HUMAN SERUM PARAOXONASE ACTIVITY DECREASES AFTER VERTICAL BANDED GASTROPLASTY*

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The aim of the study. Investigation of the effect of vertical banded gastroplasty (VBG), which is an effective method of treating patients with morbid obesity on serum paraoxonase (PON) activity.

Material and methods. Serum PON activity was measured in twenty eight morbidly obese patients 6 and 12 months after surgery. PON activity was also measured in the serum and liver of rats maintained on a restricted diet for one month.

Results. We found that VBG-induced significant reduction in body weight and serum PON activity at 6 and 12 months after surgery. Similar patterns of decreases in serum paraoxonase activity in obese patients after VBG were observed in A, AB and B paraoxonase/esterase phenotypes. After VBG, several clinically relevant events occurred: a) a decrease of serum triacylglycerol concentration was observed; b) no significant changes in total serum cholesterol and LDL-cholesterol concentrations were found; c) serum HDL-cholesterol concentration increased slightly.

Paraoxonase activity in the serum of rats maintained on a restricted diet, which induced approximately 30% and 50% of rat body weight and fat mass loss, respectively, was lower than in control animals.

Conclusions. This study indicates that after VBG significant decreases in serum paraoxonase activity occur in obese subjects. It is likely that less food ingestion and possibly a different type of food consumed by the obese subjects after VBG (compared to type of food consumed before surgery) may contribute to decreases in serum PON activity.

Key words: paraoxonase (PON) activity, vertical banded gastroplasty (VBG), obesity, BMI

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The paraoxonase enzyme family consists of three members (designed: PON1, PON2 and PON3 respectively) encoded by three different genes located on human chromosome 7q21.3-22.1 (1-3). PON1 and PON3 are associated with plasma HDL and are mainly expressed in the liver, while PON2 does not appear to be associated with plasma HDL and is expressed in many tissues (1, 2, 3). The isozymes hydrolyze various toxic aromatic carboxylic esters and organophosphates, including paraoxon (the name paraoxonase – reflects its ability to hydrolyze paraoxon), and display peroxidase, lactonase and arylolesterase activities, which destroy environmental toxins and reduce oxidative stress (1, 2, 3).

PON1, a 43-kDa glycoprotein, calcium dependent-ester hydrolase (EC 3.1.8.1) is believed to exert its antiatherogenic effects by hydrolyzing biologically active oxidized phospho-
The aim of the present study was to examine serum paraoxonase activity 6 and 12 months after VBG.

MATERIAL AND METHODS

The research was conducted in accordance with 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 for this investigation. Patients were admitted to the Department of Surgery (Medical University of Gdansk, Poland) to undergo surgery.

Patients

Twenty one morbidly obese women 39 ± 9.7 years old (range 25 to 61 years old) and 7 morbidly obese men 48 ± 5.1 years old (range 40 to 58 years old) underwent vertical banded gastroplasty (VBG). Inclusion criteria were no clinical evidence of endocrine, cardiac, hepatic, or renal failure diseases. Among the women, approximately 50% had hypertension (>150/90 mm Hg), familial obesity (57%) and 86% were premenopausal. Among the men, 6 had hypertension and 2 had familial obesity. Most of the patients displayed impaired glucose tolerance. Patients had anthropometric and laboratory parameters checked before surgery and at 6 and 12 months after VBG. Smokers were excluded from the study. After overnight fast, blood specimens were obtained for serum lipid (total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol) concentrations and serum paraoxonase activity assays.

Serum paraoxonase activity assay with phenylacetate as substrate (arylesterase activity)

Paraoxonase activity was measured in the medium containing: 100 mM Tris-HCl pH 8.0, 2 mM CaCl₂, and 2 mM phenylacetate. The reaction was initiated by the addition of a corresponding amount of serum (usually 20 ml of serum diluted 30 x) and the rate of phenol generation was determined at 270 nm and 37°C with the use of a continuously recording spectrophotometer (Beckman DU640). Blanks were included to correct for the spontaneous hydrolysis of phenylacetate. Enzymatic activity was calculated using the molar extinction coefficient 1310 M⁻¹cm⁻¹.
The serum paraoxonase activity assay was conducted with paraoxon as the substrate.

Paraoxonase activity was measured in the medium containing: 100 mM Tris-HCl pH 8.0, 2 mM CaCl₂, and 5.5 mM paraoxon (O,O-diethyl-O-p-nitrophenylphosphate). The reaction was started by the addition of a corresponding amount of serum (usually 20 ml) and the rate of nitrophenol generation was determined at 405 nm, 37°C with the use of a continuously recording spectrophotometer (Beckman DU640). Blanks were included to correct for the spontaneous (nonenzymatic) hydrolysis of paraoxon. Enzymatic activity was calculated using the molar extinction coefficient 18300 M⁻¹cm⁻¹.

It should be added that only PON1 exhibits a significant ability to hydrolyze paraoxon, while PON2 and PON3 are devoid of any significant activity with paraoxon as a substrate (23, 24, 25). Thus, paraoxonase activity determined in this paper is synonymous with PON1 activity.

Paraoxonase phenotype distribution

The phenotypic distribution of human serum paraoxonase activity was determined as described by Eckerson et al. (26, 27). Briefly, the ratio of the hydrolysis of paraoxon in the presence of 1 M NaCl to the hydrolysis of phenylacetate was used to assign individuals to one of the three possible phenotypes: A (homozygous low activity), AB (heterozygous activity), and B (homozygous high activity), which are defined by ratios of activity with the ranges 1.21±0.19 for A, 4.68±0.85 for AB, and 8.36±0.7 for B.

Rat serum paraoxonase activity assay

Rat serum paraoxonase activity with phenylacetate or paraoxon as a substrate was assayed as described above.

Rat liver paraoxonase activity

Male wistar rats, weighing approximately 460 g were randomly assigned to either control (6 rats, the animals were given ad libitum access to food and water) or food restricted animals (6 rats obtained every morning 30% of the total amount of food consumed by control group). After one month of such treatment, rats were killed by cervical dislocation between 8:00 and 10:00 am and their trunk blood was collected. Livers were immediately frozen in liquid nitrogen and stored at −80°C until analysis. The experimental protocol was approved by the Medical University of Gdansk Ethics Committee for Animal Experimentation.

0.2 g of rat livers were homogenized in a glass homogenizer with 2 ml of ice-cold homogenization buffer (20 mM Tris/HCl and 0.2% Triton x 100). The homogenate was centrifuged at 30 000 g for 20 minutes in a Sorvall RC-5B centrifuge. Supernatant was used for paraoxonase activity assay with phenylacetate or paraoxon as described above. Protein was estimated by the Lowry method.

Human serum lipids concentration analysis

Serum triacylglycerol, total cholesterol and HDL-cholesterol concentrations were analyzed by standard enzymatic procedures (Boehringer Mannheim, Mannheim, Germany). Serum LDL cholesterol concentrations were calculated according to the formula: (total cholesterol) – (HDL cholesterol) –(triacylglycerol) / 5.

Body fat mass measurement

Human fat mass was measured using the Bodystat 1500 unit (Bodystat Ltd., Douglas, Isle of Man, United Kingdom). Rat epididymal, perirenal and subcutaneous white adipose tissues were collected and rapidly weighed.

Chemicals

The chemicals, paraoxon (O,O-diethyl-O-p-nitrophenylphosphate) and phenylacetate, were obtained from Sigma Chemical Company.

Statistical analysis

Statistical analysis was performed using Microsoft Excel. Significant differences between parameters studied in patients before and after VBG were assessed by paired T-test. The statistical significance of differences between serum and liver paraoxonase activity in control and food restricted rats were assessed by a Student t-test.

RESULTS

Morbidly obese patients underwent VBG and were followed for 1 year. Significant reduction in whole body mass (women: 122±16 kg before surgery versus 87±17 kg 1 year after VBG; men: 143±17 kg before surgery versus...
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103±18kg after VBG) and body fat mass (approximately 50% one year after surgery in both men and women) was found. In most patients, changes in BMI were more pronounced during the first six months than between 6 and 12 months after VBG (fig. 1). Serum paraoxonase activity measured with phenylacetate as the substrate (arylesterase activity) decreased significantly 6 months after VBG. In most patients, changes in paraoxonase activity between 6 and 12 months were more pronounced than during the first 6 months after surgery (fig. 2). One year after VBG, the mean serum paraoxonase activity (with phenylacetate as substrate) reached approximately 25% of the activity observed before surgery (fig. 2).

These results indicate that BMI reduction (by about 35%) and body weight and fat mass reduction (by about 30% and 50% respectively) in obese subjects 12 months after VBG was accompanied by a marked (about 75%) reduction in serum paraoxonase activity. Thus, the reduction of serum paraoxonase activity was relatively larger than the reduction of BMI and fat mass. Essentially, similar patterns of decreases in serum paraoxonase activity in obese patients after VBG was found in A, AB and B paraoxonase/esterase phenotypes (fig. 3 A, B, C respectively). As expected from published data (24, 25), the phenotype B displays higher paraoxonase activity than phenotype AB, and in turn, phenotype AB displays higher paraoxonase activity than phenotype A (fig. 3).

Since serum paraoxonase activity is usually associated with HDL and potentially inhibits serum lipoprotein oxidation (2, 3), we determined the serum lipid concentrations in obese subjects before and after VBG (tab. 1). Six and 12 months after surgery, significant decreases in serum triacylglycerol were observed. No significant changes after VBG were observed in total serum cholesterol and LDL-cholesterol concentrations, while HDL-cholesterol concentration increased (tab. 1). Serum paraoxonase ac-
Activity did not show significant correlation with HDL-cholesterol level in obese patients after VBG (not shown). Moreover, there was no correlation between total serum cholesterol, LDL-cholesterol and triacylglycerol concentrations and serum paraoxonase activity (not shown).

Because our patients consumed approximately 1000 kcal/day (28) after surgical treatment, one could assume that the decrease in food intake may partly explain the decrease in serum paraoxonase activity. To support this hypothesis, we determined paraoxonase activity in the serum and liver from control and food restricted rats. Data presented in fig. 4 indicates that one month of food restriction induced the decrease in the rat body weight (fig. 4A) and adipose tissue mass (fig. 4B). Serum paraoxonase activity with paraoxon as substrate was also significantly lower in food restricted than in control animals (fig. 4C). Essentially, similar results were observed when serum paraoxonase activity was measured with phenylacetate as substrate (not shown). Hepatic paraoxonase activity (measured with either paraoxon or phenylacetate) was not significantly affected by food restriction (not shown).

**Table 1.** Serum lipids concentration in obese patients before and after vertical banded gastroplasty. Number of obese patients n = 28. Data are presented as mean ± SD, and the analysis was performed using a t test.

<table>
<thead>
<tr>
<th></th>
<th>Before surgery</th>
<th>6 months after VBG</th>
<th>12 months after VBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerole g/dl</td>
<td>200 ± 113</td>
<td>131 ± 58 **</td>
<td>93 ± 28 **</td>
</tr>
<tr>
<td>Total cholesterol g/dl</td>
<td>212 ± 43</td>
<td>193 ± 34</td>
<td>204 ± 35</td>
</tr>
<tr>
<td>HDL cholesterol g/dl</td>
<td>41 ± 13</td>
<td>50 ± 15 *</td>
<td>52 ± 9 **</td>
</tr>
<tr>
<td>LDL cholesterol g/dl</td>
<td>127 ± 29</td>
<td>125 ± 28</td>
<td>143 ± 31</td>
</tr>
</tbody>
</table>

* a value of the statistical significance between patients before and after VBG p < 0.05  
** a value of the statistical significance between patients before and after VBG p < 0.01

**Fig. 3B.** Serum paraoxonase activity in obese patients assigned as phenotype AB before and 6 and 12 month after vertical banded gastroplasty. Data are presented as mean ± SD. Number of obese patients assigned as phenotype AB: n = 7, *p<0.05  
**Fig. 3C.** Serum paraoxonase activity in obese patients assigned as phenotype B before and 6 and 12 month after vertical banded gastroplasty. Data are presented as mean ± SD. Number of obese patients assigned as phenotype B: n = 8, *p<0.05

**Fig. 4A.** Body weight of control and food restricted rats. Data are presented as mean ± SD. Number of control and food restricted rats: n = 6, *p<0.01
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DISCUSSION

The main finding of this study is that in obese subjects after VBG significant decreases in serum paraoxonase activity occurs. Moreover, we report that serum paraoxonase activity after VBG decreased more than expected from the amount of reduction in fat and BMI. Thus, these data suggest that the decrease in serum paraoxonase activity after VBG is not directly linked to weight loss (or to fat loss). In this respect, our data are in agreement with those reported by Ferre et al. (29), who found no association between serum paraoxonase activity and BMI. However, our data are in contrast to a report by Uzun et al. (21), who found a negative correlation between serum PON1 activity and BMI after Swedish adjustable gastric banding. This discrepancy may be explained by a different amount and type of food intake after surgery and/or difference in the ethnic background of the patients.

At present, we can only speculate on the mechanism of the decline in serum paraoxonase activity after VBG. Considering that after VBG, most of our patients were placed on a 1000 kcal/day, high fat diet, one can suppose that the decrease in food intake could partly explain the decrease in serum paraoxonase activity. Data presented in this paper indicate that rat serum paraoxonase activity is lower in rats kept on food restricted diets than in control animals (fig. 4C). However, food restriction did not significantly affect hepatic paraoxonase activity (not shown). Liver was chosen to assay paraoxonase activity because the majority of PON1 is synthesized and secreted by this organ (1, 2, 3). In this respect, our data are essentially similar to a recent report by Thomas-Moya et al. (30), who found that caloric restriction (40% energy restriction for 14 weeks) caused decreases in serum paraoxonase activity in rats. Serum paraoxonase activity in strain B6 mice fed high fat diet (atherogenic diet) also decreased (7). In humans, low serum paraoxonase activity was reported to be associated with a high vegetable intake (31, 32). Thus, the data discussed above suggest that the amount and type of food consumed could influence serum paraoxonase activity in obese patients after VBG.

In addition to a significant reduction of BMI, VBG results in an improvement of serum lipid profiles including a significant decrease in serum triacylglycerol and increase in HDL-cholesterol concentration (tab. 1). No significant changes after VBG were found in total serum cholesterol and LDL-cholesterol concentrations. These results are essentially similar to previous reports (28, 33). In the healthy population, serum paraoxonase activity correlated with HDL-cholesterol (34). The lack of correlation between serum paraoxonase activity and HDL-cholesterol concentration was observed under some pathological conditions, especially in the diabetic population (34). Moreover, si-
significant decreases in serum paraoxonase activity was also observed during two weeks after laparoscopic cholecystectomy (35), but HDL-cholesterol did not change in the days following surgery (35). Our results indicate that serum paraoxonase activity did not show significant correlation with HDL-cholesterol levels in obese patients after VBG (not shown). The lack of correlation between serum HDL-cholesterol concentration and paraoxonase activity in obese patients after VBG is not clear. It is possible that this is due to a perturbation of the interaction between PON1 and HDL, as has been suggested for some pathological conditions (34). HDL represents a highly heterogeneous population and PON 1 specifically associates with HDL containing apoJ (36). Thus, we cannot exclude the fact that HDL contains less apoJ after VBG.

In obese subjects, mortality is higher mainly due to the increased incidence of cardiovascular disease (37). As mentioned, weight reduction after bariatric surgery has a positive effect on many metabolic parameters (28, 33). Assuming that high serum paraoxonase activity has some beneficial effects including a cardioprotective effect, one can conclude that the decrease in serum paraoxonase activity after VBG is a rather unfavorable phenomenon. The reduction in PON1 activity could negatively affect the atheroprotective properties of HDL. This is probably partly balanced by the improvement in serum lipid profile (tab. 1) and in body and fat mass. Overall, despite the decrease in serum paraoxonase activity after VBG, bariatric surgery seems to be a safe and beneficial procedure for the treatment of morbidly obese subjects. We are in the course of studying the clinical significance of these findings in terms of antiatherogenic action of paraoxonase.

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COMMENTARY

Obesity has been recognized by the World Health Organization (WHO) as a social disease and a twenty-first century epidemic. The number of obese patients in the Far East has equaled that of malnourished human beings. In Poland, the number of patients with a Body Mass Index (BMI) >40 is nearly 250,000. Pathogenic obesity is accompanied by respiratory and circulatory diseases, type 2 diabetes mellitus, arterial hypertension, infertility, and possible neoplastic proliferation. Thus, obesity is not only an aesthetic problem but also negatively influences the social health care system, requiring economic and organizational effort to surpass the above-mentioned problem. Prophylaxis targeted toward obesity has been initiated around the world, including in Europe and Poland. Unfortunately, the above-mentioned actions are not popular in our country, their effects being non-satisfactory. Similarly, dietary,
pharmacological, and psychogenic treatment has proven unsatisfactory. Surgical management continues to be the most favorable method of therapy.

The presented study evaluated the effect of restrictive gastrectomy on body weight reduction and its influence on the activity of serum aryl esterase. The above-mentioned effects were accompanied by increased HDL cholesterol levels. Similar results were obtained in fasting rats. The investigations demonstrated that surgical limitation of nutrition not only decreased body weight, but also reduced triglyceride levels by 75%. This might be evidence of the favorable effect of bariatric operations on lipid metabolism and atherosclerosis prevention.

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