

RESEARCH ARTICLE

Influence of significant weight loss on serum matrix metalloproteinase (MMP)-7 levels

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ABSTRACT. *Background.* Matrix metalloproteinases (MMPs) and their specific inhibitors (tissue inhibitor of metalloproteinases [TIMPs]), are involved in adipogenesis, angiogenesis and remodeling of extracellular matrix. MMPs and TIMPs have been shown to be associated with various diseases such as neurological disorders, malignancies and cardiovascular disease. MMPs and TIMPs are thought to play a major role in extensive reorganization of the adipose tissue in obesity. *Methods and materials.* To test whether significant weight loss alters circulating MMPs and TIMPs, 18 morbidly obese women, who underwent bariatric surgery for weight loss, were investigated before and one year after surgery in a prospective design study. Body composition, glucose and lipid metabolism parameters were determined in all study subjects before and after weight loss. Circulating MMP-2, -3, -7 and TIMP-1, -2 and -4 serum levels were measured using commercially available, enzyme-linked immunoassays. *Results.* Pronounced weight loss was accompanied by improvements in glucose homeostasis and lipid parameters. In the mean time MMP-2 and MMP-3, as well as TIMP-1, -2 and TIMP-4 concentrations were not affected by significant weight loss, and circulating MMP-7 increased significantly after bariatric surgery, although without reaching the standard levels as determined in 18, lean, healthy women. *Conclusion.* Our data indicate that reduced MMP-7 levels in obesity might be restored by significant weight loss, suggesting that the reorganization of adipose tissue in obesity might be partially reversible by weight reduction. We hypothesize that increased circulating MMP-7 might indicate enhanced adipocyte differentiation in subjects who had undergone bariatric surgery.

Keywords: adipose tissue, matrix metalloproteinase, weight loss, obesity

Matrix metalloproteinases (MMPs) comprise a group of enzymes with particular importance in extracellular matrix (ECM) modulation. Twenty-three different MMPs, with distinct expression patterns and functions, have been described. They are divided into subgroups, *e.g.* collagenases, gelatinases and membrane-type metalloproteinases, according to location, function and structure. Expression levels of MMPs are very low, however, in cases of injury, they can be upregulated in a very short time. Their activities are regulated transcriptionally, by activation of precursors, or by tissue inhibitors of metalloproteinases (TIMPs). Four different TIMPs, with the ability to bind and deactivate MMP molecules, have been described [1]. MMPs influence ECM modulation by degrading ECM components such as collagen or gelatin, by shedding cell surface-associated molecules, and by cleaving a wide range of proteins including other MMPs. Subsequent activation or inactivation of the target molecules leads to tissue breakdown, reorganization and reconstruction as well as it supports cell-matrix and cell-cell interaction [2].

ECM modulation is involved in a variety of physiological and pathophysiological settings: it has been shown to be essential for several fundamental processes such as embryonic development, inflammatory response and wound healing. Furthermore, ECM degradation plays an important role in angiogenesis and adipogenesis [3]. Associations between MMPs and several neurological diseases (*e.g.* Alzheimer disease, multiple sclerosis), and malignancies, particularly with tumor progression and metastasis, have been described. Additionally, MMPs and TIMPs seem to play a major role in development, progression and restenosis in coronary artery disease (CAD), as well as in the expansion of aortic aneurysms and chronic heart failure [2]. As a consequence, plasma MMP-9 concentrations are considered to be a marker for cardiovascular events in patients with CAD. Remarkably, MMP-9 levels have been shown to be influenced by body weight [4, 5]. Various studies have demonstrated an important role for MMPs and TIMPs in adipogenesis and obesity. More specifically, distinct expression patterns of various MMPs and

TIMPs during differentiation of 3T3-L1 adipocytes have been found. Additionally, treatment of preadipocytes with synthetic inhibitors of MMPs suppressed adipocyte differentiation [6, 7]. Maquoi *et al.* found upregulation of MMP-3, -11, -12, -13, and -14 and TIMP-1 mRNA expression in obese mice, whereas expression of MMP-7, -9, -16, and -24 and TIMP-4 was down-regulated in obesity [8]. In another study, mRNA levels of MMP-2, MMP-3, MMP-12, MMP-14, MMP-19, and TIMP-1 were strongly induced in adipose tissue in two genetic models of obesity and in a diet-induced model of obesity when compared with lean littermates. In contrast, MMP-7 and TIMP-3 mRNA expressions were markedly decreased in obesity [6].

MMPs have also been shown to be associated with disturbances in glucose homeostasis: Derosa *et al.* found increased plasma MMP-2, MMP-9, TIMP-1 and TIMP-2 levels in diabetics, which may reflect abnormal extracellular matrix metabolism in diabetes [9]. In accordance with this hypothesis, Boden *et al.* reported that hyperinsulinemia in an euglycemic-hyperinsulinemic clamp, increases MMP-2, MMP-9 and membrane type 1-MMP activities in aortic tissues [10].

The aim of the present study was to elucidate the effect of pronounced weight loss on MMP and TIMP serum levels. We determined circulating serum levels of MMP-2, -3 and -7, as well as TIMP-1, -2 and -4 levels, in morbidly obese subjects before and one year after bariatric surgery. This design enabled us to assess MMP and TIMP levels during a period of fundamental metabolic change in a prospective setting.

METHODS

Subjects

Eighteen morbidly obese women who underwent Swedish adjustable gastric banding surgery were included in the study. Patients were tested before and one year after surgery. Standard values for MMP-7 were determined in 18, lean, healthy women (BMI range: 19-25 kg/m², age range: 20-46 y). Subjects with overt diabetes, other endocrinological disorders, severe renal or hepatic disease, cardiovascular disease, malignancies, neurological disorders, as well as subjects taking hypolipidemic, immunosuppressive or glucose-lowering drugs were excluded from the study. Informed consent was obtained from all study patients. The study protocol was approved by the local ethics committee.

Laboratory measurements

Venous blood was drawn after an overnight fast and plasma or serum was obtained by centrifugation at 3000 r.p.m. for 10 min at 4°C immediately after blood collection. Plasma or serum samples were either used immediately for analysis or were stored frozen at -80 °C. Total cholesterol, high density lipoprotein (HDL)-cholesterol (C), triglycerides (TG) and venous plasma glucose concentrations were measured using commercially available enzymatic kits. Fasting plasma insulin concentrations were

measured by a microparticle enzyme immunoassay on an IMx analyser (Abbott Diagnostics, Abbott Park, IL, USA).

Serum levels of circulating MMP-2, -3 and -7, as well as TIMP-1, -2 and -4, were determined using commercially available, enzyme-linked immuno-sorbent assays (R&D Systems, Wiesbaden, Germany). Insulin sensitivity was estimated by the homeostasis model assessment (HOMA) index [11], which was calculated by the formula:

$$\text{fasting plasma glucose (mmol L}^{-1}) \times \text{fasting plasma insulin } (\mu\text{U mL}^{-1}) / 22.5.$$

Body composition

BMI was calculated as body weight in kilograms divided by height in metres squared. Body composition (lean mass, fat mass) was determined by impedance analysis using an InBody 3.0 Body Composition analyser from Biospace Europe (Dietzenbach, Germany). Measurements were taken in the morning, after an overnight fast.

Abdominal ultrasound studies

Each patient underwent abdominal ultrasonography using a 3.0 MHz curved array transducer and a standard Acuson Sequoia 512 system (Acuson, Mountain View, CA, USA) to determine the degree of liver steatosis, as described elsewhere in detail [12]. Briefly, Level 0 was defined as a normal hepatic echo pattern, level 1 as a slight increase in echo pattern with normal visualization of vessels and diaphragm, level 2 as a moderate increase in echogenicity with reduced visualisation of portal veins and diaphragm, and level 3 as a pronounced increase in hepatic echo pattern with poor visualisation of intrahepatic vessels and posterior right lobe of the liver. To differentiate intra-abdominal from subcutaneous adipose tissue accumulation, subcutaneous and visceral fat diameters were determined as described by Pontiroli *et al.* [13]. Measurements were performed in triplicate.

Statistical analysis

Differences between parameters determined before and after surgery were calculated using Student's paired t test. The significance of differences in means between more than two groups was tested by ANOVA with the Bonferroni correction. Statistical significance was inferred at a two-tailed p-value of less than 0.05. Correlation coefficients were calculated using Pearson's method. Descriptive data are expressed as means \pm SD. SPSS for windows (version 11.0) was used for statistical analysis.

RESULTS

Clinical characteristics

Clinical characteristics of study subjects before and one year after bariatric surgery are shown in *table 1*. As expected, mean BMI and waist-to-hip ratio decreased significantly one year after bariatric surgery. The pronounced weight reduction was mainly due to significant decreases in fat mass, while lean mass, as determined by

Table 1

Clinical characteristics of study population before and one year after bariatric surgery

	Before SAGB	1 year after SAGB	P-value
Age	34 ± 6		
BMI, kg/m ²	41.5 ± 4.1	34.5 ± 3.9	< 0.001
Waist-to-hip ratio	0.79 ± 0.06	0.77 ± 0.07	0.009
Fat mass, kg	55.9 ± 9.8	38.0 ± 9.1	< 0.001
Lean mass, kg	66.9 ± 8.9	62.8 ± 9.5	0.056
Subcutaneous fat diameter, cm	4.6 ± 1.4	3.5 ± 1.1	0.007
Intraabdominal fat diameter, cm	5.3 ± 2.0	2.1 ± 1.5	< 0.001
Glucose, mg/dL	94.8 ± 9.9	93.5 ± 5.3	0.512
Insulin, µU/mL	14.5 ± 5.3	7.6 ± 3.2	< 0.001
Cholesterol, mg/dL	181.8 ± 37.6	176.0 ± 32.2	0.457
HDL-cholesterol, mg/dL	50.9 ± 9.2	54.8 ± 10.4	0.030
LDL-cholesterol, mg/dL	107.3 ± 30.8	104.8 ± 28.3	0.724
Triglyceride, mg/dL	118.6 ± 38.3	82.4 ± 34.3	0.001
HOMA Index	3.41 ± 1.46	1.79 ± 0.86	< 0.001

SAGB: Swedish adjustable gastric banding.

Statistical significance determined by paired samples t-test. Values are presented as mean ± SD.

BIA, was reduced only moderately. The mean decrease in the visceral fat area was more pronounced than the decline in the subcutaneous fat area (table 1). Furthermore, the benefits of weight loss included improvements in lipid profile and decreases in the grade of steatosis as determined by ultrasound (grade of hepatic steatosis: before weight loss: 1.4 ± 1.0, after weight loss: 0.8 ± 0.8, p = 0.001). Additionally, weight loss was accompanied by improvement in glucose homeostasis. Insulin sensitivity, as determined by the HOMA index, increased significantly after bariatric surgery.

MMP and TIMP concentrations in obese subjects

Concentrations of MMP-2, MMP-3, MMP-7, as well as of TIMP-1, TIMP-2 and TIMP-4, were determined before and one year after bariatric surgery. MMP-2 and MMP-3 levels remained unchanged after significant weight loss (MMP-2:

before weight loss: 182.08 ± 25.78 ng/mL, after weight loss: 180.49 ± 25.39 ng/mL; MMP-3: before weight loss: 8.65 ± 3.15 ng/mL, after weight loss: 8.97 ± 4.01 ng/mL). As shown in figure 1, MMP-7 increased significantly with weight loss from 2.38 ± 0.42 ng/mL to 2.61 ± 0.45 ng/mL (p = 0.04). In contrast to MMP-7, TIMP-1, TIMP-2 and TIMP-4 concentrations were similar before and after pronounced weight loss (TIMP-1: before weight loss: 170.61 ± 27.99 ng/mL, after weight loss: 160.26 ± 13.62 ng/mL; TIMP-2: before weight loss: 94.11 ± 8.67 ng/mL, after weight loss: 96.44 ± 10.02 ng/mL; TIMP-4: before weight loss 1.54 ± 0.35 ng/mL, after weight loss: 1.67 ± 0.53 ng/mL).

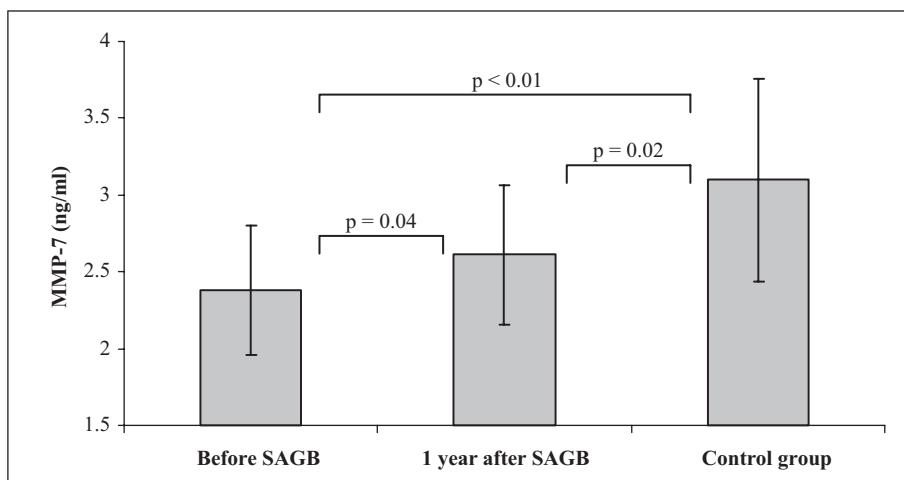
MMP-7 levels in lean, healthy subjects

In order to determine standard values for circulating MMP-7 in women, we measured its concentration in 18, lean, healthy women (MMP-7: 3.10 ± 0.66 ng/mL, BMI: 21 ± 1.5 kg/m², age: 29.2 ± 5.9 y). Mean MMP-7 levels were significantly higher in lean subjects when compared to obese subjects both before and after bariatric surgery (p < 0.01) (lean subjects *versus* obese subjects before bariatric surgery: p < 0.01 and *versus* obese subjects after weight loss: p = 0.02).

Correlations

As shown in table 2A, prior to surgery, MMP-7 serum levels correlated significantly with intra-abdominal fat diameter, as well as with the grade of hepatic steatosis and HDL-C. The significant correlations seen between MMP-7 and intra-abdominal fat diameter, hepatic steatosis and HDL-C, disappeared after bariatric surgery. MMP-3 levels correlated with glucose levels, and circulating TIMP-1 levels correlated with intra-abdominal fat diameter and BMI. Additionally, TIMP-4 serum levels correlated with HDL-C.

One year after bariatric surgery, MMP-2 levels correlated with waist-to-hip ratio, and MMP-3 serum levels correlated with BMI and fat mass, respectively. Circulating TIMP-4 serum levels also correlated with fat mass, and TIMP-1 serum levels correlated with intra-abdominal fat mass (table 2B).

**Figure 1**

Matrix metalloproteinase (MMP)-7 serum levels before and after weight loss.
SAGB: Swedish adjustable gastric banding.

Table 2

Significant Pearson correlation coefficients before (A) and after (B) weight loss

		R	P-value
A)			
MMP-3	Glucose	0.57	0.02
MMP-7	Intra-abdominal fat diameter	0.47	0.05
	Grade of hepatic steatosis	0.56	0.02
	HDL-cholesterol	- 0.74	< 0.01
TIMP-1	Intra-abdominal fat diameter	0.52	0.03
	BMI	0.52	0.03
TIMP-4	HDL-cholesterol	0.65	< 0.01
B)		R	P-value
MMP-2	Waist-to-hip ratio	- 0.56	0.02
MMP-3	Fat mass	0.49	0.04
	BMI	0.61	0.01
TIMP-1	Intra-abdominal fat diameter	0.47	0.05
TIMP-4	Fat mass	- 0.48	0.04

MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinases.

DISCUSSION

Bariatric surgery is one of the most effective options for achieving long-term weight loss in morbidly obese patients, and has become a well-established model with which to study the effects of severe obesity on metabolism, and the reversibility of these changes. It has been shown that the beneficial effects of pronounced weight loss as a consequence of bariatric surgery include not just an amelioration of several metabolic parameters, but also a significant decrease in overall mortality [14-16]. Reduced mortality in these patients might be explained by reductions in several, well-established risk factors such as dyslipidemia, hypertension and disturbances of glucose homeostasis. Additionally, reduced subclinical inflammation, as well as changes in adipocytokine pattern are thought to contribute to increased life expectancy.

Several previous studies have revealed important roles for MMPs and TIMPs in obesity. Determination of MMP and TIMP expression during adipogenesis revealed distinct time-course-adjusted expression patterns. Furthermore, animal models of diet- or genetically-induced obesity confirmed a significant role for MMPs and TIMPs in the differentiation of preadipocytes [6, 17-19]. In addition to these findings, several groups have reported altered MMP patterns in obese patients when compared with lean controls, suggesting alterations, not only in the amount of adipose tissue, but also in adipogenesis and ECM remodeling in obesity [20, 21]. The aim of this study was to elucidate the effect of pronounced weight loss on serum MMP and TIMP levels in obese patients. Recently, our group found significantly decreased MMP-9 levels after pronounced weight loss following bariatric surgery [4].

While MMP-2, MMP-3 and TIMP-1, TIMP-2, and TIMP-4 concentrations remained unaffected by weight loss, MMP-7 levels were found to be significantly increased after bariatric surgery.

MMP-7 is a protein with multifarious and partially opposing functions. It acts as an activator of plasminogen, leads to increased bioavailability of insulin-like growth factor 1

(IGF-1), increases the amount of soluble tumor necrosis factor- α (TNF- α), and is also involved in cell growth and adipocyte differentiation. Furthermore, MMP-7 mediates vasoconstriction and pro-inflammatory stimuli during substrate cleavage [1, 2]. Recently, Chavey *et al.* reported decreased MMP-7 levels in adipose tissue of obese mice, and Maquoi *et al.* showed decreased MMP-7 mRNA levels in the adipose tissue of mice fed with a high fat diet [6, 8]. The replenishment of MMP-7 might be one of the beneficial effects of pronounced weight loss acting by promoting adipocyte differentiation and thus increasing the relative number of mature adipocytes with a more favorable adipocytokine secretion pattern. Concomitantly increased MMP-7 might limit the extent of so-called dysfunctional adipose tissue comprising poorly differentiated (pre)adipocytes, which seem to play a major role in obesity-related disease [22]. The positive correlation between MMP-7 and intra-abdominal fat mass in morbid obesity could suggest that adipose tissue is not only a major target of MMP-7 action but also a major determinant of MMP-7 synthesis. Remarkably, in our study we also found that MMP-7 levels were associated with the grade of hepatic steatosis in morbid obesity. It is well known that development and progression of non-alcoholic, fatty liver disease (NAFLD) is accompanied by extensive ECM remodeling [23], suggesting that MMP-7 may play a significant role in the pathophysiology of NAFLD. Our hypothesis is supported by data from Greco *et al.* who found associations between MMP-7 gene expression and liver fat content [24]. Additionally, Alwayn *et al.* reported beneficial effects of MMP inhibition on hepatic steatosis [25]. Our data suggest that pronounced weight loss partially restores decreased MMP-7 levels in obesity. However, when compared with lean, healthy women, MMP-7 levels were still lower in weight-reduced patients. Differences might be explained by the persistently increased amount of adipose tissue in subjects following bariatric surgery and the lack of NAFLD in our control group. Among others, MMPs activities are strongly influenced by TIMPs. The importance of the inhibition of MMPs in adipogenesis has been shown in several studies [6, 18, 26]. In our study, TIMP levels were unaffected by significant weight reduction. In contrast to circulating TIMP levels, Klein *et al.* recently found decreased TIMP-1 mRNA expression in liver biopsies of patients who underwent gastric bypass surgery, suggesting tissue-specific modulation of TIMP-1 by weight reduction without influencing circulating TIMP-1 levels [27]. Moderately increased TIMP-1 concentrations in obesity have been reported in two studies [20, 28]. We thus hypothesize that oversecretion of TIMP-1 in obesity is not reversible by weight loss. TIMP-4 mRNA expression levels have been shown to be decreased in obese mice. In our study, circulating TIMP-4 remained unchanged one year after bariatric surgery, suggesting that, similarly to TIMP-1, potential alterations of circulating TIMP-4 levels in obesity might not be reversible by weight loss [8, 29]. Underlining the important role of MMPs in adipose tissue, Van Hul *et al.* reported that MMP-2^(-/-) mice on a high fat diet gained less weight than littermates on the same diet and displayed hypertrophic adipocytes [30]. Additionally, a recently published study revealed

increased plasma MMP-2 levels in obese subjects [21]. Remarkably, in our study, serum MMP-2 levels were similar before and one year after bariatric surgery. We conclude from our results that either alterations in MMP-2 metabolism are irreversible in obesity, or the contribution of adipose tissue to secreted MMP-2 was low in these patients. Similar results were found for MMP-3 in our study. MMP-3 exhibits proinflammatory effects by modulating TNF- α , a pro-inflammatory cytokine that is known to be increased in obese patients [31, 32]. On the other hand, MMP-3 seems to exert protective effects on atherosclerotic plaques by limiting plaque growth and enhancing plaque stability [33]. Moreover Traurig *et al.* found negative correlations between MMP-3 expression levels and body fat mass in Pima Indians [34].

In summary, in the present study we found that serum MMP-7 levels increase with the decline of body weight, suggesting that decreased MMP-7 levels found in obesity are reversible by weight loss. We hypothesize that increases in MMP-7 levels could indicate enhanced adipogenesis in subjects with pronounced weight loss, which could explain changes in adipocytokine secretion patterns in these patients. In contrast to MMP-7, MMP-2 and -3 and TIMP-1, -2 and -4 levels were unaffected by weight loss.

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