Serum concentrations and subcutaneous adipose tissue mRNA expression of omentin in morbid obesity and type 2 diabetes mellitus: the effect of very-low-calorie diet, physical activity and laparoscopic sleeve gastrectomy

MONIKA URBANOVÁ[1], IVANA DOSTÁLOVÁ[1], PAVEL TRACHTA[1], JANA DRÁPALOVÁ[1], PETRA KAVÁLKOVÁ[1], DENISA HALUZÍKOVÁ[1,2], MARTIN MATOULEK[1], ZDENA LACINOVÁ[1], MILOŠ MRÁZ[1], MOJMÍR KASALICKÝ[3], MARTIN HALUZÍK[1]

[1]3rd Department of Medicine, [2]Department of Sports Medicine, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

[3]Department of Surgery, 2nd Faculty of Medicine, Charles University and Military University Hospital, Prague, Czech Republic

Corresponding author:

Martin Haluzík, Prof, M.D., DS.c.

3rd Department of Medicine, 1st Faculty of Medicine

U Nemocnice 1

128 08 Prague 2

Czech Republic

Tel.: + 420 224962908; Fax: + 420 224919780

e-mail: mhalu@lf1.cuni.cz

Short title: Omentin in obesity and T2DM

Summary

Omentin is a novel adipokine with insulin-sensitizing effects expressed predominantly in visceral fat. We investigated serum omentin levels and its mRNA expression in subcutaneous adipose tissue (SCAT) of 11 women with type 2 diabetes mellitus (T2DM), 37 obese non-diabetic women (OB) and 26 healthy lean women (C) before and after various weight loss interventions: 2-week very-low-calorie diet (VLCD), 3-month regular exercise and laparoscopic sleeve gastrectomy (LSG). At baseline, both T2DM and OB groups had decreased serum omentin concentrations compared with C group while omentin mRNA expression in SCAT did not significantly differ among the groups. Neither VLCD nor exercise significantly affected serum omentin concentrations and its mRNA expression in SCAT of OB or T2DM group. LSG significantly increased serum omentin levels in OB group. In contrast, omentin mRNA expression in SCAT was significantly reduced after LSG. Baseline fasting serum omentin levels in a combined group of the studied subjects (C, OB, T2DM) negatively correlated with BMI, CRP, insulin, LDL-cholesterol, triglycerides and leptin and were positively related to HDL-cholesterol. Reduced circulating omentin levels could play a role in the etiopathogenesis of obesity and T2DM. The increase in circulating omentin levels and the decrease in omentin mRNA expression in SCAT of obese women after LSG might contribute to surgery-induced metabolic improvements and sustained reduction of body weight.

Key words: Omentin, subcutaneous adipose tissue, obesity, laparoscopic sleeve gastrectomy, very-low-calorie diet.

Introduction

Obesity has become a major contributor to the global burden of chronic diseases affecting virtually all ages and socioeconomic groups worldwide (Haslam and James 2005, Guh *et al.* 2009). It is now generally accepted that obesity increases the risk of multiple metabolic diseases, such as hyperlipidemia, insulin resistance, type 2 diabetes mellitus (T2DM), arterial hypertension, atherosclerosis and cardiovascular complications (Field *et al.* 2001, Stumvoll *et al.* 2005, Fujita *et al.* 2006).

Endocrine function of adipose tissue and its changes in obesity and T2DM have drawn a lot of research interest over the last two decades. It has been demonstrated in numerous studies that obesity markedly changes adipose tissue endocrine production (Blüher 2009, Batra and Siegmund 2012, Blüher 2012). Furthermore, functional differences of adipose tissue are associated with its anatomic distribution in subcutaneous (SCAT) and visceral omental (VAT) depots with VAT being considered more closely related to metabolic complications (Wajchenberg 2000, Després *et al.* 2008, Indulekha *et al.* 2011, Baglioni *et al.* 2012). In obese patients, adipose tissue secrets excessive amounts of proinflammatory cytokines and adipokines (e.g., resistin, leptin, TNF-α, IL-1, IL-6) that in turn contribute to chronic low-grade inflammation and the development of insulin resistance, T2DM and increased rate of cardiovascular complications (Shoelson *et al.* 2006, Hotamisligil 2006, Zeyda and Stulnig 2009, Sell *et al.* 2012).

Omentin (or intelectin-1) is a novel adipokine that is predominantly secreted by stromal vascular cells in VAT (Schäffler *et al.* 2005, Yang *et al.* 2006). Lower omentin expression levels are detectable also in SCAT (Kralisch *et al.* 2005, Barth *et al.* 2010). Serum concentrations of omentin-1, the major circulating isoform in human plasma, and mRNA expression of omentin in VAT are decreased in obese and T2DM patients (Auguet *et al.* 2011,

Pan *et al.* 2010, Jialal et al. 2013). Reduced circulating omentin-1 levels are associated with low plasma adiponectin and high-density lipoprotein (HDL-cholesterol) levels (de Souza Batista *et al.* 2007). In addition, circulating omentin-1 levels negatively correlate with serum leptin, resistin and insulin levels, body mass index (BMI), and HOMA index (de Souza Batista *et al.* 2007). *In vitro* studies have revealed that omentin has an insulin sensitizing effect on adipocytes in both visceral and subcutaneous adipose depot through increased insulin signal transduction by activation of Akt/protein kinase B (Akt/PKB). It also enhances insulin-stimulated glucose uptake in human adipocytes (Yang *et al.* 2006). These results indicate that omentin might provide a new potential target for treatment of insulin resistance/T2DM.

Little information is available with respect to changes of serum omentin levels after various weight-reducing interventions. To our best knowledge, only one study focusing on the influence of calorie restriction and weight loss on serum omentin levels has been published so far (Moreno-Navarrete *et al.* 2010). The results indicate that hypocaloric diet-induced weight loss accompanied by improvement of insulin sensitivity was associated with increased serum omentin levels. Serum omentin concentrations after regular physical exercise were explored by Saremi and colleagues who found a significant increase of serum omentin concentrations in overweight and obese men after 12 weeks of aerobic training (Saremi *et. al* 2010). Studies aiming at the effect of bariatric surgery are presently lacking.

We hypothesized that improvement of metabolic parameters after selected weight-reducing interventions could be associated with the changes in serum omentin levels or its gene expression in SCAT. To this end, we investigated the effects of three types of intervention (2-week very-low calorie diet (VLCD), 3-month regular physical activity and bariatric surgery - laparoscopic sleeve gastrectomy (LSG)) on serum omentin levels and its

mRNA expression in SCAT of obese women. Furthermore, we explored whether the presence of T2DM affects the response of serum omentin and its SCAT mRNA expression to VLCD.

Methods

Study subjects

Eleven obese women with type 2 diabetes mellitus (T2DM group), thirty-seven obese non-diabetic females (OB group) and twenty-six lean healthy women (C group) were included in the study. Twenty-three out of thirty-seven obese non-diabetic patients were on antihypertensive treatment, ten patients were treated with statins and two of them were on combined therapy with ezetimib. Eleven OB patients received thyroid hormone substitution therapy. All T2DM patients were treated either with oral antidiabetic drugs, insulin, or its combination. The antidiabetic treatment remained unchanged for at least three months prior to the start of the study. During the 2-week VLCD period, insulin and sulphonylurea doses were decreased to avoid hypoglycemia resulting from improved insulin sensitivity, decreased energy intake and body weight. Decrease in insulin/sulphonylurea doses was necessary in all patients treated with insulin, sulphonylurea or its combination. The doses of metformin were not changed throughout the VLCD period.

All of the diabetic patients were treated with antihypertensive drugs; four patients were treated with statins and one with a fibrate. Three T2DM patients were on thyroid hormone substitution therapy. Control subjects had no history of obesity and/or diabetes mellitus, arterial hypertension, or lipid metabolism disturbances and received no medication. Blood tests confirmed normal blood count, biochemical and hormonal parameters.

Ten out of thirty-seven obese and all of the diabetic patients underwent a 2-week VLCD with energy intake 2500kJ per day (600 kcal per day). During the reduction program all

patients were hospitalized at the Third Department of Medicine, General University Hospital in Prague. Thirteen obese non-diabetic patients took part in a 3-month exercise program at the Recondition Center in Prague. The patients underwent 30 minutes of aerobic exercise three times a week under the supervision of a certified coach, patients kept their usual eating habits during the exercise program. The remaining thirteen obese patients underwent LSG at the Surgical Clinic, Military University Hospital in Prague. The body weight of all study participants remained stable for at least three months before the enrollment into the study. Written informed consent was signed by all participants before beginning of the study. The study was approved by Human Ethical Review Committee, First Faculty of Medicine and General University Hospital, Prague, Czech Republic and was performed in accordance with the guidelines proposed in the Declaration of Helsinki.

Anthropometric examination, blood and adipose tissue sampling

All patients included in the reduction program (T2DM and obese non-diabetic subjects) were examined twice; at basal state before beginning of any intervention and after 2 weeks of VLCD. Obese non-diabetic patients enrolled in the physical activity program were examined at basal state before the start of regular exercise and after 3 months of the exercise program. Patients undergoing bariatric surgery intervention were examined four times – at basal state before surgical intervention and then 6, 12 and 24 months after the surgery, respectively. Normal-weight healthy subjects were examined only once. All subjects were measured and weighted, and their BMI was calculated. Blood samples for biochemical and hormonal parameters measurement were withdrawn between 07.00h and 08.00h after 12 h of overnight fasting. Blood samples were separated by centrifugation for 10min at 1000 x g within 30 min from blood collection. Serum was subsequently stored in aliquots at -80°C until further analysis. Samples of subcutaneous adipose tissue for mRNA expression analysis were

obtained from abdominal region with subcutaneous needle aspiration biopsy. Approximately 100 mg of adipose tissue was collected to 1 ml of RNA stabilization Reagent (RNAlater, Qiagen, Hilden, Germany) and stored at - 80°C until further analysis.

Hormonal and biochemical assays

Serum omentin levels were measured by a commercial ELISA kit (BioVendor, Brno, Czech Republic). Sensitivity was 0.5 ng/ml. Serum adiponectin, CRP, leptin, resistin, and insulin concentrations were measured by commercial ELISA a RIA kits as described previously (Dolinkova *et al.* 2008). The intra- and interassay variabilities for all methods were less than 5.0 and 10.0%, respectively. Biochemical parameters (glucose; total and HDL-cholesterol, triglycerides) were measured at the Department of Biochemistry of General University Hospital by standard laboratory methods. The value of LDL-cholesterol was calculated according to Friedewald formula. The homeostasis model assessment (HOMA) was calculated as HOMA-IR index using the following formula: fasting serum insulin (mIU/l) x fasting serum glucose (mmol/l)/22.5. Glycated hemoglobin was analyzed by high performance liquid chromatography (HPLC) on Variant II BioRad analyzer (BioRad).

mRNA expression determination by quantitative real-time PCR (qRT-PCR)

Samples of subcutaneous adipose tissue were homogenized on MagNA Lyser Instrument with MagNA Lyser Green beads (Roche Diagnostics GmbH, Germany). Total RNA from homogenized tissue was extracted on MagNA Pure instrument using Magna Pure Compact RNA Isolation kit (tissue) (Roche Diagnostics GmbH, Germany). The RNA concentration was determined from absorbance at 260 nm on a NanoPhotometer (Implen, Munchen, Germany). Reverse transcription was performed using 0.25 μg of total RNA to synthesize the first strand cDNA using the random primers as per the instructions of the High-Capacity

cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA). Gene expression of omentin -1 was performed on a 7500 Real-Time PCR System using TaqMan ® gene Expression Assays (Applied Biosystems, Foster City, CA, USA). For reaction a mix of TaqMan® Universal PCR Master Mix II, NO AmpErase® UNG (Applied Biosystems, Foster City, CA, USA), nuclease-free water (Fermentas Life Science, Lithuania) and specific TaqManGene expression Assays (Applied Biosystems, Foster City, CA) was used.

Controls with no template cDNA were performed with each assay and all samples were run at least in duplicate. The increase in fluorescence was measured in real time and threshold cycle (Ct) values were obtained. To compensate for variations in RNA amount and efficiency of reverse transcription, beta-2-microglobulin was used as endogenous reference and results were normalized to the mean of these values. The formula $2^{-\Delta\Delta Ct}$ was used to calculate relative gene expression.

Statistical analysis

Statistical analysis was performed on SigmaStat software (Systat Inc., Chicago, IL). Anthropometric, hormonal and biochemical results are expressed as means ± SEM (standard error of the means). Unpaired t-test or Mann-Whitney *U* test was used for group comparison as appropriate. Differences between T2DM and obese patients before and after VLCD, and obese non-diabetics before and after physical activity and LSG were evaluated using paired t-test or Wilcoxon Signed –Rank test as appropriate. Statistical significance was assigned to $p < 0.05$. The correlations between the values were estimated by Spearman correlation test. Multiple linear regression analysis was used to show the independent relationships of other parameters with serum omentin levels. A p value < 0.05 denoted statistical significance.

Results

Anthropometric, biochemical and hormonal characteristics of study subjects

Anthropometric, biochemical and hormonal characteristics of all study groups are summarized in Table 1. As expected both OB and T2DM patients had markedly increased BMI and circulating CRP levels relative to control group. Serum concentrations of leptin, insulin, glucose, HbA1c, LDL-cholesterol, triglycerides, and HOMA-index were significantly increased in both OB and T2DM patients relative to control group. On the contrary, circulating adiponectin and serum HDL-cholesterol levels were significantly decreased in both OB and T2DM patients compared with control group. Serum total cholesterol and circulating resistin levels did not significantly differ among the groups. In T2DM subjects, BMI and circulating CRP levels were significantly elevated as compared to OB patients. Serum concentrations of glucose, HbA1c and leptin were markedly elevated, whereas HDL-cholesterol levels were significantly decreased in T2DM group relative to OB group.

Serum concentrations of omentin were significantly decreased in both OB and T2DM patients relative to C subjects. Serum omentin levels did not significantly differ between T2DM and OB group (Table 1). mRNA expression of omentin (ITLN1) did not differ among the studied groups (data not shown).

The influence of VLCD on hormonal and biochemical parameters, serum omentin and its mRNA expression in SCAT in T2DM and OB patients

The influence of VLCD on hormonal and biochemical parameters in T2DM and OB patients is summarized in Table 2. Two weeks of VLCD significantly reduced BMI, circulating levels of CRP, glucose, HDL- and total-cholesterol in T2DM patients. Serum insulin, LDL-cholesterol, triglycerides, leptin, resistin, and adiponectin levels, and HOMA

index of diabetic patients were not significantly affected by VLCD. In obese non-diabetic subjects, a significant decrease of BMI, serum total cholesterol, LDL- and HDL-cholesterol was found after VLCD. The diet intervention did not significantly affect CRP, serum insulin, glucose, HOMA index, triglycerides, leptin, resistin and adiponectin levels in OB group. HbA1c was not assessed after VLCD.

VLCD had no significant effect on serum omentin levels in either T2DM or obese patients (Table 2). mRNA expression of omentin in SCAT was not affected by VLCD in any group studied (data not shown).

The effect of a 3-month regular physical activity on hormonal and biochemical parameters, serum omentin and its SCAT mRNA expression in obese non-diabetic women

Changes of the studied parameters after physical activity program are summarized in Table 3. Three months of regular exercise significantly decreased BMI, HOMA index, serum insulin, glucose and leptin levels. CRP, HbA1c, total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, adiponectin and resistin levels were not significantly affected by physical activity.

3-months regular exercise did not significantly affect either serum omentin levels (Table 3) or its gene expression in SCAT of OB group (data not shown).

The effect of LSG on hormonal and biochemical parameters, serum omentin and its SCAT mRNA expression in obese non-diabetic patients

The changes of hormonal and biochemical parameters in obese women after LSG are summarized in Table 4. Overall, LSG had no significant effect on blood glucose levels during the 2-year follow up. At month 6 after the surgery BMI, insulin levels, HbA1c and HOMA index, triglycerides and leptin levels were significantly decreased relative to baseline levels.

On the contrary, adiponectin levels markedly increased at month 6 after LSG. CRP, total-cholesterol, LDL-cholesterol, HDL-cholesterol and resistin levels were not significantly affected at month 6 after LSG as compared to baseline values. One year after the surgery, BMI was further decreased, as were LDL-cholesterol levels. CRP, fasting insulin, HbA1c, HOMA index, triglycerides and leptin levels were significantly reduced at month 12 after LSG relative to baseline values. HDL-cholesterol and adiponectin levels were markedly increased, whereas total-cholesterol and resistin levels did not significantly change 1 year after surgery. At month 24 after LSG, BMI, HbA1c, leptin and adiponectin levels significantly increased compared with those values measured at month 12 after LSG. Circulating CRP and LDL-cholesterol levels were markedly decreased at month 24 relative to pre-surgery values. On the contrary, serum HDL-cholesterol levels were significantly increased 2 years after LSG as compared to its pre-surgery levels. Serum insulin, HOMA index, total cholesterol, triglycerides and resistin levels were not significantly different from baseline values at month 24 after LSG.

Serum omentin levels significantly increased 6 months after LSG, and a sustained increase of circulating omentin levels remained significant during the 2-year follow up (Table 4). In contrast, LSG decreased omentin gene expression in SCAT. This effect was at first detected at month 6 after LSG and was most pronounced at month 12 after LSG (Figure 1). At month 24 after LSG we observed a slight non-significant increase of omentin mRNA expression in SCAT when compared with month 12, but the expression still remained markedly reduced relative to baseline values.

Relationship of serum omentin levels and its gene expression in SCAT to other studied parameters

The relationship of serum omentin levels and its mRNA expression in SCAT to other studied parameters was calculated in a combined population of healthy controls, OB and T2DM subjects at baseline (before the interventions). Serum omentin concentrations were inversely associated with BMI, CRP, serum insulin, LDL-cholesterol, triglycerides and leptin levels, whereas it positively correlated with HDL-cholesterol levels (Figure 2). We failed to find any significant relationship of baseline serum omentin to fasting blood glucose, HbA1c, HOMA-IR index, total cholesterol, adiponectin, and resistin (data not shown). Multiple regression analysis was performed with baseline serum omentin levels as dependent and other anthropometrical and biochemical parameters as independent variables. None of the factors included was identified as statistically significant independent predictor of serum omentin concentrations (data not shown). We failed to find any significant relationship of omentin gene expression in SCAT to other studied parameters including BMI, CRP, serum insulin, blood glucose, HbA1c, HOMA index, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, leptin, adiponectin and resistin levels (data not shown). Baseline serum omentin levels in the group of obese patients who underwent LSG significantly positively correlated with blood glucose ($r = 0.56$, $p = 0.046$) while no other significant relationships of serum omentin levels to other studied parameters in OB patients before LSG were found (data not shown). At month 12 after LSG, omentin mRNA expression significantly inversely correlated with CRP ($r = -0.63$, $p = 0.03$). Two years after the surgery, omentin mRNA expression positively correlated with fasting blood glucose levels ($r = 0.55$, $p = 0.049$). At month 6 after LSG, the difference in serum omentin levels (Δ serum omentin) inversely correlated with the difference in serum insulin levels (Δ serum insulin) ($r = -0.68$, $p = 0.019$) and with the difference in HOMA index (Δ HOMA) ($r = -0.71$, $p = 0.013$), respectively. 24 months after the surgery, the difference in serum omentin levels (Δ serum omentin) negatively correlated with the difference in serum CRP levels (Δ serum CRP) ($r = -0.75$, $p = 0.038$) and it was

positively related to the difference in serum LDL-cholesterol levels (*Δ* serum LDL) (r = 0.71, p = 0.013). Multiple linear regression analyses were performed with *Δ* serum omentin as dependent and the other anthropometrical and biochemical parameters as independent variables. BMI (β = 17.32, p = 0.049), insulin levels (β = -7.08, p = 0.005) and HOMA index (β = -29.62, p = 0.034) were found to be the independent predictors of changes of serum omentin levels after LSG. The results obtained from multiple linear regression analyses are summarized in Table 5.

Discussion

The most important finding of the present study is that serum omentin levels were significantly increased after LSG, whereas its mRNA expression in SCAT was significantly reduced after the surgery. 3 months of regular physical activity and 2 weeks of VLCD had no significant effect on serum omentin levels and its mRNA expression in SCAT despite its overall positive effects on anthropometric, hormonal and biochemical parameters. Furthermore, serum omentin concentrations negatively correlated with BMI, CRP, insulin, LDL-cholesterol, triglycerides and leptin levels and were positively related to serum HDL cholesterol. In contrast, omentin mRNA expression in SCAT did not correlate with any of the anthropometric and biochemical parameters studied.

In our study, we found, in agreement with other previously published papers (Auget *et al.* 2011, de Souza Batista *et al.* 2007, Yan *et al.* 2011, Jialal *et al.* 2013), markedly reduced serum omentin concentrations in both obese and T2DM patients as compared to healthy normal-weight subjects. Although we failed to find any significant differences in serum omentin levels between obese non-diabetic and T2DM patients, we cannot exclude the possibility that our findings might have been influenced by a relatively low number of T2DM

patients (11 vs. 37 in obese group) and the heterogeneity of a group of non-diabetic obese patients. Inverse correlations of circulating omentin with BMI, CRP, fasting insulin, LDL-cholesterol, triglycerides and leptin and its positive relation to HDL-cholesterol are in accordance with previous reports (de Souza Batista *et al.* 2007) and indicate that serum omentin might represent a potential marker of metabolic syndrome and endothelial dysfunction (Moreno-Navarette *et al.* 2010, Zhou *et al.* 2012). The exact factors contributing to markedly reduced circulating omentin levels in obesity and diabetes still remain to be determined. Bearing in mind the results of previous studies (de Souza Batista *et al.* 2007, Choi *et al.* 2011, Zhou *et al.* 2012), increased insulin levels typically found in patients with obesity and T2DM might be an important contributor preceding decreased omentin levels. Another possible player contributing to decreased omentin levels could be excessive adiposity and obesity-associated metabolic complications. This possibility is supported by the finding of increased circulating levels of omentin in patients with anorexia nervosa with severely reduced body fat content (Guo *et al.* 2012). Although the multiple linear regression analyses performed in our study did not lead to the identification of any from the tested parameters as the independent predictors of pre-interventions fasting serum omentin levels, we revealed that BMI, insulin and HOMA index are independent predictors of the difference in serum omentin levels (Δ serum omentin) after LSG. Both OB and T2DM groups in our study displayed markedly increased BMI, CRP, insulin, glucose, HOMA, LDL-cholesterol, triglycerides and leptin, whereas serum adiponectin and HDL-cholesterol were reduced in both groups. Such metabolic and hormonal status reflects long-term disturbances in the function of metabolically active tissues with a prominent role of endocrine dysfunction of adipose tissue. Reduced circulating omentin levels may thus reflect the overall metabolic phenotype rather than a single altered hormonal or biochemical parameter. In contrast, the increase in serum omentin

levels after LSG may be connected mainly to the reduction of body weight and fasting serum insulin levels after the surgery.

Omentin is predominantly expressed in visceral adipose tissue and its expression levels in SCAT are significantly lower (Schäffler *et al.* 2005, Kralisch *et al.* 2005, Yang *et al.* 2006). The lack of statistically significant difference in gene expression of omentin in SCAT of obese and T2DM patients as compared to lean women is in agreement with findings of Auget *et al.* (Auget *et al.* 2011). Although the study of Jialal et al. (2013) revealed significantly decreased SCAT secreted omentin protein in subjects with metabolic syndrome without T2DM, we did not examine SCAT secreted protein in our study. It is generally well-known that mRNA expression may not accurately reflect protein expression or secretion. However, it is noteworthy that a clear trend towards increased omentin mRNA expression in both OB and T2DM groups was observed in our study. It has been previously reported that decreased omentin gene expression in VAT of obese and insulin resistant or T2DM patients correlates with systemic metabolic parameters (de Souza Batista *et al.* 2011). This finding suggests that omentin produced by VAT could be a direct contributor to systemic metabolic regulations. Our results show that omentin mRNA expression in SCAT is not significantly related to systemic metabolic parameters and that omentin mRNA expression in SCAT is independent of the serum levels. Further studies focused on omentin expression in different fat depots are needed to dissect the potentially distinct roles of SCAT- and VAT-produced omentin.

In our previous works, we have demonstrated the beneficial effect of short-term calorie restriction on metabolic and proinflammatory profile of obese and diabetic patients (Mraz *et al.* 2011, Touskova *et al.* 2012). In the present study we have investigated the effect of various obesity treatment methods (VLCD, exercise, LSG) on serum omentin levels and its mRNA expression in SCAT. Moreno-Navarrete *et al.* (2010) reported that serum omentin levels significantly increased after a 4-month hypocaloric diet. Contrary to this report, in our

study 2-week VLCD and regular 3-month physical activity had no significant impact on serum omentin levels and its gene expression in SCAT. The differences between our and Moreno-Navarretes´ results can be explained by shorter duration of dietary intervention in our study.

Similarly, although the 3-month exercise explored in our study reduced both BMI and circulating insulin levels, the changes were probably not profound enough to significantly affect serum omentin concentrations. This hypothesis is further supported by the fact that more profound weight loss induced by LSG markedly increased serum omentin levels and significantly decreased omentin mRNA expression in SCAT during the 2-year follow-up. However, our results from exercise branch are in disagreement with the findings of Saremi et al., who observed a significant increase of serum omentin concentrations in overweight and obese men after 12 weeks of aerobic training (Saremi *et al.* 2010). Such a discrepancy in the results obtained by Saremi et al. and ours could be explained by the major differences in the study design; mainly the intensity of aerobic training was much higher in the study of Saremi et al. (50-60 minutes 5 times a week), which led to a more marked improvement of lipid profile compared with our study. In contrast, we observed a more profound decrease in BMI, fasting insulin levels, and HOMA IR index in our study. This difference may have been due to distinct characteristics of subjects included in both studies (males vs. females, average pre-exercise BMI 29.1 vs. 38.2).

In conclusion, we have demonstrated that obese and T2DM patients have decreased circulating omentin levels, but unchanged mRNA expression of omentin in SCAT. Our results suggest that the increase of circulating omentin levels together with the reduction of its expression in SCAT after LSG could contribute to surgery-induced metabolic improvements and sustained reduction of body weight.

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Table 1. Clinical, hormonal and metabolic characteristics of the study subjects.

	Controls	**OB**	**T2DM**
Number (n)	26	37	11
Age (years)	42.8 ± 2.17	49.2 ± 1.87	56.9 ± 2.83
BMI (kg/m2)	22.5 ± 0.35	**41.5 ± 0.82***	**52.6 ± 2.59*°**
CRP (mg/l)	0.2 ± 0.07	**1.1 ± 0.15***	**2.3 ± 0.50*°**
Fasting insulin (mIU/l)	18.1 ± 1.71	**38.7 ± 2.76***	**37.8 ± 4.43***
Fasting blood glucose (mmol/l)	4.8 ± 0.07	**6.1 ± 0.39***	**9.3 ± 1.10*°**
HbA1c (% IFCC)	3.7 ± 0.08	**4.7 ± 0.31***	**7.3 ± 0.55*°**
HOMA-IR index	3.9 ± 0.44	**10.4 ± 1.17***	**11.6 ± 2.19***
Total cholesterol (mmol/l)	4.9 ± 0.14	5.1 ± 0.21	4.5 ± 0.23
LDL-cholesterol (mmol/l)	2.1 ± 0.13	**2.9 ± 0.19***	**2.6 ± 0.22***
HDL-cholesterol (mmol/l)	2.4 ± 0.12	**1.4 ± 0.07***	**0.9 ± 0.04*°**
Triglycerides (mmol/l)	0.9 ± 0.08	**1.9 ± 0.20***	**1.9 ± 0.19***
Leptin (ng/ml)	18.8 ± 3.99	**47.7 ± 2.72***	**64.5 ± 6.95*°**
Adiponectin (µg/l)	27.8 ± 2.44	**13.6 ± 1.08***	**14 ± 2.63***
Resistin (ng/ml)	5.7 ± 0.28	6.5 ± 0.43	10.6 ± 2.26
Serum omentin (ng/ml)	565.5 ± 27.74	**397.6 ± 30.36***	**474.9 ± 44.61***

OB – obese non-diabetic, T2DM – type 2 diabetes mellitus.

Values are means ± SEM. Statistical significance is from unpaired t-test or Mann-Whitney *U* test as appropriate. * $p < 0.05$ vs. controls, ° $p < 0.05$ vs. obese non-diabetic patients

Table 2. Obese non-diabetic and type 2 diabetes mellitus patients: the effect of VLCD

	Obese non-diabetic		**T2DM**	
	Obese before VLCD	**Obese after VLCD**	**T2DM before VLCD**	**T2DM after VLCD**
Number (n)	10	10	11	11
Age (years)	60.3 ± 3.19	60.3 ± 3.19	56.9 ± 2.83	56.9 ± 2.83
BMI (kg/m2)	44.5 ± 1.75	**42.7 ± 1.63***	52.6 ± 2.59	**49.4 ± 2.34°**
CRP (mg/l)	1.4 ± 0.35	1.2 ± 0.27	2.3 ± 0.50	**1.4 ± 0.36°**
Fasting insulin (mIU/l)	35.7 ± 5.09	33.5 ± 4.19	37.8 ± 4.43	37.4 ± 6.78
Fasting blood glucose (mmol/l)	7.4 ± 1.19	6.0 ± 0.61	9.3 ± 1.09	**6.7 ± 0.75°**
HbA1c (% IFCC)	5.9 ± 0.90	Not assessed	7.3 ± 0.55	Not assessed
HOMA-IR index	11.5 ± 3.09	7.9 ± 1.90	11.6 ± 2.19	6.9 ± 1.76
Total cholesterol (mmol/l)	4.8 ± 0.32	**3.9 ± 0.28***	4.5 ± 0.23	**3.8 ± 0.19°**
LDL-cholesterol (mmol/l)	2.3 ± 0.39	**1.8 ± 0.28***	2.6 ± 0.22	2.2 ± 0.19
HDL-cholesterol (mmol/l)	1.5 ± 0.24	**1.3 ± 0.25***	1.0 ± 0.04	**0.9 ± 0.04°**
Triglycerides (mmol/l)	2.4 ± 0.64	1.8 ± 0.31	1.9 ± 0.19	1.7 ± 0.19
Leptin (ng/ml)	48.4 ± 4.89	41.0 ± 6.09	64.5 ± 6.95	53.6 ± 7.11
Adiponectin (µg/l)	15.0 ± 2.39	15.7 ± 2.51	14 ± 2.63	12.9 ± 1.77
Resistin (ng/ml)	6.9 ± 0.73	7.4 ± 1.10	10.6 ± 2.26	9.0 ± 1.20
Serum omentin (ng/ml)	403.8 ± 57.68	406.9 ± 54.20	474.9 ± 44.61	485.7 ± 42.88

Values are means ± SEM. Statistical significance is from paired t-test or Wilcoxon Signed-Rank test as appropriate. * $p < 0.05$ vs. OB before VLCD ° $p < 0.05$ vs. T2DM before VLCD

Table 3. Obese non-diabetic: the effect of a 3-month physical activity.

	Obese before PA	**Obese after PA**
Number (n)	13	13
Age (years)	49.5 ± 2.21	49.5 ± 2.21
BMI (kg/m2)	38.2 ± 1.02	**35.4 ± 1.03°**
CRP (mg/l)	0.7 ± 0.19	0.5 ± 0.14
Fasting insulin (mIU/l)	46.7 ± 5.14	**38.9 ± 4.54°**
Fasting blood glucose (mmol/l)	5.8 ± 0.18	**5.4 ± 0.13°**
HbA1c (% IFCC)	4.1 ± 0.14	3.9 ± 0.11
HOMA-IR index	12.1 ± 1.41	**8.0 ± 1.47°**
Total cholesterol (mmol/l)	5.4 ± 0.36	5,4 ± 0.29
LDL-cholesterol (mmol/l)	3.3 ± 0.33	3.29 ± 0.27
HDL-cholesterol (mmol/l)	1.3 ± 0.07	1.4 ± 0.07
Triglycerides (mmol/l)	1.7 ± 0.18	1.5 ± 0.19
Leptin (ng/ml)	39.9 ± 4.99	**31.0 ± 3.86°**
Adiponectin (µg/l)	10.9 ± 1.71	10.9 ± 2.06
Resistin (ng/ml)	4.9 ± 0.44	5.9 ± 0.46
Serum omentin (ng/ml)	432.8 ± 56.46	412.1 ± 58.25

Values are means ± SEM. Statistical significance is from paired t-test or Wilcoxon Signed-Rank test as appropriate. ° $p < 0.05$ vs. OB before PA

Table 4. Obese non-diabetic subjects: the effect of laparoscopic sleeve gastrectomy.

	Obese non-diabetic			
	Obese before LSG	**Obese after LSG_6m**	**Obese after LSG_12m**	**Obese after LSG_24m**
Number (n)	13	13	13	13
Age (years)	41 ± 2.1			
BMI (kg/m2)	42.5 ± 1.08	**33.2 ± 1.09***	**31.6 ± 1.14*°**	**33.2 ± 1.68*□**
CRP (mg/l)	1.3 ± 0.24	1.0 ± 0.25	**0.9 ± 0.2***	**0.7 ± 0.10***
Fasting insulin (mIU/l)	32.8 ± 3.31	**24.3 ± 2.45***	**25.7 ± 4.12***	30.5 ± 6.52
Fasting blood glucose (mmol/l)	5.4 ± 0.48	4.9 ± 0.21	4.9 ± 0.21	**5.2 ± 0.19□**
HbA1c (% IFCC)	4.2 ± 0.31	**3.7 ± 0.16***	**3.7 ± 0.17***	**4.0 ± 0.19□**
HOMA-IR index	7.9 ± 1.69	**5.2 ± 0.81***	**5.5 ± 1.35***	**7.1 ± 1.56 □**
Total cholesterol (mmol/l)	5.1 ± 0.4	4.7 ± 0.39	4.9 ± 0.34	4.8 ± 0.36
LDL-cholesterol (mmol/l)	3.1 ± 0.3	2.9 ± 0.33	**2.7 ± 0.3***	**2.4 ± 0.27***
HDL-cholesterol (mmol/l)	1.3 ± 0.08	1.4 ± 0.08	**1.6 ± 0.09*°**	**1.7 ± 0.12***
Triglycerides (mmol/l)	1.7 ± 0.22	**1.4 ± 0.16***	**1.3 ± 0.16***	1.4 ± 0.29
Leptin (ng/ml)	54.8 ± 3.33	**21.9 ± 3.01***	**21.8 ± 3.93***	**28.2 ± 5.95*□**
Adiponectin (µg/l)	15.3 ± 1.5	**19.0 ± 1.86***	**21.9 ± 2.14*°**	**26.1 ± 3.12*□**
Resistin (ng/ml)	7.8 ± 0.78	8.2 ± 0.73	7.3 ± 2.78	6.6 ± 0.5
Serum omentin (ng/ml)	358.1 ± 45.9	**455.7 ± 34.79***	**419.9 ± 41.2***	**449.7 ± 44.75***

Values are means ± SEM. Statistical significance is from paired t-test or Wilcoxon Signed-Rank test as appropriate. * P < 0.05 vs. OB before LSG ° p< 0.05 vs. OB after LSG_6m □ p< 0.05 vs. OB after LSG_12m

Table 5. Multiple linear regression analyses performed with *Δ* serum omentin as dependent and the other anthropometrical and biochemical parameters as independent variables in the group of obese non-diabetic patients undergoing LSG.

	Obese after LSG_6m		**Obese after LSG_12m**		**Obese after LSG_24m**	
	β	p	β	p	β	p
Δ BMI (kg/m^2)	**17.3**	**0.049**	2.1	0.60	-19.4	0.33
Δ CRP (mg/l)	13.5	0.71	-13.6	0.72	-131.8	0.19
Δ Fasting insulin (mIU/l)	**-7.1**	**0.01**	0.9	0.97	**11.6**	**0.03**
Δ Fasting blood glucose (mmol/l)	16.7	0.77	68.3	0.84	-68.2	0.6
Δ HbA1c (% IFCC)	6.5	0.63	-21.4	0.28	-51.6	0.63
Δ HOMA-IR index	**-29.6**	**0.03**	-14.7	0.91	-16.7	0.57
Δ Total cholesterol (mmol/l)	-224.4	0.12	120.3	0.61	-295.8	0.07
Δ LDL-cholesterol (mmol/l)	210.5	0.17	-137.5	0.59	328.8	0.09
Δ HDL-cholesterol (mmol/l)	299.3	0.19	-173.2	0.64	109.7	0.65
Δ Triglycerides (mmol/l)	-99.5	0.09	30.5	0.59	-276.7	0.06
Δ Leptin (ng/ml)	1.4	0.44	-0.4	0.88	-1.6	0.58
Δ Adiponectin (µg/l)	9.8	0.3	-4.3	0.7	-1.9	0.92
Δ Resistin (ng/ml)	-11.3	0.2	-14.5	0.72	3.0	0.95

Figure Captions

Figure 1. The effect of laparoscopic sleeve gastrectomy (LSG) on mRNA expression of omentin (ITLN1) in subcutaneous adipose tissue (SCAT) of obese non-diabetic women (n = 13). The patients were examined before surgical intervention and 6, 12 and 24 months after the surgery, respectively. *p < 0.001 vs. before LSG; $^{+}$p < 0.05 vs. at month 6 after LSG.

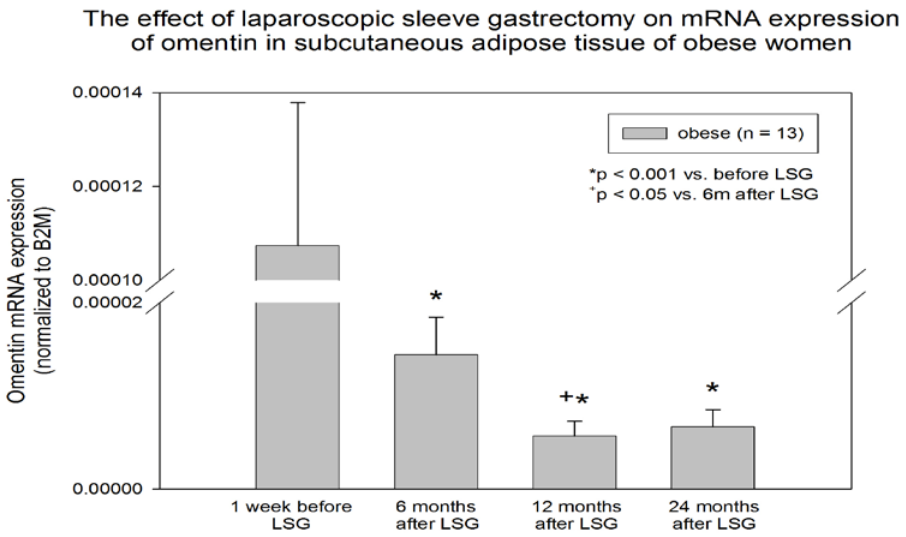

Figure 2. Significant relationships of serum omentin levels with anthropometric and hormonal parameters calculated in a combined population of normal-weight healthy women, obese non-diabetic and diabetic patients.