circumference) was inserted around the pouch 3 cm proximal to the end-to-side gastrojejunal anastomosis, in order to prevent future dilatation.

### Postoperative alimentation

Patients were instructed to take only clear low-calorie liquids during the first 15 days, followed by all types of fluids during the next two weeks. Afterwards all foods were introduced so that by 60 days most patients were on a general diet. All were advised to make small meals, and to cut all food in tiny pieces, especially meat and sausages as well as uncooked fruits and vegetables. Patients should permanently maintain low fat consumption (< 30% total energy), especially of saturated fats, refraining from energy—dense or fatty snacks and sweets such as fried items, chocolate, ice-cream, cookies and sugared beverages. A multivitamin and mineral preparation was routinely prescribed.

### Dietetic methods

Standard 24-hour food recall was performed on three different times at the end of each quarter, including one week-end, and median score was used to evaluate the intake of total calories as well as of macronutrients.

Interviews were conducted by an experienced dietitian. Patients who informed that they were ill or for some other reason changed their diet in the last 48 hours were scheduled for another date. All consumed foods and drinks were recorded, and details about ingredients and preparation technique, or food brand in case of industrialized items, were collected. Standard cups, dishware and food portions were described, and informations were discussed with the patient in case of

doubt, before registration in the questionnaire. Final verification emphasized forgotten items such as occasional cookies, sugar or alcohol containing drinks, and midnight snacks.

The commercial Nut Win software (UNIFESP, São Paulo, Brazil), which was developed for Brazilian foods and meal sizes, was employed for calculations.

#### Medical prescriptions

Consumption of therapeutic drugs was documented, with emphasis on antidiabetic and lipid-lowering agents.

#### Physical activities

Patient were encouraged to lead an active life and to avoid sedentarism, but no structured exercise protocol was utilized in this experience.

#### Laboratory assays

Documented variables included demographic and clinical information, body mass index (BMI), serum albumin, hemoglobin and lipid fractions. Clinical information concerning hospitalizations and surgical procedures, nutritional assistance, and outcome was also collected.

#### Ethical considerations

Informed consent was given for the study, and this protocol was approved by the Internal Review Board of Hospital das Clínicas.

**Table I**  
*Preliminary findings*

Variable	Perioperative	12 months
Diabetes mellitus	21.3%	7.5%*
Arterial hypertension	63.8%	41.3%*
Hyperlipidemia	23.8%	10.0%*
Use of antidiabetic drugs	15.0%	6.3%*
Use of statins	13.8%	6.3%*

#### Statistical analysis

Findings are presented as mean  $\pm$  SD. For comparison of qualitative findings (comorbidities) Chi-square test was selected whereas numerical results were compared by Analysis of Variance (ANOVA). Associations were confirmed by the Spearman correlation test (SPSS for Windows, Chicago, Ill., USA, version 14.0). P values of less than 0.05 were considered significant.

#### Results

Age of the population was  $42.6 \pm 10.8$  years, BMI was  $53.5 \pm 20.2$  and excess body weight was  $51.5 \pm 19.5$  kg. Diabetes mellitus and other comorbidities are shown in table I. All comorbidities improved concerning both absolute numbers and requirements for drug therapy.

Patients shed body mass in the expected rate, with nearly all of the first-year loss occurring within six months. Despite the precipitous drop in body weight, serum albumin suffered only minor initial adjustments and remained stable most of the time. Hemoglobin was

**Table II**  
*Nutritional and biochemical outcome*

Variable	Preoperative	3 months	6 months	9 months	12 months
BMI kg/m <sup>2</sup>	$53.5 \pm 20.2$	$45.1 \pm 9.8^*$	$35.4 \pm 8.2^*$	$34.9 \pm 7.6^*$	$33.4 \pm 9.2^*$
BMI Reduction	0%	15.7%	33.8%	34.8%	37.6%
EBW Reduction	0%	36.5%	66.3%	68.6%	71.4%
HB g/dL	$13.6 \pm 1.5$	$13.0 \pm 1.6$	$12.2 \pm 1.1^*$	$12.6 \pm 1.0^*$	$12.2 \pm 0.9^*$
Albumin g/dL	$4.4 \pm 0.5$	$4.1 \pm 0.4$	$4.1 \pm 0.3$	$4.1 \pm 0.5$	$4.1 \pm 0.3$
GLU mg/dL	$116.9 \pm 53.9$	$120.3 \pm 52.2$	$82.4 \pm 10.1^*$	$84.6 \pm 34.9^*$	$79.5 \pm 9.0^*$
CHOL mg/dL	$198.7 \pm 52.0$	$180.8 \pm 26.6$	$176.7 \pm 38.3$	$179.3 \pm 18.7^*$	$170.8 \pm 22.0^*$
HDL mg/dL	$46.3 \pm 20.2$	$49.2 \pm 13.8$	$54.5 \pm 10.1$	$52.0 \pm 11.2$	$55.8 \pm 11.6^*$
CHOL/HDL ratio	$4.3 \pm 1.7$	$3.7 \pm 0.9^*$	$3.2 \pm 0.8^*$	$3.4 \pm 0.4^*$	$3.1 \pm 0.4^*$
TRIG/HDL ratio	$2.8 \pm 1.2$	$2.6 \pm 1.1$	$2.0 \pm 0.4^*$	$2.2 \pm 0.6^*$	$1.4 \pm 0.4^*$
LDL mg/dL	$122.9 \pm 41.7$	$107.8 \pm 21.9^*$	$106.8 \pm 39.2$	$111.0 \pm 16.5^*$	$99.8 \pm 27.2^*$
VLDL mg/dL	$32.3 \pm 17.7$	$25.8 \pm 13.5$	$25.4 \pm 21.3$	$22.9 \pm 9.8^*$	$15.3 \pm 6.1^*$
TRIG mg/dL	$127.5 \pm 63.6$	$129.7 \pm 67.6$	$106.6 \pm 21.3$	$114.5 \pm 49.4$	$76.0 \pm 30.3^*$

Obs: BMI: Body mass index; BMI Reduction: Mean % decrease of preoperative BMI; EBW reduction: Mean % decrease of preoperative Excess Body Weight; HB: Blood hemoglobin; GLU: Blood glucose; CHOL: Total cholesterol; TRIG: Triglycerides; (\*):  $P < 0.05$  in comparison with preoperative results.

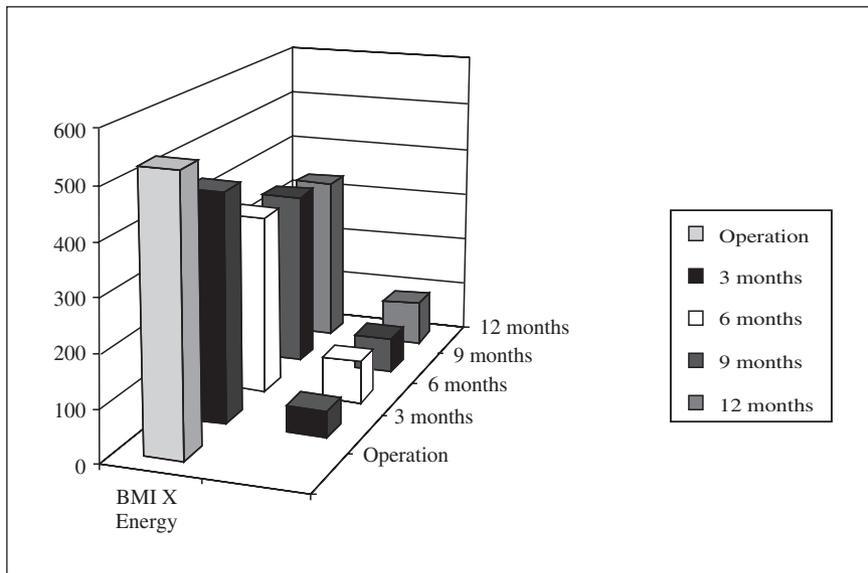


Fig. 1.—Food intake and BMI. Energy ingestion was extremely low in the first quarter, when weight loss was maximum. Values improved but not dramatically throughout the rest of the year, in parallel with less marked BMI decrease. Values presented as percentage of total intake.

more affected, with acceptable concentration in most but not all of the subjects.

Deranged glucose results came back to normal only by six months. Lipid indices recovered between three and nine months, whereas HDL elevation could not be demonstrated before a full year elapsed (table II, figs. 1 and 2).

Dietetic pattern during the first three months was consistent with semi-starvation. Absolute and relative values rose at six months and subsequent intervals, but still within the limits of a starkly hypocaloric diet. Qualitative analysis pointed out that in the beginning, carbohydrate and protein rich, fat-poor liquids predominated. Progressively this model changes towards a more lipid-based diet by the end of the year.

Protein intake was always high as recommended during this phase, eventually stabilizing around 21% of total energy (table III, figs. 3 and 4).

Body mass index correlated with the majority of the variables, as metabolic fitness is obviously weight-dependent. Perfectly parallel behavior is not the reported pattern in this setting, nevertheless very high corre-

lation was confirmed for energy intake, blood glucose and lipid fractions.

The correlation profile of energy intake was equivalent but the signal was typically negative because in the early months, when feeding was most depressed, biochemical normalization had not occurred yet. By the time lipid and glucose levels started moving down alimentation was already improving, thus significant but opposite changes continued throughout the observation period.

Triglycerides correlated with both other lipids and glucose concentration (carbohydrate metabolism), but not with total cholesterol, which was related to other lipid fractions only, as anticipated (table IV).

#### Discussion

Roux-en-Y gastric bypass is a classic but not particularly easy operation, neither for the surgeon nor for the patient, although the laparoscopic approach, which was not employed here, could eventually optimize some surgical details. An array of complications including nutritional deficits may ensue, not only in the

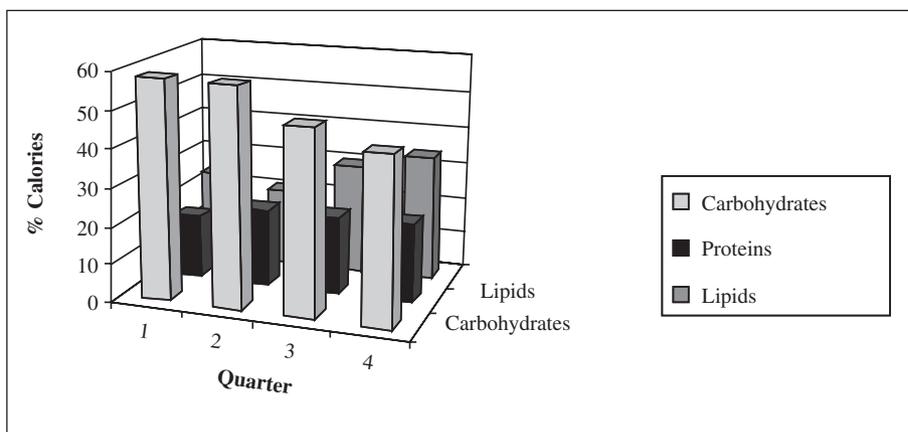


Fig. 2.—Macronutrient distribution of diet. Patients preferred carbohydrates and protein, with little fats, in the first two quarters, as diet included a large proportion of clear fluid. Meals became more solid by year end, with simultaneous increase in the proportion of lipids.

**Table III**  
Postoperative dietary pattern

Variable	Preoperative	3 months	6 months	9 months	12 months
EI (kcal/day)	2,330 ± 515	519.6 ± 306.6	836.0 ± 407.9	702.1 ± 313.1	868.8 ± 342.8*#
Kcal/kg/day	22.4 ± 6.7	6.0 ± 3.9	11.1 ± 5.7 *	10.7 ± 4.4 *	12.2 ± 4.8 *#
% Carbohydrate	49.3 ± 13.5	58.7 ± 22.3	58.2 ± 20.4	49.2 ± 14.1	44.4 ± 11.0*
% Fat	33.6 ± 10.2	23.9 ± 15.2	20.9 ± 11.3	29.8 ± 11.1	34.1 ± 7.9*
% Protein	17.1 ± 8.8	17.2 ± 10.2	21.0 ± 15.9	20.9 ± 8.3	21.4 ± 9.2

Obs: EI= Energy Intake; (\*): P < 0.05 in comparison with results of first quarter ; #: P < 0.05 in comparison with preoperative findings.

early follow-up but also after many years, even when complete physiologic adaptation should exist.<sup>17-21</sup>

Nevertheless, the prestige of this intervention as well as of others that exclude the duodenum and upper jejunum has been growing recently. Not only the very predictable weight loss is cited, reaching an average of 62% of excess body weight in the large meta-analysis by Buchwald et al.<sup>1</sup> The highly effective relief of glucose intolerance and diabetes is mostly accounted for.<sup>8,11</sup>

Indeed, several bariatric societies have recently added the word metabolic to their official name, as surgical indications for primary diabetes management in candidates with BMI < 35 are being seriously envisaged, fundamentally on the basis of surgical modalities involving upper intestinal bypass.<sup>22</sup>

In the current non-comparative investigation outcome in purely restrictive, as well as in more radically malabsorptive procedures, was not addressed. The limited aims also excluded documentation of hormonal milieu and physiologic response to insulin-resistance, metabolic syndrome and diabetes, focusing instead the general time-course of routine biochemical tests, and the consumption of standard macronutrients .

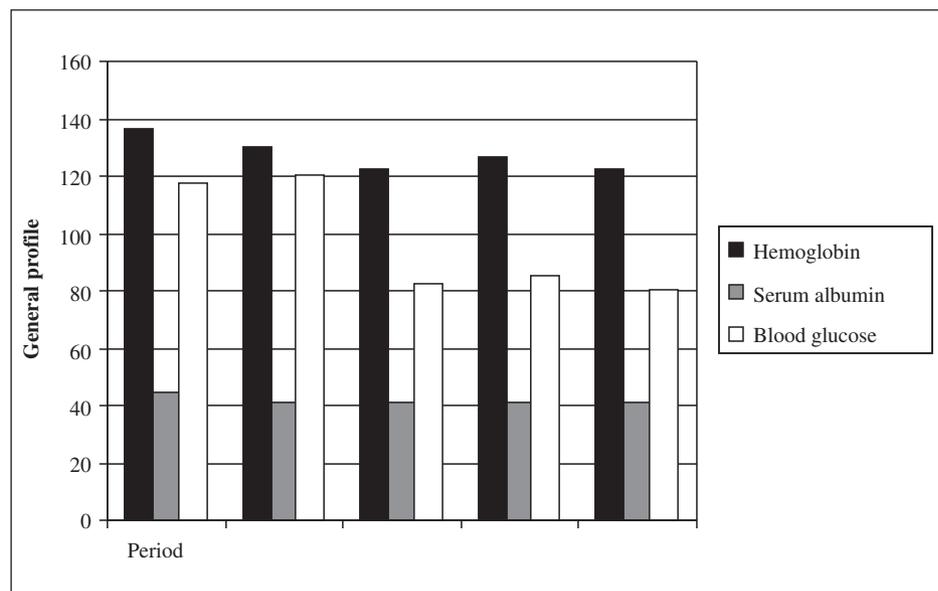
A preliminary report was presented some time ago.<sup>23</sup> Most of the early conclusions have stood the test of time, namely the very low dietary intake during the first year, which might be partially iatrogenic, in parallel with the remarkable decrease in body weight, that underpins the satisfactory regression of major comorbidities.

As shown in table II, by six months patients had already lost more than the average 62%,<sup>1</sup> at the cost of the depressed food intake, with mild anemia in some circumstances, but this should not be intrinsically risky when nutritional surveillance is available.

Restoration of glyco-lipidic biochemical markers was adequate but slower than hitherto admitted. Diabetes and hyperlipidemia significantly decreased, along with consumption of corresponding drugs, but regression did not occur in the immediate postoperative period despite the energy-poor regimen.

By 12 months more than one third of drug-dependent diabetics still required prescriptions, a proportion reminiscent of the findings of Alexandrides et al.<sup>13</sup> (34%), in contrast to those of Wickremesekera et al.<sup>9</sup> (9.7%) and Polizogopoulou et al.<sup>10</sup> (0%).

After the first quarter elapsed only values of total and LDL-cholesterol were lower, but not of glucose or of



*Fig. 3.—General biochemical profile. The first block of columns displays preoperative tests, whereas the subsequent ones reveal four quarterly results, completing one year. A modest but significant reduction of hemoglobin concentration was noticed, along with a more dramatic fall in glucose especially after six months. Serum albumin remained essentially stable.*

**Table IV**  
Correlations of body mass index, energy intake and biochemical measurements

Variable	Major correlations (P < 0.000)
Body mass index	EI r = 0.912, HB r = 0.881, GLU r = 0.992, CHOL r = 0.720, HDL r = -0.881, VLDL r = 0.606, TRIG r = 0.771
Energy intake	HB r = -0.995, GLU r = -0.933, CHOL r = -0.841, HDL r = 0.992, LDL r = -0.583, VLDL r = -0.603, TRIG r = -0.865
Total cholesterol	HDL r = -0.900, LDL r = 0.895, VLDL r = 0.906, TRIG r = 0.997
Triglycerides	GLU r = 0.742, HDL r = -0.916, LDL r = 0.858, VLDL r = 0.909

Obs: r = Pearson correlation index; BMI: Body mass index; EI = Energy Intake; HB: Blood hemoglobin; GLU: Blood glucose; CHOL: Total cholesterol; TRIG: Triglycerides.

other lipid fractions. Only around six months did metabolic fitness become more robust, however total normalization including HDL-cholesterol and triglycerides could only be demonstrated by one year.

Malabsorptive procedures, which were not present in this study, tend to be followed by more favorable responses, although results are also variable. In the series of Vazquez-Prado<sup>24</sup> et al., with duodenal-switch operation nearly 60% normalization of glucose metabolism occurred by three months (91.6% after one year). Dyslipidemia took longer to improve, with only 20% success by three months (84% after one year).

Vazquez et al.<sup>25</sup> informs that 12 months after the same operation, 90.5% of all subjects with glucose derangements recovered, whereas also triglycerides and total cholesterol displayed a significant drop by the same time.

Another observation was the progressive increase in fat calories as patients became able to adopt full voluntary alimentation. It is generally accepted that non-operated obese subjects display a preference for lipids

even when instructed otherwise, and the tendency seems to continue in the follow-up of bariatric intervention.<sup>4,26</sup>

According to Maclaughlin et al.,<sup>27</sup> this is not a dangerous development. Fat-rich alimentation, provided total calories and saturated lipids are limited, does not necessarily impair weight loss or resolution of comorbidities, and might even be advantageous regarding markers of cardiovascular disease, compared to high-carbohydrate prescriptions. Nevertheless, this is a highly debated topic. Howarth et al found out that at least in older people (> 60 years), a large fat intake was frankly deleterious for the obesity problem.<sup>28</sup>

The lack of consensus regarding bariatric nutritional management, both during routine postoperative care and in the presence of complications, is reflected in the results of an internet survey performed by the American Society for Parenteral and Enteral Nutrition.<sup>29</sup> When estimating calorie goals, 62% of the professionals used an adjusted body weight, 15% relied on ideal body weight, and for 14% the reference was actual weight. Numbers for pro-

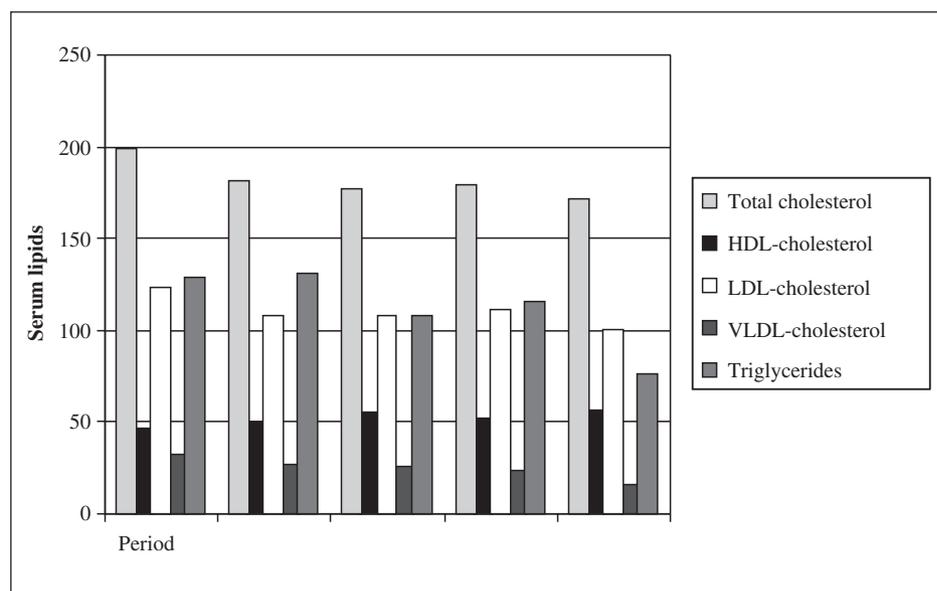


Fig. 4.—Results of serum lipids. The first block of columns displays preoperative test, whereas the subsequent ones reveal for quarterly result, completing one year. All lipid fractions diminished, except for HDL-cholesterol which gradually increased. Differences because significant around the 9<sup>th</sup> or 12<sup>th</sup> month.

tein goals were analogously divergent, underscoring the conspicuous need for clinical guidelines.

During the Enlightenment, in the XVIII Century, appetite changes and wasting were depicted as physical metaphors for melancholy, love, or religious agony.<sup>30</sup> The influence of psychologic conflicts on meal pattern and metabolic course after obesity surgery cannot be entirely ruled out, however somatic hypotheses probably deserve greater priority. These include secretion of peptide YY and assorted hormones,<sup>7-9,22</sup> along with degree of exercise and general physical activity as well as changes in resting energy expenditure.<sup>4,14-16,31,32</sup> Aberrant eating behavior and vomiting may be a problem,<sup>23</sup> whereas percentage of total body fat seems to be endowed with little influence. These facts notwithstanding, body composition might still receive attention as a marker of healthy diet and lifestyle.<sup>31,32</sup>

One intriguing report<sup>33</sup> correlates surgical outcome with size of the gastric pouch. Although 30-50 ml is the standard volume the actual number was somewhat higher,  $63.9 \pm 42.7$  ml. More importantly, values were widely scattered, with the smallest pouch about 25 ml and the biggest a staggering 250 ml, and highly significant correlation with loss of excess body weight was present at six months and one year.

Experience with both restrictive operations, which are totally reservoir-dependent, and others that include malabsorptive features, does not support straight correlation between body weight curve from one side, and metabolic outcome from the other.<sup>8,11,13,22</sup> Still, linear regression analysis in the present protocol confirmed that BMI and biochemical normalization were intimately related, therefore investigation of pouch size might be worth consideration in the future.

In fact, comparative studies of postoperative glycolipidic normalization encompassing different surgical techniques and final body mass indices are clearly recommended, as available publications address heterogeneous populations, determinants and end-points.<sup>1,11,13,15</sup>

## Conclusions

1) Energy intake after operation was insufficient, and even after 12 months a severely hypocaloric intake was unveiled; 2) Weight loss proceeded rapidly and correlated with meal pattern; 3) Decrease of glucose and lipid measurements was adequate but took several quarters to materialize; 4) Change in requirements for glucose- and lipid-lowering medication was significant but not overwhelming; 5) Fat percentage of total calories was initially low but progressively increased during the observation period, in agreement with the habits of obese subjects.

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Maria C. G. Dias and Joel Faintuch were in charge of conception and design of the protocol. Angela Ribeiro

and Maria C. G. Dias were responsible for acquisition of the data and analysis of clinical results. Joel Faintuch and Ivan Ceconello reviewed the findings and wrote the manuscript.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

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