**SERUM CYSTATIN C IN RELATION TO FAT MASS LOSS AFTER BARIATRIC SURGERY**

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Serum cystatin C concentration, generally accepted as renal function marker, is associated with cardiovascular risk and metabolic syndrome. Recent studies indicate that cystatin C increases in human obesity and that adipose tissue contributes to enhanced serum cystatin C concentration in obese subjects.

**The aim of the study** was to assess whether a reduction in body and fat mass after bariatric surgery has any impact on serum cystatin C concentrations.

**Material and methods.** Serum from 27 obese patients were tested before and 6 months after bariatric surgery. Twenty healthy subjects with normal body weight served as controls. Serum cystatin C concentrations were assayed by ELISA.

**Results.** Serum cystatin C concentrations were significantly higher in obese patients compared with non-obese subjects. Decrease of body and fat mass after bariatric surgery resulted in improvement of several parameters associated with cardiovascular risk and metabolic syndrome, like serum lipids, blood pressure and insulin sensitivity. Surprisingly the mean postoperative serum cystatin C concentration was not significantly different from that before surgery. Serum creatinine and GFR also remained unchanged.

**Conclusion.** The results presented here suggest that serum cystatin C concentration is not tightly associated with body and fat mass loss in obese patients after bariatric surgery.

**Key words:** bariatric surgery, cystatin C, fat mass, obesity, weight loss

Cystatin C – a natural inhibitor of cysteine proteases, is a low molecular weight (approx. 13 kDa), nonglycosylated, basic protein, synthesized by all nucleated cells and secreted into body fluids such as blood plasma, cerebrospinal fluid, urine, saliva, tears, and milk (1). Cystatin C has several biological functions including the regulation of extracellular proteolysis (as an inhibitor of proteases) and the modulation of the immune system (1). Several studies indicate that serum cystatin C concentration could be sensitive marker for early and mild changes in the glomerular filtration rate (GFR) used for the determination of renal function (2-6). However, some data indicates that serum cystatin C concentration should not be considered as the best marker of renal function (7).

Serum cystatin C is also associated with cardiovascular risk factors and metabolic syndrome (8-12), as well as with inflammatory markers like CRP and IL-6 during the course

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Serum cystatin C in relation to fat mass loss after bariatric surgery

of atherosclerosis (13). Several recently reported studies indicate that increased body weight and obesity are associated with elevated serum cystatin C concentrations (13-17). For instance, a positive correlation between serum cystatin C concentrations and BMI has been reported (13, 14, 15). Elevated serum cystatin C concentrations are associated with a higher waist circumference and with increased body fat percentages in healthy subjects (2, 16). Recently published data suggests that adipose tissue may contribute to elevated serum cystatin C concentrations in human obesity (18). If this is the case, a reduction in fat mass induced by bariatric surgery should lead to a decrease in serum cystatin C concentrations.

The aim of the present study was to further elucidate the role of cystatin C in obesity and to test whether elevated serum cystatin C concentrations in obese humans is reversible by pronounced body and fat mass loss after bariatric surgery.

MATERIAL AND METHODS

Twenty seven obese patients (10 males and 17 females BMI = 47 ± 2), 23-62 years of age (mean age 40 ± 2.1 years), underwent vertical banded gastroplasty at the Department of General, Endocrine and Transplant Surgery (Medical University of Gdansk, Poland). The inclusion criteria were: no clinical evidence of endocrine, cardiac, kidney or hepatic diseases. Patients had anthropometric and laboratory parameters checked before surgery and 6 months after surgery. Smokers were excluded from the study. Twenty healthy volunteers (10 males and 10 females, BMI = 24 ± 0.7), 23-58 years of age (mean age 37 ± 1.9 years) formed the control group. After overnight fast, blood specimens were obtained for further assays. The investigation conformed to the Declaration of Helsinki of the World Medical Association and was approved by the Medical University of Gdansk Ethics Committee. All patients signed an informed consent form for the investigation.

Serum cystatin C concentrations were determined using the Human Cystatin C ELISA Kit from BioVendor (Czech Republic). Serum insulin concentrations were analyzed by radioimmunoassay technique (19). Serum carbonyl groups were assayed as described previously (20). Serum glucose concentrations were measured by enzymatic method (Sigma, St Louis, MO). Serum C-reactive protein (CRP), white blood cells, creatinine, triacylglycerols, total cholesterol as well as HDL-cholesterol concentrations were assayed at the Central Clinical Laboratory of Medical University of Gdansk. The estimated glomerular filtration rate (eGRF) was calculated using the method described by Levey et al. (21). Serum LDL cholesterol concentrations were calculated as described by Friedewald et al (22). The homeostasis model assessment score (HOMA) was calculated according to the following formula: fasting glucose (mM) x fasting insulin (μU/L) / 22.5, as described by Matthews at al (23). Fat mass was measured using the Bodystat 1500 unit (Bodystat Ltd., Douglas, Isle of Man, United Kingdom).

Statistical analysis was performed using Microsoft Excel and STATISTICA. The statistical significance of the differences between studied parameters in patients before and after bariatric surgery was assessed by the paired t-test. The statistical significance of the differences between obese patients and controls was assessed by the two-tailed t-test. The linear regression coefficient was calculated to assess the correlation between selected parameters in obese subjects before and after surgery. The data are presented as mean ± SEM.

RESULTS

As expected, serum cystatin C concentrations were significantly higher in obese patients in comparison to healthy subjects of normal weight (fig. 1A). Moreover, serum cystatin C concentrations in obese patients before surgery weakly correlated with BMI (r = 0.44, p = 0.05). A significant reduction in fat mass (fig.1B) and BMI (fig.1C) 6 months after bariatric surgery resulted in a decrease in serum leptin concentrations (tab. 1) and improvement in serum glucose and lipids concentrations (tab. 1). Moreover, significant decreases in blood pressure (both systolic and diastolic), serum CRP, white blood cells count, and carbonyl groups were also found (tab. 1). Surprisingly, the mean (for all 27 patients studied) postoperative serum cystatin C concentrations were slightly higher as compared to the preoperative state (fig. 1A). However, the differences did not reach statistical significance. In 9 (33%) of the studied patients serum cystatin C concentrations significantly decreased after
bariatric surgery. However, despite the significant decrease in body and fat mass, in 12 (45%) patients, increases in serum cystatin C concentrations were observed. Bariatric surgery, despite reducing fat mass, had no significant effect on serum cystatin C concentrations in 6 patients.

Collectively, in approximately 70% of patients serum cystatin C concentrations increased or did not change after bariatric surgery. Serum creatinine concentrations and eGFR were slightly higher in obese patient before surgery as compared with controls, however, these parameters were still in their normal ranges. Bariatric surgery did not significantly affect serum creatinine concentrations and eGFR (tab. 1). Moreover, no correlation between serum creatinine concentrations (or eGFR) and serum cystatin C concentrations before and after bariatric surgery was found when all 27 patients were analyzed. Similarly, in subgroups which displayed elevated (12 patients) or lowered (9 patients) serum cystatin C concentrations as compared to the preoperative group, no correlation between serum creatinine (or GFR) and serum cystatin C was found.

DISCUSSION

Bariatric surgery is a very effective procedure for long-term weight loss in morbidly obese patients, leading not only to a significant reduction in body mass, but also to the amelioration of obesity associated diseases. This is probably due to the improvement of several parameters including plasma lipids and glucose concentrations, blood pressure, low grade inflammation and oxidative stress (20, 24, 25).

The results presented here indicate that plasma triacylglycerol concentrations decreased significantly after bariatric surgery. Moreover, HDL-cholesterol concentrations significantly increased. The ratio of LDL-cholesterol/HDL-cholesterol significantly decreased 6 months after bariatric surgery (tab. 1). Taken together, these results suggest that weight loss induced by bariatric surgery exerts beneficial effects on plasma lipids concentrations. Fasting glucose and insulin concentrations decreased after bariatric surgery, leading to significant improvements in insulin sensitivity determined by HOMA index (tab. 1). Blood pressure (both diastolic and systolic) was markedly reduced after weight loss. Additionally, serum CRP concentrations and white blood cell counts decreased 6 months after bariatric surgery, indicating a decrease of the inflammatory status. Altogether, these results indicate the beneficial effect of weight loss induced by bariatric surgery on major cardiovascular risk factors. In general the results presented here are consistent with the findings reported previously (20, 24, 25).

Several studies indicate that increased body weight and obesity are associated with elevated serum cystatin C concentrations (13, 14, 15, 17). The results presented here (fig. 1) are consistent with these findings. Thus, one would expect that serum cystatin C concentrations decreased after bariatric surgery. Surprisingly,
Serum cystatin C in relation to fat mass loss after bariatric surgery

The results presented here indicate that despite the significant effects of bariatric surgery on body and fat mass, as well as on cardiovascular risk factors, this procedure exerts some rather diverse effect on serum cystatin C concentration. In 12 (45%) patients, an increase in serum cystatin C was found. A significant reduction in serum cystatin C was found only in 9 patients (33%). The reasons behind these different effects (increase, decrease and no effect) of bariatric surgery on serum cystatin C concentrations remains unclear.

It should be noted that the percent decrease in body and fat mass were identical in both groups during the one year follow-up (not shown). Thus, changes in body and fat mass cannot contribute directly to differences in changes of serum cystatin C concentrations after surgery. Since emerging evidence indicates that obesity contributes to the development of chronic kidney disease (26), one may suppose that renal failure in obese patients is contributing to the increase in serum cystatin C concentrations. The results presented in Table 1 indicate that serum creatinine concentrations and eGFR were slightly higher, but still within the normal range, in obese patients (before surgery) as compared to control subjects. However, no changes in serum creatinine concentrations and eGFR after bariatric surgery were found. Thus, changes in serum cystatin C concentrations resembled the changes in serum creatinine concentrations. One may therefore suppose, that the increase in serum cystatin C concentrations in obese patients is the consequence of discrete renal dysfunction. The lack of effects of the bariatric surgery on serum creatinine concentrations may partly explain the lack of this treatment effects on serum cystatin C concentrations in a few patient only. However, it cannot explain the increase in serum cystatin C concentrations in some patients after bariatric surgery. Thus, it is likely that factors related to cystatin C production and/or degradation might have a greater influence on serum cystatin C concentrations than renal function. This corroborates the results reported previously, indicating that serum cystatin C concentrations are affected by factors other than renal function alone (15).

### Table 1. Selected laboratory parameters of controls and obese patients before and 6 month after bariatric surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean ± SEM</th>
<th>Obese patients before surgery Mean ± SEM</th>
<th>Obese patients 6 months after surgery Mean ± SEM</th>
<th>P&lt;sub&gt;a&lt;/sub&gt;</th>
<th>P&lt;sub&gt;b&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>20</td>
<td>27</td>
<td>27</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37 ± 1,9</td>
<td>40 ± 2,1</td>
<td>40 ± 2,1</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>72 ± 2,9</td>
<td>132 ± 5,2</td>
<td>102 ± 4,9</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>22 ± 0,36</td>
<td>46 ± 1,6</td>
<td>36 ± 2</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>122 ± 1,1</td>
<td>142 ± 2,3</td>
<td>120 ± 2,6</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>80 ± 0,8</td>
<td>89 ± 1,6</td>
<td>71 ± 1</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>117 ± 4,1</td>
<td>200 ± 16</td>
<td>123 ± 9</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>199 ± 6,1</td>
<td>213 ± 13</td>
<td>200 ± 9</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>58 ± 3,8</td>
<td>41 ± 1,6</td>
<td>51 ± 2</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>125 ± 6,5</td>
<td>130 ± 8,1</td>
<td>123 ± 7,2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LDL/HDL cholesterol</td>
<td>2,1 ± 0,12</td>
<td>3,4 ± 0,13</td>
<td>2,6 ± 0,14</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>6,4 ± 0,53</td>
<td>24 ± 2,7</td>
<td>8,9 ± 0,9</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>12 ± 1</td>
<td>32 ± 3</td>
<td>20 ± 1</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>4,9 ± 0,13</td>
<td>6,7 ± 0,39</td>
<td>5,3 ± 0,23</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>HOMA index</td>
<td>2,6 ± 0,24</td>
<td>11 ± 1,4</td>
<td>5,1 ± 0,4</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Protein carbonyl groups (nmol/mg of protein)</td>
<td>0,62 ± 0,02</td>
<td>0,99 ± 0,03</td>
<td>0,72 ± 0,02</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>44 ± 1,1</td>
<td>42 ± 0,9</td>
<td>43 ± 0,6</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>76 ± 1,1</td>
<td>76 ± 1</td>
<td>77 ± 0,8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C-reactive protein (mg/ml)</td>
<td>2,0 ± 0,44</td>
<td>8,8 ± 1,3</td>
<td>2,9 ± 0,33</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>White blood cells (x 10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>7,3 ± 0,39</td>
<td>9,6 ± 0,86</td>
<td>7,1 ± 0,35</td>
<td>&lt;0,01</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>75 ± 10</td>
<td>86 ± 15</td>
<td>86 ± 9</td>
<td>&lt;0,01</td>
<td>NS</td>
</tr>
<tr>
<td>Estimated GFR</td>
<td>109 ± 20</td>
<td>81 ± 13</td>
<td>79 ± 7</td>
<td>&lt;0,01</td>
<td>NS</td>
</tr>
</tbody>
</table>

P<sub>a</sub> – p value for differences between control subjects and obese patients
P<sub>b</sub> – p value for differences between obese patients before surgery and obese patients 6 months after surgery
BP – blood pressure
GFR – glomerular filtration rate
HOMA – homeostasis model assessment score
Recently, serum cystatin C concentration as marker of glomerular filtration after bariatric surgery was reported (27). These authors have shown that obese patients have higher serum cystatin C concentrations compared with non-obese subjects, and that the increase in cystatin C concentrations is independent of serum creatinine concentrations and eGFR. Moreover, they found that bariatric surgery improves several parameters, but had no effect on serum cystatin C concentrations measured 1 month after surgery. Thus, in this respect our results confirmed these findings (27) and, furthermore extend them. One important advantage of our study is that we studied serum cystatin C concentrations 6 months after bariatric surgery. Thus, it is unlikely that the obtained results were attributed to surgery alone. Moreover, to our best knowledge, no such longitudinal data on the association between fat mass loss and serum cystatin C concentrations after bariatric surgery has been reported.

It has been shown previously that correlation between serum cystatin C and creatinine concentrations as well as between serum cystatin C concentrations and eGFR are weak in subjects with a normal GFR (28, 29). This may explain in part the lack of a correlation between serum cystatin C and creatinine concentrations as well as between serum cystatin C concentrations and eGFR in our patients.

The cystatin C encoding gene is expressed in several human tissues/organs (18). In some tissues/organs, a higher cystatin C gene expression was observed when compared to adipose tissue (18). This suggests that serum cystatin C may have other important sources besides adipose tissue. Therefore, circulating cystatin C concentrations may be influenced not only by the production and release of cystatin C by adipose tissue, but also by other organs. This may explain in part the lack of an association between changes in fat mass and serum cystatin C concentrations after bariatric surgery. The variability of individual changes in serum cystatin C concentrations after surgery (increase in 12, lack of change in 6, or decrease in 9 patients) suggests that cystatin C production and release by adipose tissue and other organs may vary amongst patients after bariatric surgery.

A limitation in our analyses is a relatively small number of patients included in this study. However, this limitation is counterbalanced by our ability to compare serum cystatin C concentrations in the same patients before and after surgery. Further limitations of the study presented here include the fact that we did not measure serum hormones concentrations which in theory could influence serum cystatin C concentration. It has been shown that thyroid hormones (30, 31) and glucocorticoids (32) have a significant impact on serum cystatin C concentrations in humans. It should be noted that our patients did not have characteristic clinical symptoms of thyroid dysfunction and had not been treated with glucocorticoids. Finally it cannot be excluded that serum cystatin C concentration would change in longer period of time after surgery.

In conclusion, the results presented here: a) provide additional evidence that bariatric surgery has beneficial effects on several parameters, including serum lipids and glucose concentrations, blood pressure, serum CRP, carbonyl groups, and leptin concentrations; b) confirm results already reported that obese subjects have elevated levels of circulating cystatin C; and c) do not confirm a strict association between fat mass and serum cystatin C concentration in humans. It is likely that serum cystatin C concentrations could be influenced by many factors and fat mass does not appear to be more influential in regards to this than other factors. Further studies are needed to assess the factors responsible for the different effects of bariatric surgery on serum cystatin C concentrations and the possible consequence of these changes on the clinical and laboratory parameters observed in patients after surgery.

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REFERENCES


