

Serum Vaspin Concentrations in Relation to Insulin Sensitivity Following RYGB-Induced Weight Loss

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Abstract

Background Recently, vaspin was identified as a novel adipokine with insulin-sensitizing effects that might be implicated in endogenous glucose regulation. Our objective was to evaluate the impact of acute weight loss and metabolic changes on serum vaspin concentrations in morbidly obese subjects following laparoscopic Roux-en-Y gastric bypass (RYGB) surgery.

Methods Longitudinal, clinical intervention study in 33 morbidly obese subjects before and 12 months after RYGB was conducted. Fasting serum concentrations of vaspin were measured by a commercially available ELISA and correlated with BMI, parameters of insulin sensitivity, and other biochemical measurements. Fasting insulin sensitivity was estimated using the homeostasis model assessment (HOMA) of insulin resistance.

Results RYGB-induced weight loss resulted in a reduction of circulating vaspin, leptin, insulin, and C-peptide levels as well as of BMI, HbA1c, and HOMA ($p < 0.0001$, respectively). Changes in serum vaspin concentrations correlated

positively with those in HOMA, insulin, C-peptide, HbA1c, and leptin ($p < 0.05$, respectively) levels. The association between percent change of vaspin and HOMA remained significant even after the adjustment for RYGB-induced changes in BMI.

Conclusions Following RYGB surgery, changes in serum vaspin concentrations correlate significantly with the reduction of circulating leptin, insulin, and C-peptide levels and with the amelioration of insulin sensitivity. However, further studies have to elucidate whether vaspin is only a biomarker for body-weight-related changes of insulin sensitivity or whether it is implicated in the regulation of human glucose homeostasis.

Keywords Vaspin · Adipokine · Obesity · Gastric bypass · Insulin resistance · Weight loss

Abbreviations

ALT	Alanine-aminotransferase
AST	Aspartate transaminase
BMI	Body mass index
CRP	C-Reactive protein
gGT	Gamma glutamyl-transpeptidase
HDL-C	High-density lipoprotein cholesterol
HOMA	Homeostasis model assessment of insulin resistance
LDL-C	Low-density lipoprotein cholesterol
Non-HDL-C	Non-high-density lipoprotein cholesterol
OLETF	Otsuka Long-Evans Tokushima Fatty
RYGB	Laparoscopic Roux-en-Y gastric bypass
S-creatinine	Serum creatinine
TG	Triglycerides
TC	Total cholesterol
Vaspin	Visceral adipose-tissue derived serpin
WAT	White adipose tissue

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Introduction

Obesity is associated with dyslipidemia, insulin resistance, and hypertension [1, 2]. Recent evidence suggests that bariatric surgery, especially Roux-en-Y gastric bypass (RYGB), represents the most effective intervention in obesity treatment, resulting not only in major and sustained weight loss but also in an amelioration of insulin sensitivity and lipid profile [2–6]. The pathophysiological mechanisms linking obesity and insulin resistance, however, have not been completely elucidated to date. In terms of an adipoinular axis, several adipokines have been suggested to contribute to the regulation of glucose homeostasis [7–9]. Initial evidence for the metabolic activity of adipose tissue originates from the identification of leptin [10] and its regulatory function on food intake and glucose metabolism [8–11].

Recently, visceral adipose-tissue-derived serpin (vaspin) was identified as a novel adipokine that is expressed in white adipose tissue (WAT) of obese humans [12] and Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a rodent model for insulin resistance and abdominal obesity [13]. In OLETF rats, vaspin gene expression peaks at an age when obesity and insulin resistance develop and thereafter decreases with worsening of metabolic control and weight loss [13]. Administration of recombinant vaspin to these rodents was found to elicit insulin-sensitizing properties, in part, via the modulation of gene expression of glucose transporter 4 and various other adipokines known to influence glucose metabolism. Thus, vaspin might be implicated in the endogenous regulation of glucose metabolism in states of obesity-induced insulin resistance [12–15].

Despite recent studies [14, 16–19], the putative role of vaspin in human carbohydrate metabolism, however, is currently unknown. The impact of changes in body weight and associated alterations of metabolic parameters as well as of menopausal state on circulating vaspin levels has not been investigated to date.

Thus, the present study was designed to assess the relationship between serum vaspin concentrations and RYGB-induced changes in BMI and parameters of insulin resistance and secretion.

Materials and Methods

Subjects

A total of 33 Caucasian subjects with a BMI >40 kg/m² scheduled for RYGB surgery were recruited consecutively at the outpatient department of the University Clinic of Vienna. All patients showed normal hepatic and renal function as assessed by medical history, physical examina-

tion, and routine laboratory screening tests, and none was taking any medication known to influence glucose metabolism. Subjects with age <18 years, acute inflammation, diabetes mellitus, hypo- or hyperthyroidism, uncontrolled hypertension, or other chronic diseases were excluded. Anthropometric data and venous fasting blood samples have been obtained prior to and 1 year after RYGB surgery in all study participants. Of the 30 female study participants, 58.1% were in pre- and 41.9% in postmenopausal state. At the postsurgery examination, all subjects reported weight stability for at least 1 month. The study protocol has been approved by the Local Ethics Committee.

Assays and Measurements

Blood samples were collected at 8 A.M. after an overnight fast. Serum samples were stored at –80°C until subsequent analysis. Serum vaspin levels were analyzed using a commercially available ELISA (human Vaspin ELISA kit, Adipogen, Seoul, South Korea) according to the manufacturer's instructions. Intra- and inter-assay coefficients of variation were 2.76% and 3.27%, respectively, and the average recovery was 99.41%. Leptin was determined using the Fluorokine human Leptin kit and the obesity MultiAnalyte Profiling Base Kit (R&D Systems, Minneapolis, MO, USA) and plasma insulin and C-peptide concentrations by commercially available radioimmunoassays (Linco Res., St. Charles, MO, USA). All blood samples were measured in duplicate. For the evaluation of insulin sensitivity, the homeostasis model assessment (HOMA) of insulin resistance has been employed.

Statistical Analysis

All datasets were tested for normal distribution using the Kolmogorov–Smirnov test. To assess effects of RYGB, paired *t* test was used. Pearson's correlation was performed to evaluate associations between metric variables. Statistical analysis was performed using SAS Enterprise Guide 4.1 (SAS Institute, Cary, NC, USA). $P \leq 0.05$ was considered significant. Data are expressed as mean \pm SE.

Results

Pre- and Postoperative Characteristics

As expected, RYGB induced a significant decrease of BMI, serum insulin, and C-peptide concentrations as well as of HOMA-IR and HbA1c (Table 1). Postoperative state was characterized by significantly decreased serum vaspin and leptin concentrations (Table 1). No differences in circulating vaspin levels have been found

Table 1 Pre- and postoperative characteristics

	Before surgery	After surgery	<i>p</i> value
<i>n</i> (female/male)	33 (30/3)		
Age at surgery (years)	43.75±2.08		
BMI (kg/m ²)	47.12±1.18	34.84±1.09	<0.0001
TG (mg/dl)	168.0±16.2	120.4±14.0	0.021
TC (mg/dl)	195.0±5.1	158.0±6.0	<0.0001
HDL-C (mg/dl)	49.56±1.60	49.79±1.68	0.968
LDL-C (mg/dl)	116.9±5.0	86.3±4.9	<0.0001
Non-HDL-C (mg/dl)	146.3±5.1	108.2±5.6	<0.0001
S-creatinine (mg/dl)	0.83±0.02	0.79±0.02	0.004
AST (U/L)	26.41±2.51	25.00±1.87	0.543
ALT (U/L)	33.91±2.78	29.88±2.91	0.369
gGT (U/L)	38.81±3.62	25.55±3.31	0.003
CRP (mg/dl)	1.16±0.15	0.40±0.07	<0.0001
Glucose (mg/dl)	98.01±3.55	94.63±2.26	0.549
Insulin (μU/ml)	33.52±3.88	12.42±0.74	<0.0001
C-peptide (ng/ml)	4.78±0.34	2.25±0.13	<0.0001
HbA1c (%)	6.11±0.15	5.36±0.11	<0.0001
HOMA-IR	8.03±1.13	2.82±0.17	<0.0001
Vaspin (ng/ml)	0.67±0.07	0.31±0.05	<0.0001
Leptin (ng/ml)	109.0±7.7	36.4±5.3	<0.0001
TSH (μU/ml)	2.12±0.15	1.80±0.18	0.041
ft4 (ng/dl)	1.11±0.02	1.21±0.04	0.003

Age, BMI, and biochemical characteristics of 33 morbidly obese subjects before and after RYGB surgery

between female subjects in pre- as compared to postmenopausal state (before RYGB, 0.69±0.11 vs. 0.66±0.11 ng/ml; after RYGB, 0.34±0.07 vs. 0.32±0.09 ng/ml; percent change, -44.98±8.00 vs. -46.99±13.02%) or between men and women (before RYGB, 0.63±0.15 vs. 0.67±0.42 ng/ml, *p*=0.86; after RYGB, 0.46±0.68 vs. 0.30±0.24 ng/ml, *p*=0.38; percent change, -38.01±86.86 vs. -49.78±32.18%, *p*=0.61).

Correlation Analysis

Significant correlations have been observed between percent change of circulating vaspin levels and percent change of HOMA (*r*=0.567, *p*=0.004), HbA1c (*r*=0.381, *p*=0.045), insulin, C-peptide (Fig. 1) and leptin (*r*=0.552, *p*=0.004). In a multivariate linear regression model, the association between percent change of circulating vaspin levels and percent change of HOMA remained significant even after the adjustment for percent change of BMI (*B*=0.64, *p*=0.006). Δ vaspin correlated with Δ TSH (*r*=0.533, *p*=0.0014) and Δ weight (*r*=0.355, *p*=0.046) but not with Δ BMI (*p*=0.06). One year after RYGB surgery, serum vaspin concentrations correlated positively with glucose

levels (*r*=0.415, *p*=0.018). No association has been observed between percent change of circulating vaspin levels and parameters of lipid metabolism (data not shown).

A significant correlation between RYGB-induced percent changes in serum leptin concentrations has been observed with percent changes in BMI (*r*=0.793, *p*<0.0001), HOMA (*r*=0.612, *p*=0.003), insulin (*r*=0.604, *p*=0.001), C-peptide (*r*=0.702, *p*<0.0001), alanine-aminotransferase (ALT, *p*=0.568, *p*=0.004), and gamma glutamyl-transpeptidase (gGT, *r*=0.566, *p*=0.004).

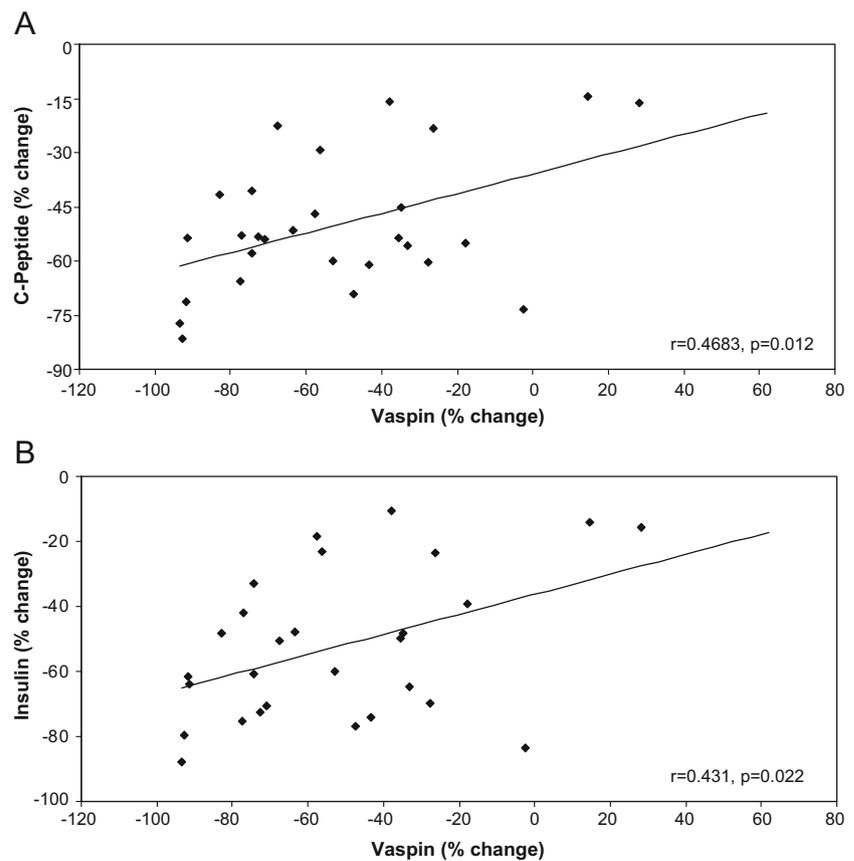
Discussion

The present study demonstrates for the first time that acute RYGB-induced weight loss is associated with a substantial decrease of serum vaspin concentrations and that these changes are positively correlated with changes in leptin, insulin and insulin sensitivity.

Recent investigations in humans showed a BMI-dependent vaspin mRNA expression pattern [12] and provided evidence for increased serum vaspin concentrations in states of obesity [13]. Consistently, our data have shown weight loss to be associated with an attenuation of circulating vaspin levels. However, no direct relationship between changes in vaspin and BMI has been observed. RYGB additionally resulted in a decrease of serum leptin, which strictly relates to the reduction of body fat mass [9]. Hence, leptin has been suggested as biomarker for the amount of adipose tissue and hepatic steatosis [20] and, according to our data, may be superior to vaspin in this respect.

As for the role of vaspin in carbohydrate metabolism, insulin-sensitizing effects of vaspin have been proposed by experimental animal data [13]. In humans, however, the association between insulin sensitivity and circulating vaspin levels remains controversial. Youn et al. [14] observed a significant BMI-adjusted correlation with the glucose-infusion rate during an euglycemic-hyperinsulinemic clamp in normal glucose tolerant subjects. Consistently, a recent study found significantly higher circulating vaspin levels in women with polycystic ovary syndrome, which decreased following 6 months of metformin therapy [17]. The attenuation of glucose levels, thereby, predicted changes in serum vaspin concentrations [17]. These findings are aggravated by reports suggesting an association between a vaspin single nucleotide polymorphism and type 2 diabetes [18] as well as between circulating vaspin levels and glycemic control [19]. In contrast, another study [16] failed to establish an association between serum vaspin concentrations and insulin sensitivity. In our study population, changes in serum vaspin concentrations were significantly correlated with the weight loss-associated decrease of

Fig. 1 a and b Pearson's correlation between RYGB-induced percent change in circulating vaspin, C-peptide, and insulin levels



circulating insulin levels and the improvement of HOMA, independent of the reduction in BMI. In addition, we have observed a correlation between changes in circulating vaspin and C-peptide levels, suggesting a relation between vaspin and the RYGB-induced attenuation of pancreatic insulin secretion. Thus, it is intriguing to hypothesize a role of vaspin in human glucose homeostasis. Regarding our as well as previous results [13, 17–19], it might be speculated that the up-regulation of circulating vaspin levels in obesity-associated insulin resistance represents an endogenous compensatory mechanism to preserve glycemic control. Similar changes have been reported for other adipokines with presumably beneficial effects on glucose homeostasis [21–23]. The assumption that vaspin might be implicated in the regulation of carbohydrate metabolism is corroborated by the predominant localization of vaspin gene expression in visceral adipose tissue [12, 13]. Although we and others have shown serum vaspin concentrations to be associated with parameters of insulin resistance, the biological relevance of vaspin is unknown to date as the reduction of serum vaspin levels may be secondary to the RYGB-associated amelioration of insulin sensitivity. Thus, we cannot exclude that circulating vaspin levels only decrease in parallel with the improved insulin sensitivity and, consecutively, rather constitute a marker

for the amelioration of insulin resistance or for the presence of visceral fat.

Weight loss is associated with a modulation of thyroid function, which, in part, might be mediated by changes in circulating leptin concentrations [24, 25]. Consistently, in our study cohort, RYGB induced a significant reduction of circulating TSH levels, which positively correlated with changes in serum concentrations of leptin and, interestingly, also of vaspin. The potential influence of thyroid function on circulating vaspin levels has not been investigated to date. It is unknown whether vaspin, in synergy with leptin, contributes to the weight-loss-associated decrease of TSH levels or whether the altered thyroid function itself or other modulating factors [26] influence release and/or clearance of vaspin.

With regard to parameters of lipid metabolism, we have observed no significant correlation with serum vaspin concentrations. This finding is in disagreement with previous reports, which have found an association between circulating vaspin levels and total as well as HDL-cholesterol [13, 16]. Differences in the results might be attributable to the missing improvement of HDL-C levels in our study population or to differences in gender distribution. Indeed, serum vaspin concentrations were reported to be higher in women as compared to men, suggesting a sex-

dependent regulation of vaspin secretion or clearance [13, 16]. It has been hypothesized that gender-related differences in amount and distribution of adipose tissue or even an estrogen-mediated induction of vaspin might account for the observed sexual dimorphism [13, 16]. Although circulating levels of total and bioavailable estrogen have not been investigated in our study population, comparison of pre- vs. postmenopausal women in our study population revealed no significant differences in serum vaspin concentrations. Thus, our data do not confirm a putative role of estrogens in the regulation of vaspin. Indeed, the reported sexual dimorphism in circulating vaspin levels is a matter of debate [13, 16] as others found no differences between female and male patients with carotid stenosis [27]. Consistently, our data indicate no sex disparities in serum vaspin concentrations but yield low statistical power due to the low sample size of male subjects.

Concerning the impact of bariatric surgery on circulating vaspin levels, it is well acknowledged that various operative procedures, e.g., RYGB, gastric banding, or biliopancreatic diversion, affect the gastrointestinal physiology and also glucose metabolism differently [28, 29]. Thus, it might be of relevance to evaluate whether differential changes in the secretion of gastrointestinal peptides following the respective bariatric surgery techniques [29, 30] modify serum vaspin concentrations and their relationship with parameters of insulin sensitivity in diverse ways.

In conclusion, a significant correlation between changes in serum vaspin concentrations, circulating insulin, and C-peptide levels and insulin sensitivity as assessed by HOMA has been observed in morbidly obese subjects following RYGB. However, it is not accessible from the present study whether vaspin directly influences insulin sensitivity [13] or whether it rather constitutes another surrogate for fat mass.

Disclosure statement All authors have nothing to disclose.

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