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Abstract

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Objective: Increased plasma levels of endothelial activation markers in obese subjects reflect the positive association between cardiovascular diseases and obesity. The pro-inflammatory state associated with obesity is thought to play a major role in endothelial cell activation in severely obese individuals. Previous studies demonstrated that long-term weight loss after bariatric surgery is accompanied by a decreased proinflammatory state. However, little is known about the long-term effects of bariatric surgery on endothelial cell activation.

Research Methods and Procedures: Plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1), soluble endothelial selectin (sE-selectin), and soluble vascular cell adhesion molecule-1 (sVCAM-1), all markers of endothelial cell activation, and of their regulators adiponectin and resistin were measured at different time-points postoperatively in 26 consecutive patients who underwent restrictive surgery, with a follow-up of 2 years.

Results: During the first 6 months after bariatric surgery, sE-selectin levels decreased. Despite substantial weight loss, sICAM-1 and sVCAM-1 plasma levels did not decrease significantly. After 24 months, sICAM-1 levels were significantly decreased, whereas sE-selectin levels were further decreased. However, sVCAM-1 levels remained elevated. Adiponectin levels did not change significantly during the first 6 months after bariatric surgery, whereas resistin levels increased. After 24 months, adiponectin levels were similar to normal-weight controls, but resistin levels remained high.

Discussion: Reductions in plasma levels of different markers of endothelial activation after bariatric surgery show different temporal patterns, suggesting that distinct mechanisms are involved in their regulation. Although not all endothelial activation markers normalize after bariatric surgery, our findings suggest that bariatric surgery can reduce endothelial activation in the long term.

Key words: endothelial function, weight loss, obesity surgery, cardiovascular risk

Introduction

Inflammation of the vascular wall is a crucial step in the pathophysiology of atherosclerosis, which causes endothelial cells to express a wide variety of endothelial adhesion molecules, including endothelial selectin (E-selectin),[1] vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1). To limit the inflammatory process, these adhesion molecules are rapidly removed from the cell surface, which occurs by proteolytic cleavage and shedding (1,2). The elevated circulating levels of products of proteolytic cleavage and shedding of adhesion molecules correlate with atherosclerosis (1). Furthermore, plasma levels of soluble ICAM-1 (sICAM-1), VCAM-1 (sVCAM-1), and E-selectin (sE-selectin) are increased in obesity (3,4).

Recently, two proteins, adiponectin and resistin, which could affect expression of endothelial activation markers by endothelial cells, were discovered. Adiponectin and resistin have opposite actions on endothelial cells, and plasma levels are respectively decreased and increased in human obesity (5,6). The adipokine adiponectin down-regulates ex-

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[1] Nonstandard abbreviations: E-selectin, endothelial selectin; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; sICAM-1, soluble ICAM-1; sVCAM-1, soluble VCAM-1; sE-selectin, soluble E-selectin.

pression of ICAM-1, E-selectin, and VCAM-1, whereas resistin, which in humans is secreted by inflammatory cells (7), up-regulates these adhesion molecules (8,9).

Weight loss in obese and severely obese individuals results in improvement of endothelial function (10) and decreased circulating inflammatory markers (11,12). This study was conducted to analyze the effects of weight loss after bariatric surgery on the levels of endothelial activation markers and their key regulators. Together with circulating plasma markers of endothelial activation (sICAM-1, sE-selectin, and sVCAM-1), plasma levels of mediators of endothelial activation (adiponectin and resistin) were studied in a follow-up of 24 months, reflecting the degree of endothelial cell activation during weight loss after bariatric surgery.

Research Methods and Procedures

Subjects and Samples

All participants gave written informed consent. The study was approved by the local ethical committee of the Academic Hospital Maastricht.

A total of 26 consecutive subjects, admitted to the Surgical Department of the University Hospital Maastricht to undergo an operation for severe obesity, were included in the study. The group comprised 23 women and three men, age 38 ± 9 years with a mean BMI of 46.3 ± 6.0 kg/m^2. These patients underwent a primary operation for severe obesity [vertical banded gastroplasty (n = 12) or laparoscopic gastric banding using Lap-Band (n = 14)]. All subjects were selected to be otherwise healthy according to history, clinical examination, and, whenever possible, laboratory findings. No overt signs of type 2 diabetes, hypertension (diastolic blood pressure, 82.12 ± 8.82 mm Hg), or other cardiovascular diseases were present. Moreover, the individuals did not use any medication apart from oral contraceptives. Ten healthy lean subjects (eight women and two men), 35 ± 14 years old, with a mean BMI of 22.5 ± 3.5, served as a control group to provide reference values (values depicted as dotted lines in Figure 1). Blood samples were collected after at least 8 hours of fasting using evacuated blood collection tubes containing EDTA 1 day before surgery and 3, 6, 12, and 24 months after surgery and processed as previously described (11).

Immunoassays

Plasma concentrations of sICAM-1 and sE-selectin were determined using sandwich enzyme-linked immunosorbent assays as described elsewhere (11,13). Plasma sVCAM-1, adiponectin, and resistin concentrations were measured using enzyme-linked immunosorbent assay development kits (R&D Systems, Minneapolis, MN). All plasma samples were analyzed in the same run. The intra- and inter-assay coefficients of variance of the various assays were <10%.

Statistical Analysis

All data are expressed as mean ± standard deviation. Due to the non-parametrically distributed data, preoperative and postoperative results were compared using repeated measurements ANOVA. A p value < 0.05 was considered statistically significant.

Results

Weight Loss after Surgery

Restrictive bariatric surgery resulted in a significant loss of BMI from 46.3 ± 6.0 to 34.7 ± 6.6 kg/m^2 ($p < 0.01$) during the 2-year follow-up period, which was not influenced by the restrictive bariatric surgical techniques used (Figure 1). During the first 6 months, a striking decrease in BMI and percentage of excess weight loss was observed, amounting to a remarkable mean 142 g body weight loss/d, which decreased to a mean weight loss of 53 g/d (6 to 12 months). Patients reached a stable weight after 1 year.

Effect of Weight Loss on Endothelial Activation Markers

Previously, we reported that inflammatory markers such as the acute phase protein lipopolysaccharide binding protein, C-reactive protein, and soluble tumor necrosis factor α receptors did not decrease during the first 6 months after bariatric surgery, even though the individuals lost substantial amounts of weight (11). In line with this, circulating levels of the adhesion molecules sICAM-1 and sVCAM-1 remained elevated (Figure 1). In contrast, plasma levels of sE-selectin rapidly declined during the first 6 months (Figure 1).

In the long term (12 to 24 months) after bariatric surgery, levels of circulating inflammatory markers decreased (11). Likewise, plasma sICAM-1 and sE-selectin levels further decreased to levels comparable with those measured in healthy controls [from 165.8 ± 32.4 to 136.8 ± 30.7 ng/mL ($p < 0.05$) and from 23.8 ± 9.5 to 15.4 ± 7.3 ng/mL ($p < 0.05$), respectively]. sVCAM-1 levels showed a trend to decrease from 1039.1 ± 290.2 to 753.0 ± 311.4 ng/mL but remained elevated as compared with healthy controls. The decrease of endothelial activation markers did not correlate with the decrease in weight after bariatric surgery.

Effect of Weight Loss on Regulators of Endothelial Activation

Despite substantial weight loss directly after bariatric surgery, only minimal changes in plasma adiponectin and resistin levels were seen. In the stable weight phase, after 12 months, adiponectin plasma levels had increased significantly from 6.4 ± 3.6 to 7.6 ± 3.0 μg/mL ($p < 0.05$) (Figure 1). After 24 months, however, adiponectin levels did not differ significantly from preoperative levels. Plasma resistin levels increased significantly during the period of

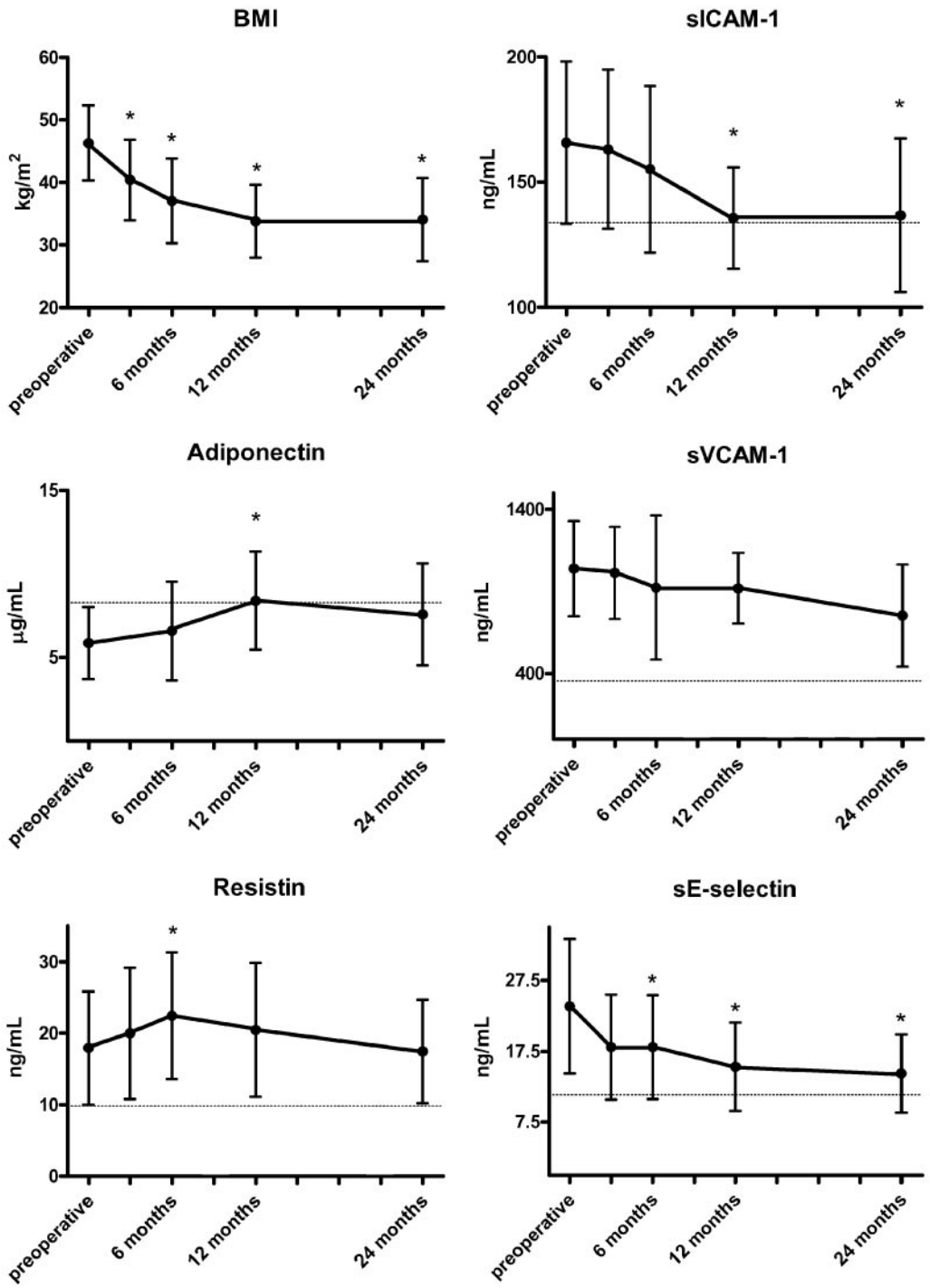

Figure 1: Effect of weight loss after bariatric surgery on BMI, endothelial activation markers, and regulators of endothelial activation. BMI of 26 severely obese subjects was determined preoperatively and after gastric restrictive surgery. BMI decreased after gastric restrictive surgery. Effect of weight loss due to gastric restrictive surgery on plasma levels of endothelial activation markers sICAM-1, sVCAM-1, sE-selectin, and their regulators, adiponectin and resistin, in 26 severely obese subjects. During the first months after bariatric surgery, adiponectin concentrations did not increase significantly, whereas resistin levels did increase. After 12 months, adiponectin levels were significantly increased, whereas resistin levels dropped to preoperative values. In contrast to plasma sE-selectin levels, sICAM-1 and sVCAM-1 plasma levels were still elevated during the first months after bariatric surgery. In the long term, sE-selectin levels further declined and sICAM-1 levels decreased significantly, but sVCAM-1 concentrations remained elevated. The dotted line represents control values. Mean values and standard deviations are: sICAM-1, 133.2 ± 22.7 ng/mL; adiponectin, 8.27 ± 4.6 μg/mL; sVCAM-1, 354.6 ± 108.2 ng/mL; resistin, 9.15 ± 2.2 ng/mL; and sE-selectin, 11.35 ± 4.7 ng/mL. * Significantly changed compared with preoperative values.

substantial weight loss, from 17.7 ± 7.5 to 22.1 ± 7.3 ng/mL after 6 months ($p < 0.05$) but decreased to preoperative values once stable weight was achieved (Figure 1).

Discussion

Cardiovascular diseases are common in severely obese individuals. Endothelial cell activation is thought to play a causative role in the development of cardiovascular diseases. In this study, we have monitored levels of endothelial cell activation markers and their main regulators in severely obese individuals up to 2 years after bariatric surgery. During the first 6 months after bariatric surgery, plasma sICAM-1 and sVCAM-1 concentrations remained high. In contrast, sE-selectin decreased significantly during this period. This suggests that endothelial cells remain in an activated state during this period, although the effects of weight loss are already reflected in components of the endothelial activation state. Because plasma adiponectin levels did not change, and resistin levels even increased during the first months after bariatric surgery, it is unlikely that these mediators of endothelial activation in vitro are responsible for the partial reduction of endothelial cell activation. Instead, inflammatory mediators may be responsible for this effect. In a previous report, we hypothesized that a severe catabolic state, brought about by substantial weight loss, could be responsible for metabolic stress, resulting in an ongoing inflammatory state during the first months after bariatric surgery (11). This may explain why the reduction in endothelial cell activation is initially only minor. In this respect, it is also noteworthy that, similar to the endothelial activation markers, there are marked differences with respect to the degree of normalization of inflammatory mediators during the first months after surgery. Plasminogen activator inhibitor-1 and the acute phase protein α1-acid glycoprotein levels, like sE-selectin levels, decrease rapidly, whereas sICAM-1 and sVCAM-1 levels, like lipopolysaccharide binding protein and C-reactive protein levels, remain elevated. These findings suggest that there is no uniform mechanism by which weight loss leads to decreased endothelial activation and inflammation, although parallel mechanisms may operate in both processes.

Once weight stabilization was reached (~12 to 24 months postoperatively), the inflammatory condition diminished. In line with this, we report substantially decreased levels of the endothelial activation markers sICAM-1 and sE-selectin and increased plasma adiponectin concentrations 12 to 24 months after surgery. On the other hand, resistin and sVCAM-1 levels remained elevated. A possible explanation can be that the subjects in our study were still obese, even at 24 months after gastric restrictive surgery (mean BMI of 34.7 kg/m^2). It has been shown that obese individuals have elevated resistin levels (14), which can explain the high levels of resistin 2 years after surgery. Analogous to this, sVCAM-1 levels in obese subjects are higher than those in normal-weight controls, as also shown by Vazquez et al. (15).

Although almost exclusively secreted by adipocytes, adiponectin levels in obese individuals are lower than levels in normal-weight control subjects and increase after weight loss (16). The low-grade inflammatory state associated with obesity is thought to be responsible for the decreased adiponectin production (17). Therefore, the ongoing inflammation during the first months after bariatric surgery may suppress an increase in adiponectin levels, despite substantial weight loss. After stable weight is achieved, the low-grade inflammation attenuates and adiponectin levels increase accordingly.

Resistin up-regulates endothelial activation markers by a nuclear factor-κB- or activator protein-1-dependent pathway (9). Adiponectin down-regulates the expression of adhesion molecules through cyclic adenosine monophosphate-dependent inhibition of nuclear factor-κB signaling (8). Moreover, endothelial activation markers have been reported to be up-regulated by proinflammatory cytokines (13). This can explain the prolonged increase of sICAM-1 and sVCAM-1 but not the rapid decrease of sE-selectin during the first months after bariatric surgery. One possible explanation for the observed discrepancy is the source of the different activation markers. sICAM-1 is not exclusively secreted by endothelial cells. The observed increased plasma concentrations of resistin could imply that monocytes are activated during the first few months after bariatric surgery. Monocytes are also capable of producing sICAM-1 (18), which could explain the prolonged increased plasma levels of sICAM-1 after bariatric surgery. Another possibility for the observed discrepancy could be the observation that sE-selectin plasma levels are more sensitive than sVCAM-1 and sICAM-1 plasma levels for the hyperinsulinemic state in individuals with impaired glucose tolerance (19). Directly after bariatric surgery, insulin levels rapidly decrease (20), which might explain the rapid decrease of sE-selectin levels during the first months after bariatric surgery, whereas sICAM-1 and sVCAM-1 plasma levels are still increased.

In summary, levels of endothelial activation markers diminish after bariatric surgery induced weight loss, leading to a decreased proatherogenic state. This underscores the importance of bariatric surgery in reducing major comorbidities of severe obesity, like cardiovascular disease.

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