Non-conventional markers of atherosclerosis before and after gastric banding surgery

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Aims
Obesity and type 2 diabetes are associated with increased cardiovascular risk and elevation of traditional and non-traditional risk markers. As bariatric surgery reduces overweight and improves metabolic derangement, we examined a cluster of established and emerging cardiovascular risk factors, such as soluble CD40 ligand (sCD40L) and lipoprotein-associated phospholipase A2 (Lp-PLA2), which might improve prediction of future cardiovascular events because of their more direct involvement in plaque destabilization.

Methods and results
Obese patients \([n = 32, \text{body mass index (BMI)}\, 46.1 \pm 5.9 \, \text{kg/m}^2]\) underwent clinical examinations and blood sampling for measurement of glucose and lipid parameters as well as non-traditional cardiovascular risk markers, i.e. high-sensitivity C-reactive protein, plasminogen activator inhibitor-1 (PAI-1), soluble cellular adhesion molecules (CAM), MMP-2, MMP-9, CD40L, and Lp-PLA2 before and after 1 year following laparoscopic adjustable gastric banding (LAGB), respectively. In patients undergoing LAGB, blood pressure \((P < 0.0001)\) and blood glucose \((P = 0.02)\) were significantly lowered by approximately 16% as well as triglyceride levels by approximately 29% \((P = 0.002)\). In addition to a decrease of the inflammatory and pro-thrombotic marker PAI-1 \((P = 0.001)\), CAMs, and MMP-9 \((P = 0.004)\) were reduced, whereas no change was observed for plasma levels of MMP-2, sCD40L, and Lp-PLA2 after LAGB, respectively. Individual changes in (ICAM-1) intercellular adhesion molecule-1 (ΔICAM-1) were related to changes in insulin \((Δ\text{fasting insulin})\) before and after LAGB \((r = 0.36\) and \(r = 0.38; \text{both}\, P = 0.04)\). E-selectin correlated positively with changes in BMI \((r = 0.38; P = 0.04\) and \(r = 0.36; P = 0.05)\), while Lp-PLA2 concentration was negatively correlated with BMI \((r = −0.41; P = 0.02)\) after 1 year. Changes were comparable in both overweight diabetic and non-diabetic subjects.

Conclusion
LAGB not only induced weight loss but also an improvement in the subclinical pro-inflammatory state. However, concentrations of most of the non-traditional risk factors for plaque instability, i.e. MMP-9, sCD40L, and Lp-PLA2 remained unchanged.

Keywords
PAI-1 • Adhesion molecules • Lp-PLA2 • CD40 ligand • Morbid obesity • Bariatric surgery • Gastric banding

Introduction
In addition to several cardiovascular risk factors, such as atherogenic dyslipidaemia, hypertension, and glucose intolerance, obesity also independently increases the risk for the development of premature atherosclerosis.1–3 Adipose tissue is an endocrine organ, which not only contributes to appetite and body weight regulation but also to the development of diabetes mellitus type 24 as well as to chronic sub-clinical inflammation.5 Fat tissue serves as a major source of pro-inflammatory adipokokines, i.e. leptin, plasminogen activator inhibitor-1 (PAI-1), or C-reactive protein, which cause endothelial dysfunction and may promote

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Atherosclerosis. Inflammatory cytokines increase vascular permeability, enhance the attachment to and migration of monocytes into the vessel wall and induce the expression of cellular adhesion molecules (CAMs) on the endothelial surface. Moreover, an imbalance of haemostatic factors, like PAI-1, may contribute to the progression and complications of atherosclerosis by promoting thrombus formation in ruptured plaques.

Recently, several non-traditional cardiovascular risk markers have attracted attention as they may predict future cardiovascular events and even mortality in otherwise healthy humans: lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) mass and activity are increased in subjects at risk for coronary heart disease (CHD) and this enzyme generates pro-inflammatory and pro-atherogenic compounds in the vessel wall from oxidized LDL like free fatty acids and phosphatidylcholine. However, it is not associated with a systemic inflammatory response and may thus be more specific for inflammation in the vasculature. Therefore, high Lp-PLA$_2$ mass and increased activity has been assumed to identify patients at high cardiovascular risk. Soluble CD40 ligand (sCD40L) is elevated in patients with unstable plaques, chronic heart failure, or diabetes mellitus. Increased concentrations of pro-atherogenic matrix metalloproteinases (MMP), MMP-2 and MMP-9, were found in patients with severe atherosclerosis and may contribute to plaque destabilization in vivo.

Bariatric surgery for severe obesity is associated with reduction of body weight and comorbidities as well as with overall mortality. The aim of this study was to investigate changes of both traditional and emerging cardiovascular risk markers, partially associated with plaque instability, after laparoscopic adjustable gastric banding (LAGB) in a severely obese population.

**Methods**

**Patients**

Between January 2000 and December 2000 consecutive obese patients (n = 510) who had been referred from general practitioners, other departments of the hospital, or the surgical department of the Sozialmedizinisches Zentrum Ost, Vienna, to our obesity clinic, were screened for a LAGB procedure. As LAGB is routinely performed and reimbursed by the general healthcare system in Austria only in severely obese patients, those (n = 38) with a body mass index (BMI) above 40 kg/m$^2$ were included and a preoperative clinical evaluation was performed. As part of a regular routine metabolic and nutritional follow-up, various biochemical tests were performed pre-operatively and were repeated 6 and 12 months after surgery. Five patients refused follow-up visits and one patient died because of septic shock not related to the surgical procedure. Informed consent was obtained from all patients before surgery and the study has been carried out in accordance with the Helsinki declaration.

Thirty-two obese patients (females/males: 29/3, mean age: 41.6 ± 9.0 years) without a history or clinical signs of cardiovascular disease were included in this study and returned to the follow-up visits at 6 and 12 months after LAGB. Pre-operatively, screening tests were performed in order to exclude patients with endocrine diseases or general conditions not related to obesity that may reduce longevity. An oral glucose tolerance test (OGTT; 75 g) was performed in all patients to identify unknown impairment of glucose metabolism, classified according to the WHO definition as normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or diabetes mellitus type-2 (DM2), respectively, at the start and during follow-up visits to detect changes in glucose metabolism during weight loss. Because patients with overt diabetes mellitus and signs of sub-clinical inflammation have been identified as subgroups with increased cardiovascular risk, patients have been divided into these subgroups for further analyses. Baseline OGTT identified 9 out of 32 patients with DM2. After 12 months only one patient still had overt diabetes and two patients had IGT, whereas six had returned to a normal glucose metabolism. None of the patients with pre-surgical NGT developed IGT or DM2 during follow-up. As a threshold for sub-clinical inflammation, the upper normal range for high-sensitivity (hs) C-reactive protein (≤10 mg/dL) in the laboratory was taken as cut-off point and patients were divided into subgroups accordingly.

At each visit, patients had their body weight (to the nearest 0.1 kg) recorded while wearing light indoor clothes and no shoes. For identifying upper body obesity waist circumference was measured at the level of the umbilicus while the patient was in a supine position. Systolic and diastolic blood pressure was measured twice on the right arm using an appropriate size cuff, with a width of at least 40% of the circumference of the arm and with the subject in the supine position after a 5 min rest at each visit. The mean of the two measurements was used to determine blood pressure. After a minimum overnight fast of 12 h, blood samples were drawn to measure traditional as well as non-traditional cardiovascular risk markers in addition to routine parameters. After surgery, regular checks including clinical examination, anthropometric measurements, and routine laboratory tests were performed every 6 months.

**Gastric banding**

The gastric banding system consists of a silicon band with an inflatable inner shell and buckle closure connected by tubing to an access port placed subcutaneously under xiphoid. The inner diameter of the band can be individually adjusted to the patient’s needs by the addition or removal of saline through the access port. The band system can be placed minimally invasive and has been shown to be a safe and effective procedure. The purely restrictive method causes early satiety by dividing the stomach and leaving only a small upper gastric pouch for food storage. The narrow outlet from the pouch slows transit of food and prolongs satiety. On leaving the gastric pouch, food passes into the main body of the stomach and an unaltered intestine.

**Laboratory assays**

Blood was rapidly centrifuged and stored at −80°C until analysis. Routine parameters including lipids and fibrinogen were measured with standard techniques, as described previously. Plasma glucose was measured with the hexokinase method applied on a modular analyzer (Roche, Basel, Switzerland). Insulin (Serono Diagnostics, Freiburg, FRG) and C-peptide (CIS Bio International, Cedex, France) were quantified with commercial radioimmunoassay kits with inter-assay coefficients of variation (CV) of <5% for insulin.

Applying the homeostasis model assessment (HOMA) to fasting concentrations of glucose and insulin yields measures of β-cell function (HOMA-%B) and insulin resistance (HOMA-R), which has been validated in DM2 and obesity. For determination of soluble CAMs samples were analysed by commercially available enzyme-linked immunosorbent assays (ELISA; British Bio-technology Product Ltd., Abdingdon, UK) as described previously, with both inter- and intra-assay CVs being <6%. PAI-1
antigen (4–49 ng/mL) was determined by means of an ELISA from Technoclone (Vienna, Austria).

sCD40L concentrations were measured using ELISA (Bender Medsystems) as described in detail elsewhere.\textsuperscript{20} Plasma levels of Lp-PLA\textsubscript{2} were determined with a commercial Lp-PLA\textsubscript{2}–ELISA kit (PLAC test; supplied by diaDexus Inc., South San Francisco, USA). The lower detection limit of Lp-PLA\textsubscript{2} in this assay is approximately 2 ng/mL. The inter-assay CV was 9.6%.\textsuperscript{21} ELISAs from R&D Systems (Wiesbaden, Germany) were used for the determination of MMPs.\textsuperscript{22}

**Data analysis and sample size calculation**

As BMI reduction is the main goal of bariatric surgery, we defined the change in BMI after bariatric surgery as the main study endpoint for the sample size estimation and made the following assumptions: we planned to include patients with a minimum BMI of 40 kg/m\textsuperscript{2}, therefore the mean baseline BMI was estimated between 40 and 45 kg/m\textsuperscript{2}. We expected a mean reduction of the BMI by 5–8 kg/m\textsuperscript{2} after bariatric surgery. Given the strict inclusion criteria and our experience on bariatric surgery during the last years, the SD for BMI was anticipated, we defined a continuous reduction of mean waist circumferences of 21.7 ± 0.9 cm 1 year after LAGB (P = 0.0001). Systolic blood pressure was significantly reduced from 151.8 ± 24.9 to 127.4 ± 10.6 mmHg (−24.4 ± 14.3 mmHg; P < 0.0001), whereas diastolic blood pressure was lowered from 91.4 ± 11.6 to 76.5 ± 5.9 mmHg (−14.9 ± 5.7 mmHg; P = 0.0001) during follow-up. The most impressive changes in lipid status were found in fasting triglyceride levels, which were significantly decreased from 165.3 ± 56.4 to 131.2 ± 45.5 mg/dL (−34.1 ± 10.9 mg/dL) after 6 months and to 118.0 ± 53.4 mg/dL (−47.3 ± 3.0 mg/dL) 1 year after LAGB. In contrast, HDL-cholesterol increased from 47.0 ± 10.9 to 50.9 ± 11.9 mg/ dL (+3.9 ± 1.0 mg/dL; P = 0.02) between months 6 and 12.

Continuous lowering of fasting blood glucose from 115.8 ± 52.5 to 95.6 ± 26.7 mg/dL (−20.2 ± 25.8 mg/dL) after 12 months (P = 0.02) resulted in a significant reduction in HbA1c from 6.48 ± 1.6% before LAGB to 5.72 ± 0.6% (−0.76 ± 1.0%) after 12 months (P = 0.003). The pre-surgical OGTT identified 9 out of 32 patients as DM2. After 12 months only one patient still had overt diabetes and two patients had IGT, whereas six returned to NGT. None of the patients with pre-surgical NGT developed IGT or diabetes during follow-up. In the whole group, HOMA-R decreased from 6.2 ± 4.1 to 2.8 ± 0.3 (−3.4 ± 2.1) after 6 months (P < 0.001)

**Results**

**Clinical and metabolic outcomes for traditional cardiovascular risk markers**

Table 1 shows body weight, BMI, waist circumference, glucose variables, arterial blood pressure, and lipid status in a group of 32 patients followed for 1 year.

<table>
<thead>
<tr>
<th></th>
<th>Pre-LAGB</th>
<th>6 months after LAGB</th>
<th>P-value</th>
<th>12 months after LAGB</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>129.7 ± 18.1</td>
<td>109.7 ± 16.0</td>
<td>&lt;0.001</td>
<td>97.6 ± 15.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>46.1 ± 5.9</td>
<td>39.0 ± 5.4</td>
<td>&lt;0.001</td>
<td>34.5 ± 5.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>133.9 ± 14.6</td>
<td>121.3 ± 13.4</td>
<td>&lt;0.001</td>
<td>112.2 ± 13.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>115.8 ± 52.5</td>
<td>103.3 ± 30.4</td>
<td>0.02</td>
<td>95.6 ± 26.7</td>
<td>0.02</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.5 ± 1.6</td>
<td>5.9 ± 0.8</td>
<td>0.01</td>
<td>5.7 ± 0.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>26.37 ± 13.34</td>
<td>17.76 ± 7.18</td>
<td>0.01</td>
<td>13.5 ± 4.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-R</td>
<td>6.2 ± 4.1</td>
<td>2.8 ± 2.0</td>
<td>&lt;0.001</td>
<td>3.2 ± 1.9</td>
<td>0.06</td>
</tr>
<tr>
<td>HOMA-%B</td>
<td>78.4 ± 48.3</td>
<td>39.7 ± 26.4</td>
<td>0.04</td>
<td>52.2 ± 30.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>151.8 ± 24.9</td>
<td>131.3 ± 11.3</td>
<td>&lt;0.001</td>
<td>127.4 ± 10.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>91.4 ± 11.6</td>
<td>80.9 ± 4.9</td>
<td>&lt;0.001</td>
<td>76.5 ± 5.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>165.3 ± 56.4</td>
<td>131.2 ± 45.5</td>
<td>0.002</td>
<td>118.0 ± 53.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>207.4 ± 27.7</td>
<td>201.7 ± 28.6</td>
<td>0.42</td>
<td>202.4 ± 37.0</td>
<td>0.55</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>48.1 ± 10.0</td>
<td>47.0 ± 10.9</td>
<td>0.61</td>
<td>50.9 ± 11.9</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>123.9 ± 24.4</td>
<td>123.5 ± 28.6</td>
<td>0.94</td>
<td>122.7 ± 37.7</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Data are means and SD analysed with Student’s t-test. P-values compare baseline and respective follow-up visits. BMI, body mass index; HbA1c, haemoglobin A1c; HOMA-R, homeostasis model assessment-insulin resistance; HOMA-%B, β cell function.
Markers of atherosclerosis before and after gastric banding

and to $3.2 \pm 1.9$ ($-3.0 \pm 2.2$) after 12 months ($P = 0.06$; baseline
vs. 12 months) thus indicating improved fasting insulin resistance.
HOMA-R decreased from $12.5 \pm 5.8$ to $3.8 \pm 4.2$ ($-8.7 \pm 1.6$)
in the diabetic subgroup and from $5.2 \pm 2.9$ to $3.1 \pm 1.6$
($-2.1 \pm 1.3$) in non-diabetics (both $P < 0.001$). Although a sub-
stantial fall of HOMA-%B was observed from $78.4 \pm 48.3$ to
$39.7 \pm 26.4$ ($-38.7 \pm 21.9$) after 6 months and to $52.2 \pm 30.7$
($-26.2 \pm 17.6$) after 12 months in the study population, the
decline in β-cell function did not reach statistical significance
($P = 0.04$ after 6 months and $P = 0.07$ after 12 months). In
addition, no statistically different changes were seen for
HOMA-%B comparing the diabetic and the non-diabetic sub-
groups: HOMA-%B decreased from $63.7 \pm 31.7$ to $40.1 \pm 36.8$
($-23.6 \pm 5.1$; $P = 0.07$) in diabetics and declined from
$89.9 \pm 56.5$ to $55.7 \pm 26.2$ ($-34.2 \pm 30.3$; $P = 0.09$) in non-
diabetics 1 year after LAGB.

A clinical but not statistically significant higher reduction of 9 kg
body weight in non-diabetics was not followed by pronounced
changes in baseline parameters and traditional cardiovascular risk
factors compared with diabetics 12 months after LAGB.

Non-traditional cardiovascular risk markers

Figures 1–3 show various non-traditional cardiovascular risk
factors in the whole patient cohort at baseline and at the two
follow-up visits.

Markers of chronic inflammation

High-sensitivity C-reactive protein tended to, but did not significantly,
decrease from $7.74 \pm 6.99$ to $6.0 \pm 5.09$ mg/dL and $6.14 \pm 4.74$ mg/
dl ($-1.74 \pm 1.9$ mg/dL; $P = 0.05$ and $-1.6 \pm 1.25$ mg/dL; $P = 0.08$)
after 6 and 12 months, respectively. Compared with baseline, PAI-1
concentration decreased from $57.0 \pm 43.9$ to $38.4 \pm 15.6$ ng/mL
($-18.6 \pm 28.3$ ng/mL) after 6 months ($P = 0.01$) and remained
similar with $41.3 \pm 18.1$ ng/mL after 12 months ($-15.7 \pm 25.8$
ng/mL; $P = 0.001$).

Lp-PLA$_2$ concentration was comparable before and after
LAGB ($310.9 \pm 52.2$ vs. $315.9 \pm 53.9$; $-5.0 \pm 1.7$ ng/mL) vs.
$308.6 \pm 73.6$ ng/mL ($-2.3 \pm 21.4$ ng/mL).

Cellular adhesion molecules

Statistically significant decreases of all CAMs were observed when
compared before and during the follow-up after LAGB: ICAM-1
concentrations were reduced from $312.2 \pm 241.1$ to
$253.2 \pm 78.1$ ng/mL ($-59.0 \pm 64.0$ ng/mL) after 6 months ($P =
0.01$) but tended to increase later ($284.6 \pm 85.9$ ng/mL), resulting
in a final reduction of $-27.6 \pm 38.2$ ng/mL ($P = 0.06$). Furthermore,
VCAM-1 levels were only significantly lowered 1 year after LAGB
($663.9 \pm 1115$ vs. $619 \pm 86.9$ ng/mL; $-44.9 \pm 24.6$ ng/mL; $P =
0.009$). E-selectin concentrations were significantly reduced after
both follow-up visits: $40.4 \pm 13.9$ ng/mL ($-17.1 \pm 15.4$; $P < 0.001$)
after 6 months and $43.2 \pm 17.8$ ng/mL ($-14.3 \pm 11.5$ ng/mL;
$P < 0.01$) after 1 year compared with pre-surgical values
($57.5 \pm 29.3$ ng/mL) and showed the most pronounced decrease
(approximately 25%) after LAGB.

sCD40L increased from $1.4 \pm 1.3$ before LAGB to $1.6 \pm 1.5$
after 6 months ($P = 0.68$) and to $2.0 \pm 1.7$ ng/mL after 1 year
(a significant increase of $0.6 \pm 0.4$ ng/mL over the whole study
period; $P = 0.01$). In contrast, CD40 decreased significantly from
$152.7 \pm 111.5$ to 121.7 $\pm 104.4$ ($-31.0 \pm 7.1$) and to 95.5 $\pm 59.1$
($-57.2 \pm 52.4$) within 6 and 12 months after LAGB, respectively
($P = 0.01$ for both).

Metalloproteinases

Controversial effects were found for matrix-metalloproteinases
MMP-2 and MMP-9: While MMP-2 concentrations were in the

Figure 1 Time-dependent changes of the proinflammatory markers high-sensitivity C-reactive protein and plasminogen activator inhibitor-1 in severely obese patients before laparoscopic adjustable gastric banding (LAGB) and after 6 and 12 months. $^aP < 0.01$ baseline vs. 6 or
12 months LAGB.
same range at baseline (163.4 ± 25.8) and 6 months after LAGB (170.1 ± 33.0 ng/mL, +6.7 ± 7.2), MMP-2 increased significantly from 6 months up to 1 month (182.3 ± 30.2 ng/mL), which represents a change in concentration of +18.9 ± 4.4 ng/mL over the whole time period after LAGB (P = 0.004). In contrast, MMP-9 significantly decreased from 766.4 ± 350.9 before LAGB to 606.2 ± 302.9 after 6 months (−2160.2 ± 48.0 ng/mL; P = 0.002) and to 616.3 ± 348.9 ng/mL after 12 months (−2150.1 ± 2.0 ng/mL; P = 0.004), respectively.

Comparison of non-traditional cardiovascular risk factors in diabetics and non-diabetics

The following risk factors were statistically different between subgroups of diabetics (d) and non-diabetics (non-d): E-selectin (d: 77.4 ± 34.0; non-d: 49.4 ± 23.3; difference: −28.0 ± 10.7; P = 0.01) and PAI-1 (d: 83.8 ± 67.2; non-d: 44.1 ± 15.3; difference: −38.9 ± 4.4; P = 0.02) was elevated in diabetics when compared with non-diabetics and also remained higher during the follow-up period after LAGB (Table 2). At baseline hs-C-reactive protein concentrations were insignificantly higher in diabetics (d: 8.4 ± 1.8; non-d: 5.7 ± 0.6 mg/dL; difference: −2.7 ± 1.2; P = 0.06) but decreased both after LAGB by also reducing the difference between groups (d: 6.4 ± 0.3; non-d: 5.5 ± 0.4; difference −0.9 ± 0.1 mg/dL; P = 0.56).

Comparison of non-traditional cardiovascular risk factors in patients with high or low level of sub-clinical inflammation

Based on a threshold of ≤10 mg/dL hs-C-reactive protein (upper normal range) patients were divided into subgroups. Ten patients (including three diagnosed diabetics) had signs of sub-clinical inflammation and exhibited a hs-C-reactive protein concentration of 15.1 ± 6.42 mg/dL, while the remaining patients had no signs of inflammation and a hs-C-reactive protein of 3.6 ± 1.8 mg/dL at baseline (P = 0.01). Six and 12 months after LAGB, hs-C-reactive protein concentration remained elevated in patients with signs of inflammation when compared with patients without (after 6 months: 10.5 ± 5.8 mg/dL vs. 3.5 ± 2.4 mg/dL; difference: 7.0 ± 3.4 mg/dL; P < 0.0001; after 12 months: 12.9 ± 10.2 mg/dL vs. 4.7 ± 3 mg/dL; difference: 8.2 ± 6.5; P = 0.003; Table 3). Non-traditional risk markers were not affected by a milieu of increased inflammation except sCD40L, which was significantly higher in this subgroup when compared with patients without signs of sub-clinical inflammation 12 months after LAGB (2.8 ± 1.8 ng/mL vs. 1.5 ± 1.4 ng/mL; difference: 1.3 ± 0.4; P = 0.03).

Correlations

A statistically significant positive correlation between metabolic parameters and non-traditional cardiovascular risk factors could
be demonstrated for changes in fasting insulin and CD40 concentrations ($r = 0.432; P = 0.015$). Moreover, systolic blood pressure showed a weak positive correlation with sCD40L ($r = 0.40; P = 0.03$) at baseline. Furthermore, weak positive correlations were observed between fasting insulin and ICAM-1 concentrations before LAGB ($r = 0.36; P = 0.04$), as well as 6 months ($r = 0.38; P = 0.048$) and 12 months ($r = 0.41; P = 0.04$) thereafter.

### Table 2 Revised changes in non-traditional cardiovascular risk factors in diabetic and non-diabetic obese subjects before and after laparoscopic adjustable gastric banding (LAGB)-induced weight loss

<table>
<thead>
<tr>
<th></th>
<th>T2DM ($n = 9$)</th>
<th>Non-T2DM ($n = 23$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before LAGB</td>
<td>12 months after LAGB</td>
</tr>
<tr>
<td></td>
<td>128.9 ± 17.7</td>
<td>94.2 ± 16.0</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>131.9 ± 20.0</td>
<td>105.9 ± 9.2</td>
</tr>
<tr>
<td></td>
<td>0.73</td>
<td>0.05</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>141.8 ± 14.4</td>
<td>121.1 ± 14.9</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>371.6 ± 157.9</td>
<td>291.9 ± 111.6</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.83</td>
</tr>
<tr>
<td>VCAM-1 (ng/mL)</td>
<td>693.6 ± 129.9</td>
<td>640.0 ± 88.2</td>
</tr>
<tr>
<td></td>
<td>0.47</td>
<td>0.42</td>
</tr>
<tr>
<td>E-selectin (ng/mL)</td>
<td>77.4 ± 34.0</td>
<td>51.5 ± 20.1</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>High-sensitivity C-reactive protein (mg/dL)</td>
<td>8.4 ± 1.8</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.04</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>83.8 ± 67.2</td>
<td>55.0 ± 22.8</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.003</td>
</tr>
<tr>
<td>Lp-PLA$_2$ (ng/mL)</td>
<td>317.1 ± 48.5</td>
<td>257.3 ± 67.6</td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>0.01</td>
</tr>
<tr>
<td>CD40 ligand (ng/mL)</td>
<td>1.0 ± 0.7</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>0.26</td>
<td>0.33</td>
</tr>
<tr>
<td>CD40</td>
<td>108.0 ± 43.4</td>
<td>101.4 ± 62.2</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.72</td>
</tr>
<tr>
<td>MMP-9 (ng/mL)</td>
<td>771.2 ± 396.4</td>
<td>633.0 ± 406.1</td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td>0.86</td>
</tr>
<tr>
<td>MMP-2 (ng/mL)</td>
<td>169.0 ± 14.9</td>
<td>182.6 ± 23.8</td>
</tr>
<tr>
<td></td>
<td>0.41</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Data are means and SD analysed with Student’s t-test. $P$-values are compared between diabetic and non-diabetic subgroups before LAGB and 12 months thereafter. ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; T2DM, type 2 diabetes mellitus; PAI-1, plasminogen activator inhibitor-1; Lp-PLA$_2$, lipopolysaccharide-associated phospholipase A$_2$. 

Figure 3: Time-dependent changes of the non-traditional cardiovascular risk markers CD40 ligand, lipopolysaccharide-associated phospholipase A$_2$, MMP-p, and MMP-2 in severely obese patients before laparoscopic adjustable gastric banding (LAGB) and after 6 and 12 months. $^aP < 0.05$ baseline vs. 6 or 12 months LAGB; $^bP < 0.01$ baseline vs. 12 months LAGB.
correlated positively with changes in BMI after 6 and 12 months (r = 0.38; P = 0.04 and r = 0.36; P = 0.05), while changes in BMI over time showed an inverse correlation with Lp-PLA2 mass (r = −0.41; P = 0.02).

Discussion

This study demonstrates a decrease in several established as well as in a number of non-traditional cardiovascular risk factors in severely obese patients following LAGB. Whereas markers of a systemic inflammatory response, i.e. PAI-1, were reduced, Lp-PLA2, as marker for vascular inflammation, remained unchanged. Since CAMs but not sCD40L, which is known to be associated with higher risk for future myocardial infarction in association with plaque rupture, significantly improved after weight loss, these results might indicate the presence of a very early stage of atherosclerosis in our study population. Moreover, an upregulation of hs-C-reactive protein and PAI-1 levels, independent of a diabetic metabolic state, was not followed with an increase in other non-traditional risk factors. The observed increase of soluble CAMs in all obese patients, mainly of E-selectin, might reflect an early stage of endothelial dysfunction, induced by an impairment of blood glucose and insulin. Following considerable weight loss, sCAMs decreased significantly, indicating an amelioration of the pro-inflammatory state, which was independent of C-reactive protein in these subjects. As described before, surgical and conservative weight management in general show beneficial effects on lowering CAMs, although the results may vary. Interestingly, elevated levels of E-selectin and PAI-1 remained higher after weight loss in diabetics compared with non-diabetics in our hands, indicating a greater activation of subclinical chronic inflammation in this subgroup.

Lp-PLA2 has been identified as a novel cardiovascular risk marker with pro-inflammatory properties. Yang et al. demonstrated an independent correlation between non-significant coronary artery disease (<30% stenosis) and Lp-PLA2, while others were not able to find such an association with extra coronary atherosclerosis. Lp-PLA2 primarily circulates attached to LDL cholesterol and acts on oxidized forms of lipids. Elevated Lp-PLA2 concentrations (>310 ng/mL) may identify individuals at higher risk for CHD despite LDL levels <130 mg/dL. Lp-PLA2 concentration was neither affected by weight loss, presence of diabetes, or LDL concentration nor by a pro-inflammatory milieu in our study. In 21 patients with low LDL levels and with a mean value of Lp-PLA2 of 318 ng/mL, we could not find any statistical

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Table 3 Differences in non-traditional cardiovascular risk factors in patients without signs of subclinical inflammation [high-sensitivity (hs) C-reactive protein <10 mg/dL] and with subclinical inflammation (hs-C-reactive protein >10 mg/dL) before and after laparoscopic adjustable gastric banding (LAGB)-induced weight loss

<table>
<thead>
<tr>
<th>Patients with normal hs-C-reactive protein (n = 21)</th>
<th>Patients with elevated hs-C-reactive protein (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before LAGB</td>
<td>12 months after LAGB</td>
</tr>
<tr>
<td>hs-C-reactive protein (mg/dL)</td>
<td>3.6 ± 1.8</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)</td>
<td>43.6 ± 14.9</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>328.1 ± 143.1</td>
</tr>
<tr>
<td>VCAM-1 (ng/mL)</td>
<td>683.3 ± 115.9</td>
</tr>
<tr>
<td>E-selectin (ng/mL)</td>
<td>60.2 ± 35.5</td>
</tr>
<tr>
<td>Lp-PLA2 (ng/mL)</td>
<td>304.3 ± 45.1</td>
</tr>
<tr>
<td>CD40 ligand (ng/mL)</td>
<td>1.4 ± 1.4</td>
</tr>
<tr>
<td>MMP-9 (ng/mL)</td>
<td>771.2 ± 396.4</td>
</tr>
<tr>
<td>MMP-2 (ng/mL)</td>
<td>165.5 ± 24.8</td>
</tr>
</tbody>
</table>

Data are means and SD analysed with Student’s t-test. P-values are compared between subgroups of patients with normal or elevated hs-C-reactive protein concentrations before LAGB and 12 months thereafter. ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; Lp-PLA2, lipopolysaccharide-associated phospholipase A2.

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Markers of atherosclerosis before and after gastric banding

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Conclusions

The present study provides evidence that both, established and non-traditional cardiovascular risk factors are frequently elevated in extreme obesity, suggesting an increased risk for premature atherosclerosis. While risk factors related to pro-inflammatory states such as PAI-1, ICAM-1, and E-selectin, as well as MMP-9 significantly decreased after extensive weight loss, markers indicative of atherosclerosis in progression such as sCD40L and Lp-PLA2 remained unchanged. Despite obesity, which was associated with increased traditional risk factors, i.e. BMI and blood pressure, only signs for unspecific systemic subclinical inflammation were found indicating a very early stage of disease in our patients, which could be reduced by LAGB-induced weight loss. In summary, gastric banding is capable of reducing pro-atherogenic and pro-inflammatory risk factors before profound atherosclerosis and related clinical signs has developed in overweight patients and should therefore be offered early and more frequently to extremely obese patients.

Conflict of interest: none declared.

References


