Impact of weight loss on inflammation and red blood cell biomarkers after laparoscopic gastric banding surgery

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ABSTRACT

Adipose tissue produces several adipokines that are enrolled in different metabolic and inflammatory pathways that may disturb iron metabolism and erythropoiesis. Considering that laparoscopic adjustable gastric banding (LAGB) has not been associated with a long-term risk of malabsorption, we performed a 13-month follow-up study in severe obese patients submitted to LAGB in order to clarify its impact on inflammation, iron metabolism and on red blood cell (RBC) biomarkers. Twenty obese patients were enrolled in the study, being clinical and analytically assessed before (T0) and 13 months after LAGB intervention (T1). Inflammation, iron bioavailability and RBC biomarkers were evaluated at T0 and T1. At T1, weight and anthropometric indices decreased significantly; patients showed a significant increase in mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration (MCHC), and a reduction in red cell distribution width, ferritin, hepcidin, tumor necrosis factor-α, interleukin-6 (IL-6) and C-reactive protein. Before LAGB, IL-6 correlated negatively with iron, hemoglobin concentration and MCHC; hepcidin correlated inversely with transferrin. Our data show that 13 months after LAGB, the weight loss is associated with an improvement in inflammation, namely a reduction in IL-6 that may reduce hepcidin production, improving iron availability for erythropoiesis, as shown by more adequate erythrocyte hemoglobinization.

Significance of this study

What is already known about this subject?
- Adipose tissue produces adipokines that are enrolled in inflammatory pathways.
- Adipokines may disturb iron metabolism and erythropoiesis.
- Weight loss improves inflammatory process.

What are the new findings?
- Weight loss, achieved through laparoscopic adjustable gastric banding, reduces IL-6 production.
- The reduction in IL-6 seems to trigger a decrease in hepcidin production.
- This improves iron availability for erythropoiesis as shown by more adequate erythrocyte hemoglobinization.

How might these results change the focus of research or clinical practice?
- Studies that allow understanding the direct effects of adipose tissue and iron metabolism in obesity are needed.
- In obese subjects and those submitted to weight loss, iron status should be evaluated.

INTRODUCTION

Adipose tissue is an active endocrine organ that secretes adipokines known to interfere with insulin resistance, glucose and lipid metabolisms, as well as with oxidative stress and inflammatory processes.[1] Obesity is associated with low-grade chronic inflammation, shown by increased circulating levels of several proinflammatory cytokines and acute phase reactants, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and C-reactive protein (CRP).[2 3] Another common feature in obesity is hypoferremia, which appears to be a consequence of the chronic low-grade inflammation state. Hepcidin, an acute-phase protein, is a major regulator of iron metabolism, inhibiting iron intestinal absorption and reducing iron mobilization from macrophages of the reticuloendothelial system. Hepcidin binds to the iron exporter channel ferroportin,[4] at the membrane surface of enterocytes, hepatocytes and macrophages, inducing its internalization and degradation, and thus reducing iron availability for erythropoiesis. The continuous inflammatory stimuli in obesity may trigger hepcidin expression.[5] It is known that proinflammatory adipokines, such as IL-6 and leptin, upregulate hepatic hepcidin expression.[5–7]

Proinflammatory cytokines, as TNF-α and interferon-γ,[8 9] are also able to inhibit erythropoiesis. TNF-α induces transcription of the erythropoiesis inhibitor GATA in CD34+ hematopoietic stem/progenitor cells, inhibiting erythroid cell development.[10] TNF-α also induces macrophages, hepatocytes and adipocytes to produce ferritin,[11] an iron storage protein that

is also an acute-phase protein. Moreover, TNF-α is able to inhibit erythropoietin (Epo) production[12] and to reduce Epo receptors and Epo-induced erythroid progenitor cell proliferation.[13]

Bariatric surgery is recommended for patients with a body mass index (BMI) >40 kg/m^2 and for patients with 35–40 kg/m^2, when presenting obesity-related comorbidities, such as hypertension, diabetes or hypercholesterolemia.[14 15] Laparoscopic adjustable gastric banding (LAGB) is minimally invasive and, when compared with other laparoscopic bariatric procedures, shows lower rates and severity of associated complications, lower hospital readmission and lower mortality.[16 17] Surgical injury induces a systemic inflammatory metabolic-endocrine response that seems to be proportional to the severity of the surgical stress. A study by Zengin *et al*[18] evaluated IL-6 and CRP at 12, 24 and 48 hours after LAGB and after open stoma-adjustable silicone band application, and reported a significantly lower inflammatory response for laparoscopic gastric banding. LAGB has not been associated with malabsorption or with disturbances in iron metabolism, as the area of iron absorption (duodenum) is not bypassed.[19] However, as the other bariatric procedures, it has been associated with acquired postoperative food intolerance,[20–22] eventually to red meat, an important dietary source of iron. LAGB leads to substantial weight loss, as occurs with other bariatric procedures.[23]

Over the past years, as the incidence of obesity increased worldwide, the use of bariatric surgery to treat severe obesity has increased. Considering that LAGB has not been associated with a long-term risk of malabsorption, we performed a 13-month follow-up study in severe obese patients who were submitted to LAGB in order to clarify the impact of weight loss on inflammation, iron metabolism and on red blood cell (RBC) biomarkers.

MATERIALS AND METHODS

Subjects

Patients were invited to participate and enrolled in the study after informed and written consent. Patients who were more than 18 years old, with a BMI >40 kg/m^2 or a BMI 35–40 kg/m^2 when presenting obesity-related comorbidities, such as arterial hypertension, diabetes and/or hypercholesterolemia, were the criteria of inclusion in the study. One of the patients included in the study had a BMI of 34.2 kg/m^2 and obesity-related comorbidities, arterial hypertension and dyslipidemia.

Twenty obese patients were assigned to the follow-up study, 18 women and 2 men, with ages ranging between 31 and 57 years old. Patients were clinically and analytically evaluated before (T0) and 13 months after (T1) LAGB (LAP-BAND, Allergan, Irvine, California, USA).

Sociodemographic and clinical evaluation included weight, height (Ht), waist circumference (WC) and hip circumference (HC); the ratios between WC and Ht (WC:Ht) and between WC and HC (WC:HC) were calculated, as well as BMI.

Collection and preparation of blood samples

Blood was collected after an overnight fast, by venipuncture, into tubes with and without anticoagulant (EDTA), in order to obtain whole blood and plasma, and serum, respectively. Samples were processed within 2 hours of collection; aliquots of plasma and serum were prepared and immediately stored at −80°C until assayed.

Analytical assays

Plasma levels of IL-6, hepcidin, soluble transferrin receptor, TNF-α and Epo were evaluated by enzyme immunoassays (Quantikine ELISA Human IL-6 Immunoassay, Quantikine ELISA Human Hepcidin Immunoassay, Quantikine IVD Soluble Transferrin Receptor ELISA, and Quantikine HS ELISA Human TNF-alpha Immunoassay, R&D Systems, Minneapolis, Minnesota, USA; eBioscience Human Erythropoietin Platinum ELISA (Short Incubation), Bender MedSystems, Vienna, Austria, respectively).

Iron concentration was determined using a colorimetric method (Iron, Randox Laboratories, North Ireland, UK), whereas ferritin, transferrin and CRP were measured by immunoturbidimetry (Ferritin, Randox Laboratories, Northern Ireland, UK; Transferrin, Randox Laboratories, Northern Ireland, UK; CRP (Latex) High-Sensitivity, Roche Diagnostics, Basel, Switzerland, respectively). Transferrin saturation (TS) was calculated according to the formula TS (%)=70.9 × serum iron levels (μg/dL)/transferrin (mg/dL). RBC count, hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), and total and differential white blood cell (WBC) counts were evaluated by using an automatic blood cell counter (Sysmex XT-1800i; Sysmex, Hamburg, Germany).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS V.22.0) for Windows. The parametric variables are presented as mean±SD and the non-parametric variables are presented as median (IQR). Differences before and after LAGB intervention were tested using Wilcoxon signed-ranks test and the paired Student's t-test, in accordance with the Gaussian distribution of the variables. Spearman's rank correlation coefficient was used to evaluate relationships between sets of data. A p value lower than 0.05 was considered as statistically significant.

RESULTS

Thirteen months after LAGB intervention, weight, BMI, WC, HC, WC:Ht and WC:HC ratios decreased significantly (table 1). At T0, 5% (n=1) of patients presented a BMI between 30.0 and 34.9 kg/m^2, 60% (n=12) between 35.0 and 39.9 kg/m^2, and 35% (n=7) a BMI >40 kg/m^2; 65% of patients presented one or more comorbidities, namely arterial hypertension (55%), dyslipidemia (40%) and type 2 diabetes (10%). After 13-month LAGB (T1), the obese patients presented a mean weight reduction of 13.5 kg and a mean BMI reduction of 5.2 kg/m^2; moreover, at T1, 15% of the patients presented a BMI between 25.0 and 29.9 kg/m^2, 45% between 30.0 and 34.9 kg/m^2, 35% between 35.0 and 39.9 kg/m^2, and 5% a BMI >40 kg/m^2.

Concerning hematologic evaluation, at T1, obese patients presented a significant decrease in RBC count, Hb and Hct, alongside with a significant increase in MCV, MCH and MCHC, and in Epo levels; the reduction in RDW was not

Table 1 Anthropometric data before (T0) and 13 months after (T1) laparoscopic adjustable gastric banding (n=20)

	T0	T1	p
Weight (kg)	100.6±11.0	87.1±11.3	<0.001
Height (m)	1.60±0.07	1.60±0.07	–
BMI (kg/m^2)	39.4±2.9	34.2±4.3	<0.001
WC (cm)	116.5±7.6	101.8±9.2	<0.001
HC (cm)	125.2±11.6	115.5±9.8	0.006
WC:Ht	0.73±0.05	0.64±0.06	0.001
WC:HC	0.94±0.08	0.88±0.06	0.009

Values are presented as mean±SD or as median (IQR).
BMI, body mass index; HC, hip circumference; WC, waist circumference; WC:HC, waist circumference to hip circumference ratio; WC:Ht, waist circumference to height ratio.

statistically significant (table 2); total WBC, neutrophil and monocyte counts presented a significant reduction.

The study of iron metabolism showed a significant decrease in ferritin and hepcidin at T1. The inflammatory markers, TNF-α, IL-6 and CRP, presented a significant decrease at T1 (table 2).

We found that at T0, IL-6 was positively correlated with CRP (r=0.479; p=0.032), and negatively correlated with Hb concentration (r=−0.453; p=0.045), MCHC (r=−0.525; p=0.017) and iron (r=−0.659; p=0.002); hepcidin was negatively correlated with Epo (r=−0.617; p=0.004) and transferrin (r=−0.537; p=0.015), and positively correlated with ferritin (r=0.473; p=0.035); a significant positive correlation was also found between TNF-α and ferritin (r=0.506; p=0.023). All these correlations, observed before gastric banding intervention, were lost at T1.

DISCUSSION

Thirteen months after LAGB, the achieved weight loss was associated with an improvement in obesity-associated inflammation that seems to lead to a more adequate iron availability and erythrocyte hemoglobinization.

The increasing oxygen demands, due to the high BMI in obesity, triggers erythropoiesis to maintain an adequate number and hemoglobinization of circulating erythrocytes. After LAGB, the reduction in BMI was followed by a significant reduction in RBCs, Hb concentration and Hct, explaining the significant increase in Epo levels. The reduction in TNF-α (an inhibitor of Epo production) may also contribute to the Epo increment.

Our data confirmed the importance of LAGB on the promotion of weight loss (table 1), as shown by the significant decrease observed in body weight, BMI, hip and waist circumferences, and in the anthropometric indices. The distribution of BMI values before and after LAGB also strengthens this improvement.

The significant reduction of IL-6 and CRP, following weight loss induced by LAGB, showed improvement in the inflammatory state (table 2). The reduced vascularization

Table 2 Hemogram values, Epo, iron metabolism and inflammatory markers levels in patients (n=20), before (T0) and 13 months after (T1) laparoscopic adjustable gastric banding

	T0	T1	p
RBC (×10^2/L)	4.94 (4.81–5.18)	4.25 (4.09–4.45)	<0.001
Hemoglobin (g/dL)	14.35±1.13	12.99±1.00	<0.001
Hematocrit (%)	43.62±2.87	38.24±3.56	<0.001
MCV (fL)	86.63±5.13	88.55±4.71	0.005
MCH (pg)	28.48±1.66	30.13±1.65	<0.001
MCHC (g/dL)	32.90±1.21	34.02±1.09	0.004
RDW (%)	13.50±0.90	13.12±0.91	0.050
WBC (×10^9/L)	7.68 (6.78–8.61)	5.75 (5.25–6.63)	<0.001
Neutrophils (×10^9/L)	4.55 (3.73–5.35)	3.27 (3.08–4.14)	<0.001
Eosinophils (×10^9/L)	0.20 (0.10–0.20)	0.11 (0.05–0.16)	0.005
Basophils (×10^9/L)	0.05 (0.00–0.10)	0.02 (0.00–0.07)	0.308
Lymphocytes (×10^9/L)	2.45 (2.20–2.70)	1.98 (1.69–2.09)	<0.001
Monocytes (×10^9/L)	0.40 (0.30–0.48)	0.26 (0.21–0.34)	<0.001
Epo (mUI/mL)	5.20 (2.50–6.16)	5.43 (3.22–7.85)	0.005
Iron (µg/dL)	103.2±37.5	88.3±33.2	0.095
Transferrin (mg/dL)	274.8 (240.4–326.4)	268.7 (233.8–334.0)	0.911
Transferrin saturation (%)	24.57 (20.98–30.37)	21.06 (14.15–31.38)	0.079
sTfR (nmol/L)	19.03 (14.36–26.21)	17.72 (13.02–25.76)	0.313
Ferritin (µg/L)	164.9±95.8	119.4±84.4	<0.001
Hepcidin (ng/mL)	54.04 (27.59–65.70)	23.11 (6.66–32.86)	0.001
CRP (mg/dL)	0.66 (0.32–1.02)	0.30 (0.12–0.48)	0.001
IL-6 (pg/mL)	2.71 (1.69–4.68)	1.60 (1.00–2.72)	0.001
TNF-α (pg/mL)	1.11 (0.88–1.34)	0.58 (0.45–0.75)	<0.001

Values are presented as mean±SD or as median (IQR).
CRP, C-reactive protein; Epo, erythropoietin; IL-6, interleukin-6; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red cell distribution width; sTfR, soluble transferrin receptor; TNF-α, tumor necrosis factor-α; WBC, white blood cell.

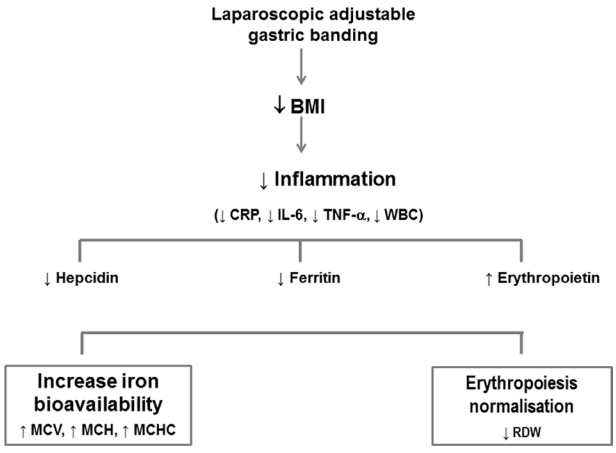

Figure 1 Impact of weight loss on inflammation, erythropoiesis and iron bioavailability markers after laparoscopic gastric banding surgery. BMI, body mass index; CRP, C-reactive protein; IL-6, interleukin-6; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, red cell distribution width; TNF-α, tumor necrosis factor-α; WBC, white blood cell.

of the increased hypertrophic adipocytes in obesity leads to a hypoxic environment that enhances production of proinflammatory cytokines, creating an inflammatory milieu, able to interact with different metabolic pathways. The hepatic production of CRP may be triggered by IL-6, TNF-α and IL-1 produced by the increased adipose tissue. The adipose tissue itself is able to produce CRP, and its expression has been correlated with IL-6 expression.[24] In obesity, the large amount of body fat may produce an important part of circulating CRP. Thus, both hepatic and adipose tissues might contribute to the high plasma CRP levels found in obesity, probably, however, with different contributions. Indeed, we found a positive and significant correlation between IL-6 and CRP before LAGB that was not observed after weight loss, suggesting that the adipose tissue may have a contribution for their circulating levels, according to the severity of obesity.

Besides the significant reduction in the inflammatory markers (TNF-α, IL-6 and CRP), we also found a significant reduction in hepcidin and ferritin, as well as in total WBC, neutrophil and monocyte counts (table 2), further showing the reduction in obesity-associated inflammation. Our data are in accordance with other studies, reporting a decrease in CRP,[25 26] IL-6[27], TNF-α[25] and hepcidin[28] after weight loss induced by bariatric surgery. Concerning hepcidin, there are few studies evaluating the impact of LAGB-induced weight loss on its levels. As far as we know, this is the first report addressing the concomitant evaluation of these variables, allowing the study of the interplay between them.

Low levels of iron are common in obesity and may result from inadequate dietary iron ingestion to face the increased iron requirements for a higher body mass and increasing blood volume.[29 30] However, the chronic low-grade inflammatory state of obesity may trigger a functional iron deficiency that in the more severe cases of inflammation may lead to a chronic inflammatory anemia. It is known that IL-6 triggers hepcidin synthesis, altering iron bioavailability and erythropoiesis. Indeed, the increased production of hepcidin, by triggering a reduction in iron absorption and mobilization from iron stores, reduces iron availability for Hb synthesis in erythroid cells, and thus leads to the formation of smaller and less hemoglobinized RBCs. In accordance, we found that weight loss, induced by LAGB, was associated with a significant increase in MCV, MCH and MCHC and a reduction in RDW (table 2), showing an improvement in iron availability for erythropoiesis (figure 1). Moreover, the significant decrease in ferritin (an acute phase protein) was also observed, accompanying the reduction in inflammation and the reduction in the functional iron deficiency, as shown by a better mobilization of iron (from iron storages) for erythropoiesis (figure 1). In spite of adequate iron stores, in case of functional iron deficiency, there is a reduced iron absorption and a failure in iron release for erythropoietic demands.[31]

The close relationship between the inflammatory process, iron metabolism and erythropoiesis is supported by the negative correlations of IL-6 with iron, Hb concentration and MCHC, and of hepcidin with transferrin, before weight loss induced by LAGB. Actually, the adipose tissue seems to have a crucial role in the crosstalk between the triad erythropoiesis–inflammation–iron metabolism. In order to clarify the interplay between weight loss and these players, it would be important to evaluate their changes with non-surgical weight loss.

The studies evaluating the effect of weight loss after LAGB on biomarkers of inflammation, iron metabolism and RBC biomarkers are few and often controversial. This might result from the enrollment of patients with different degrees of obesity (achieving different weight loss), coexistence of different comorbidities and different follow-up periods of evaluation. In the present study, although a significant decrease in weight and BMI was achieved (a mean decrease of 13.5 kg and 5.2 kg/m^2, respectively), 85% of patients were still obese after 13 months following LAGB intervention. Moreover, the adhesion of patients to a more healthy diet and lifestyle habits is not often observed.

Considering that the relationships between hepcidin, iron status, erythropoietic activity and inflammation are complex, further studies are needed to understand the direct effects of adipose tissue and iron metabolism in obesity; however, it seems that a functional iron deficiency should be considered in case of obese patients. Apparently, in obese patients, despite adequate iron stores, as defined by conventional criteria, iron may not be released rapidly enough to effectively support erythropoiesis. According to our data, weight loss improves iron metabolism, suggesting that this could be a suitable therapeutic option for functional iron deficiency, in obesity.

In summary, our data show that 13 months after LAGB, weight loss was associated with an improvement in inflammation, namely a reduction in IL-6 that, by reducing hepcidin production, improves iron availability for erythropoiesis, as shown by more adequate erythrocyte hemoglobinization.

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