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ORIGINAL ARTICLE

Hepatic left lobe volume is a sensitive index of metabolic improvement in obese women after gastric banding

M Giannetti[1], P Piaggi[2], G Ceccarini[1], S Mazzeo[3], G Querci[1], P Fierabracci[1], G Salvetti[1], G Galli[1], I Ricco[1], S Martinelli[1], C Di Salvo[4], M Anselmino[4], A Landi[2], P Vitti[1], A Pinchera[1] and F Santini[1]

BACKGROUND: Nonalcoholic fatty liver disease is a common finding in obese subjects. Increasing evidence has been provided suggesting that it represents the hepatic component of the metabolic syndrome.
OBJECTIVE: Aim of this longitudinal study was to evaluate the relationships between several anthropometric measures, including the hepatic left lobe volume (HLLV), and various indicators of the metabolic syndrome in a cohort of severely obese women before and after laparoscopic adjustable gastric banding (LAGB).
STUDY DESIGN AND RESULTS: Seventy-five obese women (mean age 45 ± 10 years and body mass index (BMI) $42.5 \pm 4.8\,kg\,m^{-2}$) underwent LAGB and completed an average ($\pm$ s.d.) post-surgical follow-up of 24 ± 6 months. Determination of HLLV, subcutaneous and intra-abdominal fat (IAF) was based on ultrasound. The principal component statistical analysis applied to pre-operative measurements, highlighted HLLV as a parameter that clustered with serum insulin, IAF, serum glucose and uric acid, along with triglycerides (TGs), alkaline phosphatase and high-density lipoprotein cholesterol. After LAGB, the average reduction of BMI was 23%, 12% for subcutaneous fat (SCF), 42% for HLLV and 40% for visceral fat. Among body weight, BMI, SCF, IAF and HLLV, reduction of the latter was an independent predictor of reduction of serum transaminases and γ-Glutamyltransferase, glucose, insulin and TGs.
CONCLUSIONS: In severely obese women: (i) HLLV is a sensitive indicator of ectopic fat deposition, clustering with parameters defining the metabolic syndrome; (ii) weight loss achieved by LAGB is associated with a reduction of liver volume as estimated by HLLV; (iii) among various anthropometric parameters measured, reduction of HLLV that follows LAGB represents the best single predictor of improvement of various cardiometabolic risk factors.

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Keywords: nonalcoholic fatty liver disease; bariatric surgery; metabolic syndrome; hepatic left lobe volume; gastric banding

INTRODUCTION

With the growing epidemic of obesity and the associated increasing prevalence of the metabolic syndrome,[1-4] much interest has arisen on the hepatic manifestations that develop as a consequence of visceral fat accumulation.[5-7] Nonalcoholic fatty liver disease (NAFLD) is now recognized as the most common cause of abnormal liver tests. NAFLD is defined by a lipid accumulation $>5\%$ in hepatic tissue in the absence of chronic alcohol consumption.[8] NAFLD is characterized by a wide spectrum of liver damage ranging from simple steatosis to advanced fibrosis and to cryptogenic cirrhosis through steatohepatitis (NASH), and, ultimately, to hepatocellular carcinoma.[9,10] The prevalence and severity of NAFLD increases with increments of body mass index (BMI),[11-13] and steatosis is observed in up to 86% of severely obese patients.[11,14]

NAFLD is usually asymptomatic, and hepatomegaly can be the only objective sign. No combination of clinical or biochemical abnormalities can accurately differentiate the spectrum of NAFLD, and only liver biopsy can establish with certainty the diagnosis. However, whole-hepatic enlargement is proportional to the severity of the metabolic syndrome, and various imaging methodologies can be used for the estimation of liver volume.[15,16] We have recently introduced an ultrasound technique that measures the hepatic left lobe volume (HLLV), calculated by the ellipsoid formula, and we have shown that HLLV is tightly correlated to, and thus an excellent indicator of, visceral adiposity.[17]

Treatment of obesity is based upon low-calorie diets, behavioral modifications and physical activity, the pharmacological treatment being considered as further approach. Unfortunately, particularly in patients with severe obesity, the results of these approaches are often disappointing, with many patients either losing an inadequate amount of weight or experiencing total weight regain within a short period of time. Several surgical procedures have therefore been developed that have proven to be effective in achieving: (1) a long-term weight loss, (2) reducing the mortality rate and (3) improving the co-morbidities associated with the disease.[18-23]

The aim of this longitudinal study was to evaluate the relationships between several anthropometric measurements, including the HLLV, and various indicators of the metabolic syndrome in a cohort of severely obese women before and after bariatric surgery. Of particular interest to us, were the effects of weight loss on liver volume, in parallel with amelioration of various co-morbidities, in obese women treated by laparoscopic

[1]Department of Endocrinology and Kidney, University Hospital of Pisa, Pisa, Italy; [2]Department of Energy and Systems Engineering, University of Pisa, Pisa, Italy; [3]Department of Radiology, University Hospital of Pisa, Pisa, Italy and [4]Bariatric Surgery Unit, University Hospital of Pisa, Pisa, Italy. Correspondence: Professor F Santini, Department of Endocrinology and Kidney, University Hospital of Pisa, Via Paradisa 2, Pisa, 56124, Italy.
E-mail: ferruccio.santini@med.unipi.it
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adjustable gastric banding (LAGB). LAGB was chosen because it produces a sustained weight loss without major alterations of the anatomy and physiology of the gastrointestinal tract.[24,25]

SUBJECTS AND METHODS

In this study, 75 consecutive obese women were included, who underwent LAGB and completed an average ($\pm$ s.d.) post-surgical follow-up of 24 ± 6 months. Patients of our study belonged to a cohort of 145 women who underwent gastric banding during the same time period. Among them, 33 women could not be included because of the selection criteria described below. Thirty women could not be enrolled because of missing information due to unintentional events that precluded the collection of the entire dataset, whereas seven subjects were lost at follow-up.

The mean age of the 75 patients was 45 ± 10 years (range 22–67 years), the mean body weight was 110.7 ± 14.8 kg (range 78.5–148 kg) and the mean BMI was 42.5 ± 4.8 kg m^{-2} (range 33.9–55.7 kg m^{-2}).

None of the patients was taking hypoglycemic, hypolipemic or hypouricemic agents (18 were taking anti-hypertensive drugs). Additional exclusion criteria were: self-reported alcohol consumption >20 g daily, use of illicit drugs or hepatotoxic medications, viral hepatitis as assessed by conventional serum markers, pregnancy or breast feeding within the 12-month period before enrolment. Clinical, hematological and instrumental examinations of each patient were performed following the Italian guidelines for obesity, and each patient was treated according to the appropriate protocols for her condition. Anthropometric measures were determined after an overnight fasting. Body weight was measured to the nearest kilogram, whereas height and abdominal circumference were determined to the nearest centimeter.

Venous blood samples were obtained after an overnight fasting for measurement of serum glucose, triglycerides (TGs), total cholesterol, high-density lipoprotein (HDL) cholesterol, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-Glutamyltransferase (GGT), alkaline phosphatase (ALP), erythrocyte sedimentation rate (ESR) and insulin. The homeostasis model of insulin resistance (HOMA) was calculated based on fasting serum glucose and insulin concentrations.[26] Ultrasound examination for determination of HLLV, subcutaneous and intra-abdominal fat (IAF) was performed as previously described.[17] Briefly, the ellipsoid formula (width $\times$ height $\times$ length $\times$ 0.52) was applied to calculate the HLLV. The height of the lobe was obtained by an epigastric longitudinal scan, considering the distance between the diaphragm and the lower margin of the left lobe. The length of the lobe was calculated on the axial scan by drawing a line between the round ligament and the lateral margin of the hepatic lobe. Thickness was obtained on both the axial and the longitudinal scans, measuring the distance between the anterior and the posterior borders of the liver. Thickness of the abdominal subcutaneous fat (SCF) was taken 1-cm over the transversal umbilical vein, by measuring the distance between the skin and the external face of the muscular fascia, while IAF thickness was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta.

All patients underwent LAGB (Swedish Adjustable Gastric Band by Ethicon Endo-Surgery, Johnson and Johnson, New Brunswick, NJ, USA) performed by the same surgeon. At the time of post-surgical evaluation, the same clinical, hematological, and instrumental examinations were repeated as already described.

STATISTICAL ANALYSIS

The principal component analysis (PCA)[27] based on standardized parameters (that is, anthropometric and hematological measurements) was applied in order to identify groups of correlated variables. Namely, PCA is a dimensional reduction technique, which seeks the best linear combinations of variables (the *principal components* (*PCs*)), that account for most of the variance of the total data. PCs are found to be uncorrelated from each other and ordered in decreasing way of explained variance, so that the first PC is the best linear combination of variables that accounts for most of the variability, the second PC for most of the residual variance (uncorrelated with the first PC) and so on until all variability is explained by all calculated PCs.

The number of significant PCs was determined using the *scree* test criterion[27] by plotting the percentage of explained variance by each PC versus their rank (that is, the *scree plot*) and further confirmed by the parallel analysis statistical approach.[28] The Pearson's correlation coefficient (that is, PCA *loading*) and the squared cosine index were employed to quantify the contribution of each variable on extracted PCs in order to highlight the clusters of correlated variables.

Student's *t*-test for paired data was used to evaluate differences before and after LAGB intervention for all variables. Logarithmic transformations of skewed variables were employed, as needed.

For each parameter (dependent variable), a multiple linear regression analysis using a forward stepwise selection algorithm was employed to determine the significant predictors among independent variables (P-value <0.05). The R^2 statistic was employed to quantify the contribution of explained variance by the significant predictor selected at each step.

Data analysis was performed using Matlab (MathWorks, Natick, MA, USA) and statistical significance was assumed for P-values <0.05. Data are presented as mean $\pm$ s.d.. Statistical analysis was conducted by PP and AL from the Department of Energy and Systems Engineering, University of Pisa.

RESULTS

Anthropometric and hematological parameters before and after LAGB

Table 1 shows the values of anthropometric and metabolic measurements obtained before and 2 years after LAGB in our cohort of 75 severely obese female patients.

As expected, after bariatric surgery, a marked reduction of body weight was observed. Weight loss was associated with a significant reduction of SCF and with an even more pronounced reduction of IAF and HLLV.

Significant reductions of serum glucose, uric acid, TGs, ESR, ALP, AST, ALT and GGT were also noticed, together with a marked reduction of serum leptin and fasting insulin. Insulin resistance (as assessed by the HOMA index) decreased, whereas HDL cholesterol significantly increased.

PCA of data obtained before LAGB

As a result of PCA, scree plot and parallel analysis, all parameters measured before surgery could be clustered in two components (PCs) explaining together 38% of the total variance.

HLLV ($r=0.73$), HOMA index ($r=0.71$), insulin ($r=0.66$), IAF ($r=0.62$), GGT ($r=0.56$), serum glucose ($r=0.55$) and uric acid ($r=0.52$) were mainly represented on the PC1 (squared cosines on PC1 $>60\%$, $P<0.01$) that accounted for 26% of the total variance; furthermore, smaller but significant contributions to this cluster of variables were given by TGs ($r=0.40$), ALP ($r=0.38$) and, inversely, by HDL cholesterol ($r=-0.34$).

Body weight ($r=0.62$) along with small contributions by total cholesterol ($r=-0.33$) and leptin ($r=0.31$) was mostly associated with the PC2 (squared cosines on PC2 $>60\%$, $P<0.05$) that explained an additional 12% of the total variance.

BMI ($r_{PC1}=0.60$, $r_{PC2}=0.60$; squared cosine $=50\%$ on both PC1 and PC2), AST ($r_{PC1}=0.57$, $r_{PC2}=-0.66$; squared cosines $=43\%$ on PC1 and 57% on PC2), ALT ($r_{PC1}=0.60$, $r_{PC2}=-0.64$; squared cosines $=47\%$ on PC1 and 53% on PC2) and ESR ($r_{PC1}=0.34$, $r_{PC2}=0.30$; squared cosines $=57\%$ on PC1 and 43% on PC2) were associated with both PCs.

PCA loadings are shown in Figure 1 where the absolute values of the correlation coefficients between each variable and the PC1 (x axis) are plotted against those related to the PC2 (y axis). HLLV, HOMA index, insulin, IAF, serum glucose, uric acid, ALP, TG and

Table 1. Anthropometric (A) and laboratory (B) parameters measured in our cohort of 75 obese women, before and after LAGB

	Before	*After*	*Mean variation (%)*	*Statistical significance*
(A) Anthropometric measures				
Body weight (kg)	110.7 ± 14.8	85.2 ± 11.9	−25.6 (−23.1%)	$P<0.001$
BMI (kg m^{-2})	42.5 ± 4.8	32.9 ± 4.1	−9.7 (−22.7%)	$P<0.001$
SCF (mm)	40.2 ± 10.4	35.6 ± 11.6	−4.6 (−11.5%)	$P<0.001$
IAF (mm)	79.2 ± 20.3	47.5 ± 17.7	−31.7 (−40.0%)	$P<0.001$
HLLV (cc)	401.9 ± 192.8	232.5 ± 86.4	−169.4 (−42.1%)	$P<0.001$
(B) Laboratory measures				
Serum glucose (mg dl^{-1})	96.8 ± 18.9	83.5 ± 8.3	−13.3 (−13.7%)	$P<0.001$
Uric acid (mg dl^{-1})	5.4 ± 1.1	4.1 ± 1.2	−1.3 (−24.2%)	$P<0.001$
TG (mg dl^{-1})	139.1 ± 65.2	94.1 ± 35.6	−45.1 (−32.4%)	$P<0.001$
Total cholesterol (mg/dl)	215 ± 44.6	213.5 ± 41.5	−1.5 (−0.7%)	N.S.
HDL cholesterol (mg dl^{-1})	54.5 ± 10	65.8 ± 14	+12.7 (+23.2%)	$P<0.001$
ESR (mm h^{-1})	25.2 ± 14.3	19.4 ± 11.7	−5.6 (−22.3%)	$P<0.001$
AST (U l^{-1})	22.5 ± 11.4	15.5 ± 4.9	−7 (−30.9%)	$P<0.001$
ALT (U l^{-1})	29.3 ± 22.7	14.6 ± 6.6	−14.8 (−50.4%)	$P<0.001$
GGT (U l^{-1})	26.6 ± 18.8	14.1 ± 9.5	−12.5 (−46.8%)	$P<0.001$
ALP (U l^{-1})	188.7 ± 46.9	152.6 ± 46.1	−36.1 (−19.1%)	$P<0.001$
Insulin (mU l^{-1})	16.8 ± 9	7.6 ± 4.5	−9.4 (−56.1%)	$P<0.001$
Leptin (ng ml^{-1})	57.3 ± 27.6	20.6 ± 11.6	−37.1 (−64.8%)	$P<0.001$
HOMA index	4.1 ± 2.6	1.6 ± 1	−2.6 (−62.9%)	$P<0.001$

Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; ESR, erythrocyte sedimentation rate; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein; HLLV, hepatic left lobe volume; HOMA, homeostasis model of insulin resistance; IAF, intra-abdominal fat; N.S., not significant; SCF, subcutaneous fat; TG, triglyceride. HLLV, SCF and IAF were assessed by ultrasound. Data are presented as mean ± s.d. Mean variations (calculated by subtracting data obtained after LAGB to those measured before LAGB) are expressed also as % variation with respect to pre-operative values.

HDL cholesterol best clustered together, whereas body weight, BMI, AST, ALT and GGT were grouped in a different cluster. ESR, total cholesterol, leptin and SCF clustered in a third distinct group.

In summary, these results suggest that HLLV is an anthropometric parameter that clusters with those strictly related to the metabolic syndrome and is a sensitive indicator of visceral adiposity.

Multivariate analysis of changes after LAGB

The relationship between improvements of various serum parameters (dependent variables) and reduction of various anthropometric measures (independent variables) was analyzed by multiple regression analysis using a forward stepwise algorithm. Reductions of liver enzymes AST ($R^2=23\%$, $P<0.01$) and ALT ($R^2=23\%$, $P<0.01$) were related only to reduction of HLLV, whereas reduction of GGT was correlated to both reduction of HLLV ($R^2=14\%$, $P<0.01$) and reduction of BMI (additional $R^2=6\%$, $P<0.05$).

The reductions of serum glucose ($R^2=16\%$, $P<0.01$), HOMA index ($R^2=16\%$, $P<0.01$) and TGs ($R^2=10\%$, $P<0.01$) were independently associated only with reduction of HLLV (Figure 2), whereas reduction of fasting insulin was related to both reduction of HLLV ($R^2=13\%$, $P<0.01$) and reduction of body weight (additional $R^2=5\%$, $P<0.05$).

The post-surgical reductions of ALP ($R^2=18\%$, $P<0.01$), ESR ($R^2=9\%$, $P<0.01$), leptin ($R^2=6\%$, $P<0.05$) and the increase of HDL ($R^2=13\%$, $P<0.01$) were independently associated only with reduction of body weight. Finally, reduction of uric acid was associated both with reduction of IAF ($R^2=18\%$, $P<0.01$) and reduction of BMI (additional $R^2=5\%$, $P<0.05$). Various results from multivariate analyses are summarized in Figure 3.

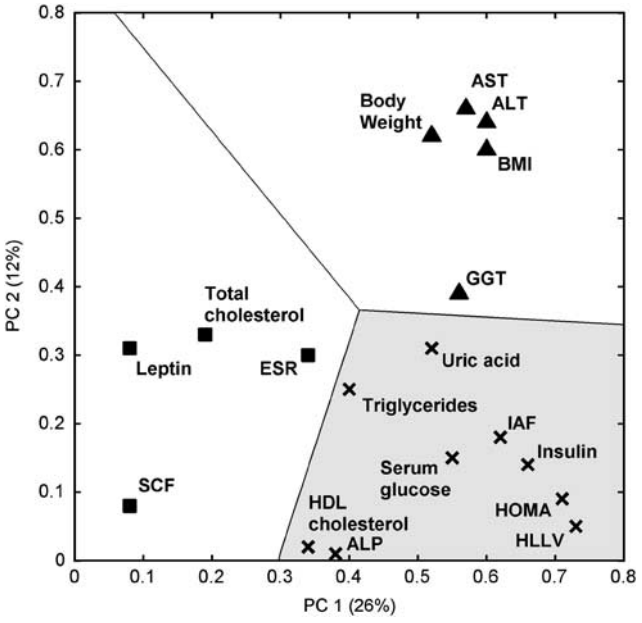

Figure 1. Graphical plot of *loadings* obtained from the PCA of anthropometric and hematological measurements of 75 obese women before LAGB. The correlation coefficients (absolute values) between single variables and the first PC (*x* axis) are plotted against those related to the second PC (*y* axis). The hierarchical clustering algorithm (using the Ward's method), applied to the absolute values of loadings, identified three clusters of correlated variables as the optimal number of distinct groups. The related Voronoi diagram was also drawn (black lines) to highlight the separation borders of the three clusters. HLLV, HOMA index, insulin, IAF, serum glucose, uric acid, ALP, TGs and HDL cholesterol best clustered together (black crosses, gray area), whereas body weight, BMI, AST, ALT and GGT were grouped in a different cluster (black triangles). ESR, total cholesterol, leptin and SCF clustered in a third distinct group (black squares).

DISCUSSION

BMI is the measure commonly used to define and classify obesity. However, BMI does not fully predict the morbidities associated

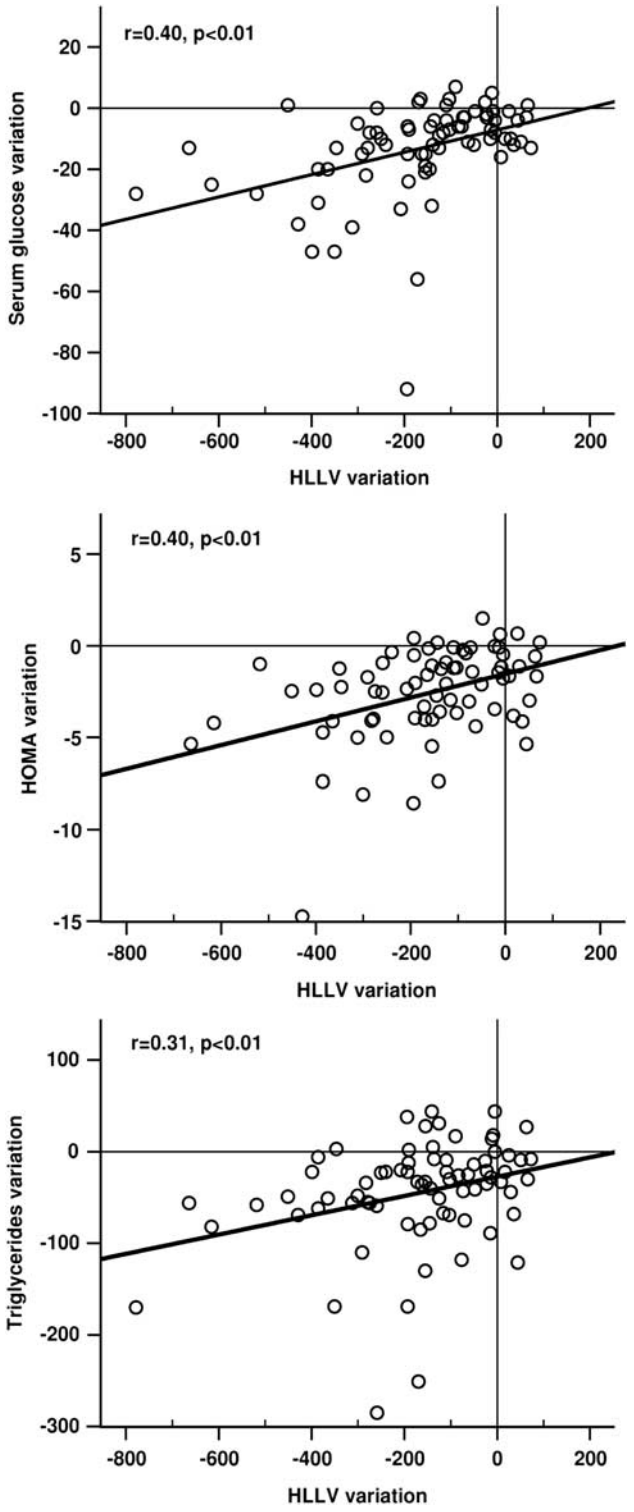

Figure 2. Correlations between the post-surgical reduction of HLLV and those of serum glucose (upper panel), HOMA index (middle panel) or TGs (lower panel) in 75 obese women after LAGB.

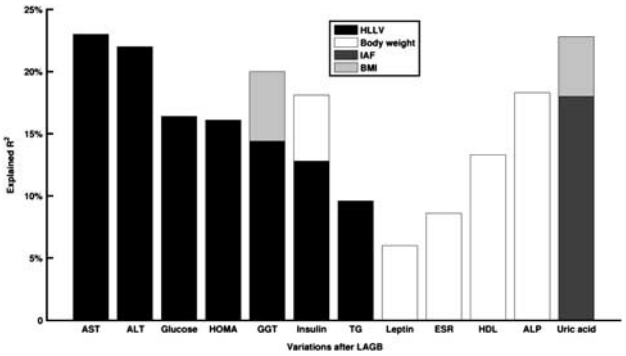

Figure 3. Explained R^2 in multivariate regression analyses between improvement of each hematological parameter (dependent variable) and anthropometric measures (independent variables), selected as the most significant by the forward stepwise algorithm. For each hematological parameter (plotted on the *x* axis), the variance (that is, R^2 statistic expressed as percentage, *y* axis) that is explained by various independent predictors is graphed using different colors. Reductions of AST, ALT, serum glucose, HOMA index, GGT, insulin and TGs were mostly related to HLLV reduction (shown in black), whereas leptin, ESR, HDL cholesterol and ALP variations were mostly related to weight loss (shown in white). Reduction of uric acid was associated with reduction of intra-abdominal fat (dark gray) and with reduction of BMI (light gray).

with abnormal fat accumulation. Abdominal obesity is more closely associated with cardiovascular risk than gluteo-femoral obesity is, and a large body of studies indicates that waist circumference and the waist-to-hip ratio are better predictors of cardiovascular risk, morbidity and mortality than BMI,[29-31] particularly in severe obesity.[32] A failure in the capacity of SCF to accumulate additional energy might be associated with storage of the lipid surplus in ectopic fat depots. Lipid deposition in tissues, such as liver, skeletal muscle and visceral fat, is thought to be involved in the pathogenesis of insulin resistance, atherogenic dyslipidemia and a chronic low-grade systemic pro-thrombotic inflammatory state.[7] Yet, simple measurements of abdominal circumferences, though extremely powerful in epidemiological studies, may not be sufficient to identify the single patient at high risk of cardiovascular disease and diabetes mellitus, in particular in severely obese subjects in whom the excess of SCF may lead to an overestimation of IAF depots. The need of proper assessment of regional fat distribution by imaging techniques has been advocated to establish the relationship between ectopic fat deposition and relevant metabolic markers, and to facilitate the identification of obese individuals who are at increased risk of cardiovascular disease, and thus better candidates for interventions, including bariatric surgery.[7] With this aim, we have recently developed an easy, safe, repeatable and low-cost technique that allows a precise determination of HLLV during routine abdominal ultrasound examination,[17] and we have shown that HLLV is a sensitive indicator of visceral adiposity in obese subjects.

In the present study, we have expanded our previous observation by applying a statistical analysis (PCA) able to identify clusters of correlated variables in obese patients scheduled for bariatric surgery. PCA confirmed a strong association between HLLV, visceral fat, various parameters of the metabolic syndrome and liver enzymes, whereas body weight, BMI and leptin co-segregated within a different component. At 2 years after bariatric surgery, our patients achieved a significant weight loss and a concurrent reduction of IAF and HLLV was demonstrated. A parallel improvement of various cardio-metabolic risk factors was also observed. When the single components of the metabolic syndrome were analyzed (serum glucose, TGs, fasting insulin and HOMA), their reduction was independently associated only with the reduction of HLLV. Clamp studies would have been more informative to assess peripheral insulin resistance and their lack is a limitation of our study. Yet, we believe that the correlations observed in our large cohort of patients reinforce the role of hepatic volume as a powerful index of visceral adiposity and

associated insulin-resistance. Reduction of serum transaminases and GGT in association with reduction of liver volume confirms the amelioration of liver disease observed in patients who have undergone LAGB.[33] Reduction of serum HDL was related only to reduction of body weight, indicating that regulation of this lipoprotein may be influenced by other factors beside ectopic fat accumulation. Similar explanations can be proposed for uric acid and ESR, whereas serum leptin was found reduced secondary to the drop of body weight, and thus of total body fat. The independent association between reduction of ALP and body weight is intriguing and may be related to a composite origin of increased ALP levels in obese women.[14]

In conclusion, results of this study show that in severely obese women: (i) HLLV is a sensitive indicator of ectopic fat deposition, clustering with parameters defining the metabolic syndrome; (ii) weight loss achieved by LAGB is associated with a reduction of liver volume as estimated by HLLV; (iii) among various anthropometric parameters measured, reduction of HLLV that follows LAGB represents the best single predictor of improvement of various cardiometabolic risk factors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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