

Effect of Surgically-Induced Weight Loss on Inflammatory Mediators and Peripheral Blood Monocyte CD11b Expression in Morbid Obesity

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Obesity is characterized by a state of chronic mild inflammation, with raised circulating levels of inflammatory markers. Expression and release of inflammation-related adipokines, generally, rise as adipose tissue expands. In the present study we evaluated the level of serum mediators concerned in inflammation and monocyte activation (TNF- α , hs-CRP, MCP-1) together with percentage of CD11-b expression on monocytes in a group of morbidly obese individuals (n=20) before and (3-6 months) after restrictive surgery, and in 15 healthy normal weight individuals. Serum MCP-1, TNF- α and hs-CRP were assayed by enzymatic immunoassay, while the percentage of CD11b expression on monocytes was assayed by flow cytometry. The total lipid profile and random blood glucose levels were also assessed. Morbidly obese individuals (before surgical weight loss) had significantly increased levels of MCP-1, TNF- α , hs-CRP, CD11b expression on monocytes as compared to controls ($P < 0.01$). Levels of MCP-1, TNF- α , hs-CRP were significantly decreased 3 to 6 months after restrictive surgery than before the operation ($P < 0.01$). hs-CRP, MCP-1 and TNF- α were positively correlated versus each other. TNF- α and hs-CRP also showed positive correlation with the body mass index. Our data suggested that the studied serum and monocyte parameters may link obesity with systemic inflammation and metabolic disorders. The interactions of MCP-1, CD11b and other inflammatory parameters might provide the basis for development of new therapies for this syndrome.

Obesity is now considered epidemic throughout the world and represents a major risk factor for a variety of life-threatening diseases, such as heart attack, stroke, diabetes, cancer and chronic diseases like asthma (Takahashi *et al.*, 2003; Castro-Giner *et al.*, 2009). The adipose tissue is a highly complex tissue and consists of mature adipocytes, preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, resident monocytes/macrophages, and lymphocytes (Schäffler and Büchler, 2007). White adipose tissue is no longer considered an inert tissue mainly devoted to energy storage but is emerging as an active participant in regulating physiologic and pathologic processes, including immunity and inflammation (Fantuzzi, 2009). Obesity is characterized by a state of chronic mild inflammation, with raised circulating levels of

inflammatory markers. The expression and release of inflammation-related adipokines generally rises as adipose tissue expands (Trayhurn & Wood, 2005). Macrophages are components of adipose tissue and actively participate in its activities. Furthermore, cross-talk between lymphocytes and adipocytes can lead to immune regulation. Adipose tissue produces and releases a variety of proinflammatory and anti-inflammatory factors, including the adipokines leptin, adiponectin, resistin, and visfatin, as well as cytokines and chemokines, such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and monocyte chemoattractant protein - 1 (MCP-1) (Fantuzzi, 2009).

Circulating monocytes exist in a proinflammatory state in obese persons known to be at increased risk of developing diabetes, heart disease or both. These cells

enter the artery and set up atherosclerosis, activate fat cells to produce more pro-inflammatory factors and interfere with insulin signaling, causing insulin resistance (Ghanim and Dandona, 2004). In obesity, macrophages are the major source of TNF- α in adipose tissue. It reduces adiponectin secretion by adipocytes and is involved in the pathogenesis of inflammation and insulin resistance (Hivert, 2008). TNF- α stimulates adhesion of monocytes to the surface of endothelial cells by enhancing the expression of adhesion molecules as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), stimulates pre-adipocytes and endothelial cells to produce MCP-1, which belongs to the family of chemokines (Wellen and Hotamisligil, 2003). MCP-1 is a key mediator of monocyte trafficking and promotes macrophage recruitment, as its receptor (CCR2) is expressed on circulating monocytes (Thalman and Meier, 2007). Additionally CD11b is expressed during activation and differentiation of monocytes. Its expression is an essential step for the attachment of monocytes to endothelial surface and their subsequent migration from the peripheral blood into the target tissues (Kim and Vaziri, 2007).

In the present study we evaluated the level of serum mediators concerned in inflammation and monocyte activation: TNF- α , highly sensitive C-reactive protein (hs-CRP), MCP-1 together with % of CD11b expression on monocytes in a group of morbidly obese individuals before and after weight loss by restrictive surgery.

Subjects and Methods

Subjects

This study comprised 20 morbidly obese patients (6 males and 14 females) with ages ranged between; 30 \pm 9 years (mean \pm SD). The study also included 15 non obese individuals (4 males and 11 females) as a control

group, their ages ranged between, mean \pm SD, 28 \pm 2.5 years. The patients were enrolled from the obesity outpatient clinic, Ain Shams University hospital, Cairo, Egypt. The patients were followed up (for a period ranging from 3-6 months) after weight loss as the result of restrictive bariatric surgery. All patients gave written informed consent to participate in the study and the study was approved by the Ain Shams Medical Ethical Committee.

Methods

Blood samples

Six milliliters of venous blood were collected from each subject and were divided in 2 tubes, EDTA tube for estimation of CD11b by flow cytometry, and the other tube was plain to obtain serum which was separated into two aliquots one was used immediately for assaying serum glucose, lipid profile, and the other aliquot was stored frozen at -20°C until time of assay of MCP-1, TNF- α , and hs-CRP.

Analytical methods

- Analysis of monocyte cell surface markers was done on lysed whole blood using PE-labeled CD14 and FITC-labeled CD11b monoclonal antibodies (Becton-Dickinson, San Jose, CA, USA) using Coulter EPICS- XL flowcytometer (Beckman Coulter, Inc. Mervue, Galway, Ireland).
- Highly sensitive CRP (hs-CRP) levels were assayed using an enzyme immunoassay (ELISA using high sensitivity CRP Monobind Inc. Lake Forest, USA) according to the manufacturer instructions.
- MCP-1 was assayed using an enzyme immunoassay (human MCP-1 ELISA BMS281 kit).
- TNF- α was assayed using an enzyme immunoassay (Orgenium, Finland www.Orgenium.com).
- Serum glucose and lipid profile (serum cholesterol, serum triglycerides, HDL, LDL) were performed using Synchron CX9 (Beckman instrument Inc. Brea, California, USA).

These parameters were determined in the control group and in the patient group before and after weight loss by surgery.

Statistical Methods

The results were analyzed using the SPSS computer program (statistical program for social science version 12). The data were presented as mean and standard deviation (SD). Student test was used to compare

quantitative variables between both groups and paired t-test was used to compare quantitative variables in the same group before and after intervention. Chi-square test was used to compare qualitative variables between both groups. Correlation coefficient test was used to rank different variables against each others either positively or inversely. Probability of error less than 0.05 was considered statistically significant (Miller and Knapp, 1992).

Results

Comparative statistics of studied parameters (MCP-1, TNF- α , hs CRP, % of CD11b positive monocytes, total cholesterol and triglycerides) between morbidly obese individuals (before and after weight loss) and normal weight controls, revealed a significant increase in all parameters in morbidly obese before and after intervention except for the % of CD11b positive monocytes which was statistically significant only in morbidly obese patients before intervention (Tables 1 & 2).

The studied parameters (MCP-1, TNF- α , hsCRP, % of CD11b positive monocytes, total cholesterol and triglycerides) were compared between morbidly obese patients before & after weight loss. There was a significant decrease in the all parameters in morbidly obese patients after intervention except for the % of CD11b positive monocytes, decreased after weight loss but did not reach a statistically significance (Table 3).

Correlation between hs CRP, MCP-1, % of CD11b positive monocytes, and TNF- α versus each others and versus other variables among cases before intervention, showed a positive correlation between hsCRP, MCP-1. Also TNF- α and hsCRP were positively correlated versus BMI and WC and versus each others (Table 4).

Table 1. Comparison between morbidly obese patients (before intervention) and controls with regard to studied parameters

Variables	Morbid obese (N=20) mean \pm SD	Controls (N=15) mean \pm SD	P value
hs CRP (mg/dl)	7.9 \pm 0.9	0.96 \pm 0.4	<0.01
Total cholesterol (mg/dl)	221 \pm 39	163 \pm 17	<0.01
TG (mg/dl)	140.5 \pm 38	79 \pm 8	<0.01
HDL (mg/dl)	42 \pm 7	64 \pm 8	<0.01
LDL (mg/dl)	150 \pm 35	83 \pm 18	<0.01
% of CD11b+ve monocytes	93 \pm 4	82 \pm 22	<0.01
MCP-1 (Pg/ml)	59 \pm 25	16 \pm 4	<0.01
TNF- α (Pg/ml)	138 \pm 93	8 \pm 3	<0.01

hs CRP: highly sensitive CRP; TG: triglycerides; HDL: high density lipoproteins

LDL: low density lipoproteins; MCP-1: monocyte chemoattractant protein-1; TNF- α : tumor necrosis factor- α

P < 0.05 is significant.

Table 2. Comparison between morbidly obese patients (after intervention) and controls with regard to studied parameters

Variables	Morbid obese (N=20) mean±SD	Controls (N=15) mean±SD	P value
hs CRP (mg/dl)	4.9±1.9	0.96±0.4	<0.01
Total cholesterol (mg/dl)	179±30	163±17	<0.01
TG (mg/dl)	120±16	79±8	<0.01
HDL (mg/dl)	53±5	64±8	<0.01
LDL (mg/dl)	97±23	83±18	<0.01
% of CD11b+ve monocytes	89±5	82±22	NS
MCP-1 (Pg/dl)	34±15	16±4	<0.01
TNF-α (Pg/dl)	34±16	8±3	<0.01

hs CRP: highly sensitive CRP; TG: triglycerides; HDL: high density lipoproteins

LDL: low density lipoproteins; MCP-1: monocyte chemoattractant protein-1; TNF-α: tumor necrosis factor-α

P < 0.05 is significant. NS: not significant.

Table 3. Comparison between studies variables before and after intervention among the studied cases.

Variables	Before (N=20)	After (N=20)	% change	P value
	mean±SD	mean±SD		
hs CRP (mg/dl)	7.9±0.9	4.9±1.9	37%	<0.01
Total cholesterol (mg/dl)	221±39	179±30	19%	<0.01
TG (mg/dl)	140.5±38	120±16	14%	<0.01
HDL (mg/dl)	42±7	53±5	26%	<0.01
LDL (mg/dl)	150±35	97±23	33%	<0.01
RBS (mg/dl)	122± 57	94±16	18%	<0.05
% of CD11b+ve monocytes	93±4	89±5	3%	NS
MCP-1 (Pg/dl)	59±25	34±15	42%	<0.01
TNF-α (Pg/dl)	138±93	34±16	75%	<0.01

hs CRP: highly sensitive CRP; TG: triglycerides; HDL: high density lipoproteins; LDL: low density lipoproteins.

RBS: random blood sugar; MCP-1: monocyte chemoattractant protein-1; TNF-α: tumor necrosis factor-α

P < 0.05 is significant. NS: not significant

Table 4. Correlation between studied parameters among cases before intervention

Variables	MCP-1	% of CD11b+ve monocytes	TNF- α	hs CRP
hs CRP (mg/dl)	0.66*	0.17	0.56*	-
Total cholesterol (mg/dl)	0.17	0.03	-0.19	0.11
TG (mg/dl)	0.18	0.13	0.06	0.05
HDL (mg/dl)	0.14	0.16	-0.21	0.17
LDL (mg/dl)	0.15	0.10	0.14	0.16
SBP (mmHG)	0.20	0.10	0.19	0.15
DBP (mmHG)	0.19	0.15	0.15	-0.08
% of CD11b+ve monocytes	0.14	-	-	-
MCP-1 (pg/dl)	-	-	-	-
TNF- α (pg/dl)	0.18	0.11	-	-
RBS (mg/dl)	0.08	-0.14	0.12	0.20
BMI	0.21	0.02	0.84*	0.66*
WC	0.32	-0.14	0.67*	0.65*

SBP: systolic blood pressure; DBP: diastolic blood pressure.

RBS: random blood sugar; BMI: body mass index.

WC: waist circumference.

Discussion

The present study revealed that MCP-1 level is significantly higher in obese patients than in the control group. Comparable results were reported by others (Malavazos *et al.*, 2005; Kim *et al.*, 2006). In addition, Kim *et al.* (2006) suggested that human adiposity with BMI of more than 30 kg/m² can influence the level of circulating MCP-1 since it has been demonstrated, in their study, that MCP-1 was significantly increased in obese subjects with BMI >30 kg/m² compared with non obese controls with BMI < 25 kg/m².

A number of possible mechanisms may explain the increase in MCP-1 in obese

patients. Obese patients show definite increase in serum levels of pro-inflammatory cytokines as TNF- α , released from macrophages like MCP-1. Indeed, CRP has also been reported to enhance MCP-1 expression (Devaraj *et al.*, 2004). MCP-1 was shown to be secreted by adipocytes and adipose tissue and its level increases with increasing fat mass (Kim *et al.*, 2006). Moreover, adipocytes may attract and activate macrophages which are the main source of MCP-1 (Curat *et al.*, 2004).

In the current study levels of MCP-1, were significantly decreased by about 42% 3 to 6 months after surgical weight loss than before surgery ($P < 0.01$). Other studies have observed a wide range (20%-47%) of

reduction in serum MCP-1, relative to the level before intervention (Christiansen *et al.*, 2005; Schernthaner *et al.*, 2006).

Herder *et al.* (2006) concluded that weight loss or exercise can decrease systemic MCP-1 concentrations and attributed this to a complex mechanism. Weight loss is associated with a decrease in the proinflammatory cytokines IL-6, IL-1 β and TNF- α and also with a decrease in fat mass (Esposito *et al.*, 2003). Moreover, weight loss leads to a general and systemic attenuation of low-grade inflammation which parallels the weight loss and includes reduction of MCP-1 release from stromal-vascular cells accounting for most of the adipose tissue-derived MCP-1 (Herder *et al.*, 2006). This supports our finding of lower MCP-1 levels in the patients after significant weight loss than before.

C-reactive protein is an acute-phase reactant synthesized mainly in the liver and is regulated by circulating levels of IL-6, IL-1 and TNF- α which originate from adipose tissue (Ode *et al.*, 2009).

Hs-CRP levels showed statistically significant higher levels in obese patients as compared to normal controls. This is in accordance with several studies (Malavazos *et al.*, 2005; Kim *et al.*, 2006; Adriana *et al.*, 2007; Gokalp *et al.*, 2007).

In the current study hs-CRP levels, also, showed a significant decline after surgical weight loss. Similarly, it was reported that even small weight loss and metabolic control were associated with a reduction in hs-CRP levels, supporting the hypothesis that lifestyle modification reduces inflammation and the risk of cardiovascular diseases (Yuji *et al.*, 2005).

Hs-CRP levels were positively correlated with MCP-1 and TNF- α in our patients before surgical weight loss. The study made by Kim *et al.* (2006); showed that the serum levels of CRP were positively correlated with the serum levels of MCP-1 providing a link

between MCP-1 and the low-grade inflammatory condition found in obesity. Moreover, CRP enhances expression of MCP-1 in the human endothelial cell via increased secretion of IL-6 and endothelin-1, a potent endogenous vasoconstrictor (Devaraj *et al.*, 2004).

In the present study a significant elevation of the ratio of CD11b positive monocytes was found in morbidly obese cases (before weight loss) when compared to normal healthy controls. The explanation for this increase in the ratio of CD11b expression in obesity may be due to elevated levels of LDL that are known to induce over-expression of this antigen through the phosphoinositide-dependent signaling pathway leading to an increase in cytosolic-free calcium (Han *et al.*, 2003). This may also be due to the possible stimulation of monocytes to induce expression of CD11b expression by MCP-1, which is known to be increased in obesity (Takahashi *et al.*, 2003). Another explanation might be due to elevated levels of CRP which is known to be increased in obesity and induces CD11b expression on monocytes (Woollard *et al.*, 2006).

After surgical weight loss the ratio of CD11b positive monocytes were decreased in our patients, however, this decrease did not reach a statistical significant level when compared to the ratio of expression before weight loss. In accordance with this finding, Takahashi *et al.* (2003) and Kim *et al.* (2007); found that CD11b-positive monocytes in circulating blood are increased in obesity and decreased after surgery.

Our study showed high TNF- α levels in patients before and after weight loss in comparison to its level among the normal weight controls. Also Liang and his co-workers (2007) and Nieto-Vazquez *et al.* (2008) reported that the concentrations of systemic and local TNF- α were significantly elevated in obese animals. Consistent with

these results, TNF- α has been reported to be over expressed in the adipose tissue of rodents and humans with obesity and insulin resistance (Hotamisligil *et al.*, 1993; Hotamisligil *et al.*, 1995). Moreover, a study by Enzo and his colleagues (2006) showed that the soluble proinflammatory factor, TNF- α , decreases the amount of energy burned by a cell and increases the amount of weight gained by obese rodents.

In the current study TNF- α levels showed a significant decline after surgical weight loss. Similarly, it was found that plasma TNF- α concentration was high, when compared with controls and fell significantly after weight loss. The magnitude of weight loss and fall in TNF- α were related to basal body weight and basal TNF- α concentrations (Dandona *et al.*, 1998). Also, Giugliano (2004) found that after six months of stable body weight after liposuction, women were less insulin resistant, had reduced concentrations of IL-6, IL-18, TNF- α and CRP.

Our study showed significant positive correlation between TNF- α level and BMI. Expression of TNF- α was reported to be in strong positive correlation with the degree of obesity (Uysal *et al.*, 1997). These results suggest that TNF- α may have a direct or indirect effect on the increase of adipose tissue mass. TNF- α potentially augments in vivo adipogenesis, because it may function as a growth factor for preadipocytes (Hotamisligil and Spiegelman, 1994).

The group of morbidly obese patients before surgery showed significant higher mean random blood sugar than its level after weight loss by surgery. High blood glucose levels before weight loss may be due to increased levels of inflammatory markers such as TNF- α , which interfere with insulin-receptor signaling (Tilg and Machen, 2008).

Impressive shift towards normalized glucose tolerance after weight reduction due to improvement of insulin resistance after

weight loss with subsequent decrease in blood glucose (Kopp *et al.*, 2003).

In the current study statistical comparison between obese patients and controls showed statistically significant higher mean total cholesterol, triglycerides, LDL and lower mean HDL in patient group than in the control group.

When the lipid profile was compared in the morbidly obese cases before and after weight loss surgery, statistically significant higher cholesterol, triglycerides, LDL and lower mean HDL was found before than after surgery. These findings were previously recorded by Tzotzas *et al.* (Tzotzas *et al.*, 2006); who found that lipid profile in obese patients return to normal levels after substantial weight loss.

So we concluded that, MCP-1 and activated monocytes, as represented by increased expression of CD11b, in concert with other proinflammatory cytokines such as TNF- α link obesity with systemic inflammation and metabolic disorders. Significant weight loss after restrictive bariatric surgery resulted not only in significant reduction of blood pressure, triglycerides and LDL but also in systemic inflammation, MCP-1 level, CD11b expression on monocytes and TNF- α . This suggests that the latter parameters may play an important role in the pathogenesis of metabolic syndrome accompanying obesity. The interactions of MCP-1, CD11b and other inflammatory parameters might provide the basis for development of new therapies for this syndrome.

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