RESEARCH ARTICLE

Short-term effect of weight loss through restrictive bariatric surgery on serum levels of vaspin in morbidly obese subjects

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ABSTRACT. Background. The aims of this study were to evaluate the short-term effects of laparoscopic restrictive bariatric surgery (LRBS) on plasma levels of vaspin and the potential associations of changes in vaspin levels with changes in anthropometric indices, insulin-resistance and dietary intake. Methods. Thirty, severely obese subjects (21 female; mean age, 32.5 years) with a mean body mass index (BMI) of 44.1 ± 4.9 kg/m² underwent LRBS. Measurements of anthropometric indices, dietary intakes, physical activity and plasma vaspin concentrations were performed prior to, and six weeks after LRBS. Insulin-sensitivity was estimated using the homeostasis model assessment of insulin-resistance (HOMA-IR). Results. Six weeks after LRBS, BMI decreased to a mean of 38.4 ± 4.9 kg/m². Significant reductions were also observed in waist circumference (WC), daily intakes of calorie, fat and protein, and plasma concentrations of triglyceride. No significant change was observed in fasting levels of insulin, blood sugar or HOMA-IR. Vaspin decreased significantly (0.26 ± 0.17 vs 0.36 ± 0.20, p=0.048) following surgery. While the percentage change of vaspin was not correlated with percent changes in anthropometric indices and HOMA-IR, it correlated positively with the percentage change in intake of calories, fat and protein: this correlation remained significant even after adjustment for sex and changes in WC and HOMA-IR. Conclusion. Our study suggests that LRBS decreases the serum vaspin concentrations in parallel with the restriction of dietary intake. Furthermore, decreased levels of vaspin early after LRBS seem more likely to result from decreased dietary intake rather than weight-loss-induced insulin sensitivity improvement.

Key words: obesity, weight reduction, laparoscopic adjustable gastric banding, laparoscopic total gastric vertical plication, visceral adipose tissue-derived serine protease inhibitor

Obesity is recognized as a continuously growing, global public health problem. It is associated with increased risk of a variety of acute and chronic disorders, such as type II diabetes mellitus, dyslipidemia, and cardiovascular disease (CVD) [1,2]. In addition to storing excess energy, adipose tissue secretes several bioactive peptides, termed “adipokines”, that play important roles in metabolic homeostasis through endocrine, paracrine, or autocrine pathways [3-8]. Thus, to explain the mechanisms for the epidemiological relationship between obesity and increased metabolic risk, the main focus has been on adipokine secretion and action.

Vascular adipose tissue-derived serine protease inhibitor (vaspin) is a newly-discovered adipokine with insulin-sensitizing properties, and was originally isolated from the visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats [9], a rodent model for insulin resistance and abdominal obesity, and was then found to be expressed in white adipose tissue of obese humans [10]. Although controversial, there is evidence from both animal and clinical studies that vaspin mRNA expression in adipose tissue, as well as its circulating levels, correlate with

Abbreviations

CVD cardiovascular
Vaspin visceral adipose tissue-derived serine protease inhibitor
OLETF Otsuka Long-Evans Tokushima Fatty
RYGB Roux-en-Y gastric bypass
BMI body mass index
FPG fasting plasma glucose
LAGB laparoscopic adjustable gastric banding
LTGVp laparoscopic total gastric vertical plication
WC waist circumference
WHR waist-to-hip ratio
TG triglyceride
TC total cholesterol
LDL-C low density lipoprotein-cholesterol
HDL-C high density lipoprotein-cholesterol
HOMA-IR homeostasis model assessment for insulin resistance

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insulin resistance and obesity [9-20]. Hence, it has been proposed that elevated serum vaspin levels in obese subjects might be a compensatory response to obesity-induced insulin resistance [21]. Until now, molecular target(s) of vaspin and its mode of action have remained unelucidated [22]. However, based upon previous data on vaspin action, it might be postulated that vaspin acts through inhibition of a protease that degrades a hormone or molecule with a direct or indirect glucose-lowering effect [22]. Chang et al. [11] recently reported decreased levels of serum vaspin after a modest weight loss in obese subjects that was accompanied by improvements in parameters of insulin sensitivity. Similarly, Handisurya et al. [14] recently reported a reduction in circulating levels of vaspin following laparoscopic Roux-enY gastric bypass (RYGB) surgery in morbidly obese subjects. However, the effect of restrictive bariatric surgery on circulating vaspin levels had not yet been investigated. Moreover, in an animal study, Wang et al. [23] recently reported that dietary intake is related to serum and adipose tissue concentrations of vaspin. The relationship between dietary intake and vaspin concentrations in humans, and its potential for explaining the effect of bariatric surgery on serum vaspin concentrations have not been clearly documented. The aims of this study were two-fold: firstly, to investigate the possible short-term effects of restrictive bariatric surgery on serum levels of vaspin and other metabolic variables relevant to insulin sensitivity, and secondly, to evaluate the possible relationship between dietary intake and serum vaspin concentrations in obese subjects.

METHODS AND SUBJECTS

Study population

Between September 2009 and October 2010, severely obese patients older than 15 years, with a body mass index (BMI) of more than 40 kg/m² consecutively submitted to restrictive bariatric surgery, were enrolled in the study. Exclusion criteria were the following: diabetes mellitus; hypo- or hyperthyroidism; Cushing syndrome, hypertension and/or use of anti-hypertensive drugs, a history of convulsion and/or use of antiepileptic drugs; liver disease; kidney disease; ischemic heart disease; cancer; rheumatoid arthritis; symptoms of acute or chronic infection; current smoking and alcohol intake; pregnancy or lactating; use of hormonal contraception, lipid-lowering drugs (statins, fibrates) and/or antihyperglycemic medications (metformin, thiazolidinediones, insulin). The study protocol was approved by the ethics committee of Tehran University of Medical Sciences and written, informed consent was obtained from all subjects.

Study protocol

Patients underwent a clinical assessment including medical history, physical examination and co-morbidity evaluation by a multidisciplinary consultation team. In addition, for all patients, assessment of dietary intake, physical activity, anthropometric measurements and blood sampling for biochemical assays were performed prior to, and six weeks after surgery.

Bariatric surgery

All patients met the criteria detailed by the National Institutes of Health for patient selection to undergo bariatric surgery for morbid obesity [24]. Patients underwent restrictive bariatric surgery using one of two techniques: laparoscopic adjustable gastric banding (LAGB) and laparoscopic total gastric vertical plication (LTGVP). Patient preference determined the type of surgery performed. All patients were operated on by a single team. LAGB surgeries were performed according to the pars-flaccida technique described elsewhere [25]. This technique involves the placement of an adjustable, silicone, gastric band containing an inflatable inner balloon that can be adjusted by adding or removing saline via a small, subcutaneous access port. LTGVP was performed according to the technique described by Talebpour and Amoli [26]; briefly, patients were placed in the supine position with a 30-degree reverse Trendelenburg position. After the release of the greater curvature, continuous suturing from the fundus of the stomach to the antrum, making one or two layers of plication from the anterior wall of the stomach to its posterior wall, was performed.

Measurements of anthropometric indices and blood pressure

Qualified, trained staff measured anthropometric indices and blood pressure prior to, and six weeks after surgery. Body weight was measured to the nearest 0.1 kg using a calibrated manual weighing scale (Seca 709, Les Mureaux, France). Height was measured to the nearest 0.5 cm on a standardized wallmounted height board. Waist circumference (WC) was measured at the minimum circumference between the iliac crest and the rib cage at minimal respiration. Hip circumference was measured at the maximum protuberance of the buttocks, and the waist-to-hip ratio (WHR) was calculated. BMI was defined as weight in kilograms divided by height in meters squared (kg/m²). For measuring the blood pressure, the participants remained at rest for at least 15 minutes, then the same staff measured blood pressure using the right arm and in a sitting position.

Dietary intake and physical activity assessment

All enrolled subjects received instructions to record their daily dietary intake for three days (two non-consecutive weekdays and a weekend). Records were reviewed and analyzed by a dietitian. The nutrient composition program, Food Processor, version 2.0 (ESHA Research, Salem, OR, USA) was used to estimate macro- and micronutrient intakes. Subjects self-reported physical activity using the International Physical Activity Questionnaire-Short form for the previous seven days [27].

Laboratory assays

Between 8:00 and 10:00 a.m., peripheral venous blood specimens were collected from an antecubital vein after 10-12 hours overnight fasting. Blood samples were centrifuged at 3,000 g for 10 min and the resultant plasma samples were stored at -80 °C until analysis.
Serum vaspin concentrations were determined using an enzyme-linked immunosorbent method (Human vaspin ELISA kit, AdipoGen Pharmaceuticals, Seoul, South Korea) with a detection limit of 12 pg/mL and an intra-assay and inter-assay variability of 1.3-3.8 and 3.3-9.1%, according to the manufacturer. Fasting plasma glucose was measured using the glucose-oxidation method (Pars Azmoon, Tehran, Iran), and total cholesterol (TC), triglyceride (TG), and low density lipoprotein-cholesterol (LDL-C) were determined by enzyme colorimetric assay (Pars Azmoon, Tehran, Iran) using an Eppendorf autoanalyzer (Eppendorf Corp., Hamburg, Germany). High density lipoprotein-cholesterol (HDL-C) was measured using a precipitation-based method. Serum insulin level was assayed using an immunoradiometric method (Biosource Europe SA, Belgium). The insulin sensitivity was determined using the Homeostasis Model Assessment (HOMA) index with formula: HOMA-IR=fasting insulin (µU/mL) × fasting glucose (mmol/l)/22.5 [28].

Statistical analysis
The Kolmogorov-Smirnov test was applied to examine normal distribution. Data are presented as mean ± SD for numeric variables, and absolute frequencies with percentages in parentheses for categorical variables. Numeric variables were compared using the paired sample t-test or the equivalent non-parametric procedure (Wilcoxon test) whenever the presumption of normality was not met. To determine the relationship between the various metabolic and anthropometric parameters, and plasma levels of vaspin at baseline as well as after surgery, Pearson’s correlation was used. Linear regression analysis was performed to identify independent predictors of serum vaspin concentrations. For the statistical analysis, the statistical package SPSS version 13.0 for Windows (SPSS Inc, Chicago, Illinois, USA) was used. All p values were 2-tailed with a statistical significance defined by p ≤0.05).

RESULTS
Out of a total of 30 study subjects compatible with our selection criteria (mean age of 32.5 ± 9.2 years), 21 (70.0%) were women. Fifteen patients underwent LAGB and the remaining subjects underwent LTGVP surgery. The clinical and nutritional characteristics of the study participants at baseline and six weeks after surgery are presented in table 1.

Patients lost an average of 16.4 ± 5.8 kg (13.1%) during the six weeks following surgery. Restrictive bariatric surgery resulted in significant reductions in BMI, WC and WHR during the 6-week follow-up period. Daily intake of total calories (2,258.0 ± 803.4 vs 621.7 ± 301.0, p=0.0001) and all macronutrients were also decreased after bariatric surgery. We observed decreased levels of physical activity in study participants six weeks after surgery (table 1).

Table 1 demonstrates the blood biochemistry profile of the study participants before and after the bariatric surgery. No significant change was observed in lipid profiles, apart from TG, which significantly decreased following bariatric surgery (180.4 ± 118.9 vs 135.7 ± 51.8, p=0.03).

At baseline, vaspin concentrations did not significantly correlate with parameters of insulin resistance, anthropometric indices, lipid profile, dietary intake or physical activity. Following bariatric surgery, although circulating levels of vaspin did not show any significant correlation with parameters of insulin resistance, anthropometric indices, lipid profile and physical activity, it correlated significantly with total daily calorie intake (r=0.397, p=0.030)

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Before surgery</th>
<th>6 weeks after surgery</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>32.5 ± 9.2</td>
<td>32.7 ± 8.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>126.2 ± 19.9</td>
<td>109.7 ± 18.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>44.1 ± 4.9</td>
<td>38.4 ± 4.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>122.3 ± 15.1</td>
<td>110.1 ± 13.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.90 ± 0.10</td>
<td>0.87 ± 0.10</td>
<td>0.017</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>117.0 ± 13.7</td>
<td>109.3 ± 25.8</td>
<td>0.262</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.2 ± 10.7</td>
<td>71.4 ± 17.0</td>
<td>0.307</td>
</tr>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily calorie intake (kcal)</td>
<td>2258.0 ± 803.4</td>
<td>621.7 ± 301.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>76.6 ± 24.6</td>
<td>28.8 ± 14.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lipid (g)</td>
<td>90.9 ± 38.3</td>
<td>24.9 ± 17.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>289.0 ± 126.0</td>
<td>73.7 ± 41.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>16.7 ± 8.2</td>
<td>5.9 ± 4.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IPAQ score</td>
<td>1.07 ± 0.25</td>
<td>1.04 ± 0.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* All plus-minus values are mean±SD. IPAQ, International Physical Activity Questionnaire.
as well as daily intake of fat ($r = 0.371, p = 0.043$) and protein ($r = 0.477, p = 0.008$). We also observed a significant correlation between percentage change in vaspin serum levels and percent change in total daily calorie intake ($r = 0.389, p = 0.046$), as well as percentage change in daily intakes of fat ($r = 0.505, p = 0.010$) and protein ($r = 0.384, p = 0.050$). In a multivariate linear regression model, the association between percentage change in circulating vaspin levels and percentage change in total daily calorie intake ($β = 1.71, p = 0.055$) as well as percent changes in daily intakes of fat ($β = 2.47, p = 0.004$) and protein ($β = 1.23, p = 0.053$) remained significant even after adjustment for sex and percent changes in WC and HOMA-IR.

While HOMA-IR did not correlate with anthropometric indices, lipid profile, dietary intakes or physical activity at baseline, it showed significant correlations with male gender ($r = 0.378, p = 0.039$), WC ($r = 0.497, p = 0.005$), WHR ($r = 0.477, p = 0.008$), plasma levels of TG ($r = 0.407, p = 0.026$), and daily carbohydrate intake ($r = 0.383, p = 0.037$) following bariatric surgery.

### DISCUSSION

The present study demonstrates, for the first time, that restrictive bariatric surgery is accompanied by a significant decrease in serum vaspin concentrations. Our study agrees with previous studies that investigated the effect of weight reduction/lifestyle modification programs on circulating vaspin levels in obese subjects. Handisurya et al. [14] reported decreased levels of vaspin in the serum of morbidly obese subjects 12 months after RYGB surgery. Chang et al. [11] observed that a 12-week weight reduction program through restriction of daily energy intake by 500 kcal, regular exercise and treatment with the anti-obesity drug Orlistat®, significantly reduced serum vaspin in obese subjects. Lee et al. [16] have also shown reduced levels of vaspin following seven days of intensive lifestyle modification in overweight and obese children. Oberbach et al. [17] observed that vaspin levels decreased significantly after both short-term and long-term physical activity.

Currently, data on the association between circulating vaspin and obesity are conflicting. While Kloting et al. [10], Cho et al. [13], Kornet et al. [15], and Youn et al. [20] reported a positive correlation between obesity and serum levels of vaspin and/or its mRNA expression in adipose tissue, the correlation was more subtle or indeed absent in more recent studies [14, 19, 29, 30]. Although Chang et al. [12] observed a positive correlation between serum vaspin concentrations and the visceral adipose tissue area in insulin-resistant subjects, they observed no correlation between the visceral adipose tissue area and BMI or WC. In another study by the same group [11], a significant correlation between changes in vaspin concentrations and BMI and WC, in insulin-resistant patients who had undergone a weight reduction program was observed. In our study, we found no correlation between serum vaspin concentrations and anthropometric indices. Moreover, the changes in circulating vaspin levels did not correlate with the changes in BMI or WC following bariatric surgery. In concordance with our findings, Handisurya et al. [14] reported no significant correlation between serum vaspin concentrations and BMI after RYGB surgery. The exact reason for such a discrepancy remains to be elucidated. However, it might be explained by the findings of Aust et al. [29]. They observed a U-shaped relationship between body mass and plasma vaspin concentrations in elderly, overweight subjects with carotid stenosis. Von Loeffelholz et al. [19] similarly observed that circulating vaspin levels increased with BMI only in males, while in the group of females, they paradoxically observed the highest serum levels in normal weight subjects and accordingly, no significant correlation between vaspin and BMI was detectable in subjects with a BMI ≥ 25 kg/m². Hence, a potential association between plasma vaspin levels with body fat distribution could be of interest in future studies.

Administration of recombinant vaspin to obese mice has been shown to improve glucose tolerance, insulin sensitivity, and to alter gene expression of candidate genes for insulin resistance [9]. In addition, Kloting et al. [31] were able to demonstrate that central vaspin administration leads to reduced food intake and has a sustained blood glucose-lowering effects. Hence, vaspin has become an attractive candidate for drug development and subsequently there have been substantial research efforts to identify its importance in insulin resistance in humans. However, the relationship between circulating vaspin levels and the parameters of insulin sensitivity and glucose metabolism remains controversial in human [15, 19]. In this study, we found no correlation between vaspin and fasting plasma glucose, fasting insulin and HOMA-IR. Similar results

### Table 2

<table>
<thead>
<tr>
<th>Serum biochemistry profile of study subjects before and six weeks after restrictive bariatric surgery*</th>
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<tbody>
<tr>
<td>Before surgery</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
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<tr>
<td>Fasting glucose (mg/dL)</td>
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<tr>
<td>Fasting insulin (mu/L)</td>
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<tr>
<td>HOMA-IR</td>
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<tr>
<td>Vasin (ng/mL)</td>
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</tbody>
</table>

*All plus-minus values are mean±SD. LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance.
were also obtained in several recently published studies [11, 12, 19]. Von Loeffelholz et al. [19] demonstrated that vaspin does not correlate with insulin resistance measured using both euglycemic-hyperinsulinemic clamps and HOMA-IR, in a cross-section of 108 non-diabetic volunteers. Moreover, they found no effect of short-term lipid-induced insulin resistance due to a 300 min intravenous lipid challenge on serum vaspin. More recently, Oberbach et al. [17] demonstrated that short- and long-term exercise training decreases vaspin serum concentration through increased oxidative-stress, whereas changes in insulin-sensitivity do not seem to regulate circulating vaspin. Conversely, several studies reported a significant correlation between plasma levels of vaspin and HOMA-IR [10, 13-16, 18, 20]. Handisurya et al. [14] have shown that following RYGB surgery, changes in serum vaspin concentrations are significantly associated with the reduction in plasma levels of insulin and C-peptide, and with the amelioration of insulin sensitivity. Our data are contradictory to their data and the reasons for these discrepancies are unclear [14, 20]. Our subjects underwent restrictive bariatric surgery while Handisurya et al. performed a malabsorptive surgery (RYGB). There is evidence that these two types of bariatric surgery differ to some extent with respect to both efficacy, and the underlying mechanisms of action in insulin homeostasis [32]. Moreover, we assessed the effect of bariatric surgery on metabolic profile and vaspin levels in a short-term follow-up (six weeks after surgery), while they evaluated their subjects after 12 months. In other words, improvement in parameters of insulin-resistance might require longer periods of restricted intake following bariatric surgery.

The novel aspect of our study was that for the first time we specifically assessed the short-term effect of restrictive bariatric surgery on plasma levels of vaspin. Furthermore, we attempted to investigate the potential effect of dietary intake on circulating vaspin that had not been addressed in previous studies [10-20, 30]. Wang et al. [23] recently showed that a 16-week, high-fat diet could induce metabolic syndrome and accordingly lower vaspin levels in serum and peripipidylfat in rats, while pioglitazone and a 4-week calorie-restriction could increase the production of vaspin. However, they emphasized that further studies are needed to clarify whether dietary control accelerates or decelerates the production of vaspin and its mechanism of regulation in humans. In the present study, we demonstrated for the first time that independent of sex and changes in WC and HOMA-IR, the changes in serum vaspin concentrations are significantly associated with the reduction in plasma levels of insulin and C-peptide, and with the amelioration of insulin sensitivity. Our data are contradictory to their data and the reasons for these discrepancies are unclear [14, 20]. Our subjects underwent restrictive bariatric surgery while Handisurya et al. performed a malabsorptive surgery (RYGB). There is evidence that these two types of bariatric surgery differ to some extent with respect to both efficacy, and the underlying mechanisms of action in insulin homeostasis [32]. Moreover, we assessed the effect of bariatric surgery on metabolic profile and vaspin levels in a short-term follow-up (six weeks after surgery), while they evaluated their subjects after 12 months. In other words, improvement in parameters of insulin-resistance might require longer periods of restricted intake following bariatric surgery.

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One limitation of our study was the use of the surrogate index, HOMA-IR, instead of a euglycemic-hyperinsulinemic method, the standard method used to define insulin resistance. However, estimates using the HOMA-IR correlates well with those obtained from the euglycemic-hyperinsulinemic clamp (r=0.83, P<0.01) and have an acceptable degree of reproducibility [33].

In conclusion, our findings show that restrictive bariatric surgery in morbidly obese subjects leads to reduction in plasma concentrations of vaspin. Furthermore, our study demonstrated that decreased dietary intake might explain the reduction in vaspin levels following bariatric surgery, at least in part, whereas changes in insulin-sensitivity do not seem to be accountable for the decreased levels of vaspin early after bariatric surgery.

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Contributions AG contributed to designing and performing the study, and revised the article. NT contributed to data collection. FM analyzed the data and wrote the article. ZK contributed to performing the study. MT performed the bariatric surgeries. MH was the epidemiological consultant and analyzed the data. MJH was the chief researcher and contributed to idea formation, designing and managing the research project.

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