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ORIGINAL ARTICLE

Body composition changes after laparoscopic adjustable gastric banding: what is the role of −174G > C interleukin-6 promoter gene polymorphism in the therapeutic strategy?

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Background: There is growing evidence that interleukin-6 (IL-6) is linked to the regulation of fat mass (FM). Our previous data define the common −174G > C IL-6 polymorphism as a marker for 'vulnerable' individuals at risk of age- and obesity-related diseases. An association between −174G > C IL-6 polymorphism and weight loss after bariatric surgery has been demonstrated.
Objective: We investigated the impact of −174G > C IL-6 polymorphism on weight loss, body composition, fluid distribution and cardiometabolic changes in obese subjects, after laparoscopic adjustable gastric banding (LAGB) surgery.
Design and Outcome measures: A total of 40 obese subjects were studied at baseline and at 6 months follow-up after LAGB surgery. Cardiometabolic and genetic assessment of −174G > C IL-6 polymorphism, anthropometric, body composition and fluid distribution analysis were performed.
Results: After LAGB surgery, significant reductions in weight ($\Delta\% = -11.66 \pm 7.78$, $P<0.001$), body mass index ($P<0.001$), total and trunk FM (kg, %) ($\Delta\%$ of total FM $= -22.22 \pm 12.15$, $P<0.01$), bone mineral density (*T*-score) ($P<0.001$), resting metabolic rate (RMR) ($P<0.01$), and total body water and intracellular water (TBW, ICW) ($P<0.05$) were observed. At baseline, C(−) carriers of IL-6 polymorphism had a significantly higher RMR ($P<0.05$), free FM (kg), but less total and trunk FM (%), higher body cell mass (BCM), content of TBW (L) and ECW (extracellular water)/ICW ratio compared with C(+) carriers ($P<0.001$). After LAGB, C(+) carriers had a significantly stronger reduction of total FM (kg), but lower bone density, compared with C(−) carriers ($P<0.05$).
Conclusions: Beyond the relationship between −174G > C IL-6 polymorphism and body composition, this study provides first evidence about the association of IL-6 variant with fluid distribution, at baseline, and FM and bone density loss in obese subjects at 6 months follow-up after LAGB surgery. LAGB was less effective if the subjects were carrying risk genotypes, C(−) carriers, for obesity, suggesting a role of genetic variations on bariatric surgery outcomes.
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Keywords: body composition; laparoscopic adjustable gastric banding; −174G > C IL-6 polymorphism

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Introduction

Adipose tissue is one of the main sources of inflammatory mediators, with interleukin-6 (IL-6) among them. In circulating blood, 15–35% of total (noninflammatory) IL-6 concentration has been estimated to originate from adipose tissue.[1] The adipocytes themselves can produce and secrete IL-6 but they may only contribute to a fraction of total IL-6 released by the adipose tissue as non-fat cells are also capable of producing it.[2–5] Adipose tissue-derived IL-6 may have an effect on metabolism through several mechanisms, including adipose tissue-specific gene expression, triglyceride release, lipoprotein lipase downregulation, insulin sensitivity, and so on.

Recent studies have shown that IL-6-deficient mice develop mature onset obesity with high leptin levels in the circulation.[6] Moreover, intra-cerebro-ventricular IL-6 treatment decreases body fat and increases energy expenditure in rodents.[7,8] Thus, besides regulating the immune system, IL- 6 also has a role in the regulation of body fat and energy expenditure.[9]

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Although high systemic levels of inflammatory mediators are cachectogenic and/or anorexic, today it is a widely propagated thesis that in the background of obesity, a low level of chronic inflammation can be found, with IL-6 being one of the many suggested mediators. As the adipose tissue expands, there is insufficient vasculature to maintain normoxia throughout the organ, clusters of adipocytes then become relatively hypoxic, and an inflammatory response ensues that serves to increase blood flow and to stimulate angiogenesis.[10] Obesity is also associated with mechanical stress, excess lipid accumulation, abnormalities in intracellular energy fluxes and nutrient availability, that can in turn exacerbate the vicious circle linking obesity, oxidative stress, inflammation and metabolic disorders.[11–14]

In addition to inducing inflammatory signals, IL-6 plasma concentration also depends on endogen transcription regulation, like the presence of single-nucleotide polymorphisms. The common −174G>C IL-6 single-nucleotide polymorphism, which is located within the negative regulative domain of the IL-6 gene promoter, has been found to affect transcriptional regulation. Fishman *et al*. (1998)[15] observed that the −174 C allele was associated with decreased plasma IL6 levels in healthy subjects.

Some comparatively small studies indicate that the functional −174G>C IL6 polymorphism is connected with increase of fat mass (FM), and its surrogated parameters, such as body mass index (BMI) and leptin levels.[16–18] Such variation may be sensed by the homeostatic feedback system that controls energy balance and may in turn contribute to some disturbances in weight regulation, particularly in a situation of negative energy balance, like induced weight loss.[19]

Among several anti-obesity approaches, bariatric surgery seems to represent a valid strategy to treat severe obesity and related diseases. Laparoscopic adjustable gastric banding (LAGB) is a restrictive comparable procedure, reversible and showing a very low rate of complication.[20] Previous data demonstrated that genetic factors, such as −174G>C IL-6 polymorphism, which have an important role in the regulation of body weight (BW), may account for differences in the therapeutic response to LAGB.[21]

Because of several, but not really exhaustive, data regarding the relevance of IL-6 on body composition and adipose tissue homeostasis regulation, we investigated the role of −174G>C IL-6 polymorphism on the efficacy of weight loss, body composition and fluid distribution changes in obese subjects, evaluated by dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA), at 6 months follow-up after LAGB surgery.

Patients and methods

Patients' recruitment

The study group consisted of 62 Italian Caucasians with obesity, that is, grade II–III obesity according to the World Health Organization criteria,[22] consecutively recruited at the San Camillo-Forlanini Hospital (Rome, Italy) from November 2008 to November 2009. Patients desiring surgical intervention for the treatment of obesity were referred to the San Camillo-Forlanini Hospital, which has experience in the care of obese and bariatric patients, to determine the patients' eligibility for surgery on the basis of the international guidelines.[23] Only patients with BMI > 40 kg m^{-2} or with BMI > 35 kg m^{-2} with comorbidities, in whom all appropriate non-surgical measures failed to achieve or maintain adequate weight loss for at least 6 months, without any psychiatric problem and/or drug or alcohol addiction were admitted to surgery. Exclusion criteria were secondary causes of obesity, pregnancy, antipsychotic medication, eating disorder, severe altered self body image and non-realistic expectations about weight reduction. Psychiatric problems were evaluated on the basis of tests such as eating disorder examination, body dysmorphic disorder examination and other tests related to general psychopathology.[24] The Binge Eating Scale, an easily administered 16-item questionnaire with a range between 0–42, was used to assess symptoms of binge eating. Higher scores indicate greater degree of binge eating severity.[25] Participation in the study included a complete medical history to gather information about health status, current medication history, including supplements of vitamin and mineral, social habits, like alcohol drinking and smoking, appetite,[26] physical activity (PA) [27] and family history for chronic diseases.

Patients were instructed to not modify PA during 6 months of follow-up after LAGB. None of the patients was receiving drug treatments at the time of the assessment. If a subject was eligible, an operation was scheduled after an informed consent was obtained. A total of 50 subjects were eligible and admitted to the intervention, and underwent LAGB surgery, an adjustable gastric band (Lap-Band, Inamed, Santa Barbara, CA, USA). A multidisciplinary team (an internist, a cardiologist, an endocrinologist, a gastroenterologist, a psychiatrist, a surgeon and a nutritionist) met with each patient, and provided an educational session regarding risk and benefits of bariatric surgery and a nutrition and meal-planning guidance. A total of 40 patients (28 females and 12 males) gave consensus for the study assessments, completed the screening for anthropometry, body composition and fluid distribution 6 months after surgery and were successfully genotyped for the −174G>C IL-6 polymorphism (Figure 1).

All patient assessments were conducted in collaboration between San Camillo-Forlanini Hospital and the University of Rome Tor Vergata, Human Nutrition Unit. The collection of DNA as well as the experiments were approved by the Ethical Commission of the University of 'Tor Vergata', Rome, Italy. A statement of informed consent was signed by all participants in accordance with principles of the Declaration of Helsinki.

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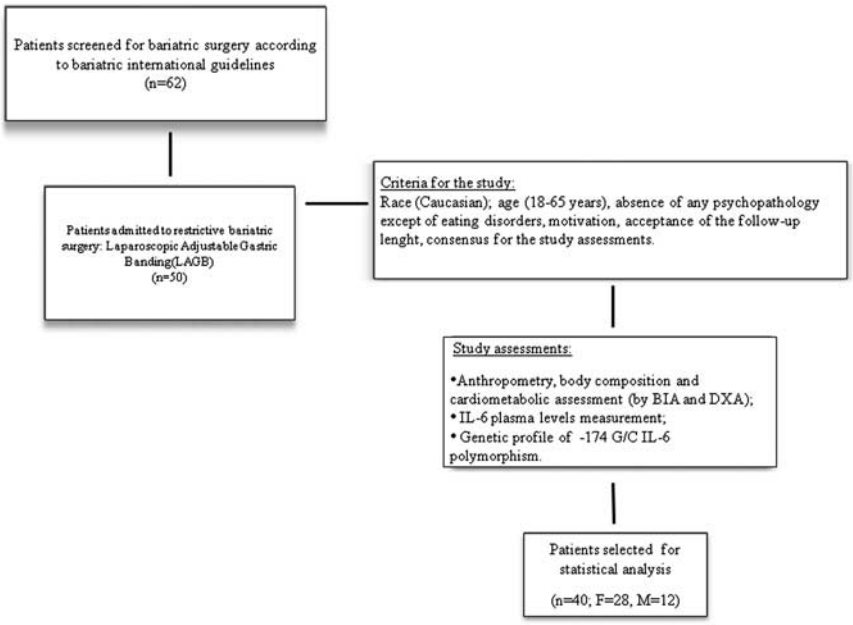

Figure 1 Selection flowchart and experimental design.

Dietary intervention following LAGB

A standardized protocol for meal progression after LAGB was assigned to patients. The acute postoperative diet (first and second day) consisted of non-carbonated clear liquids with no calories, no sugar and no caffeine. For the first month after LAGB, a semiliquid diet of 850 kcal d^{-1} was prescribed (33% proteins, 19% lipids, 48% carbohydrates). One month after LAGB, a solid diet was reintroduced, and the suggested diet was 1200 kcal d^{-1}; iron was supplemented on the basis of blood examinations performed during the second month. Diet included 48% carbohydrates (starch or bread), 33% proteins (fat-free parts of different animals and fish) and 19% lipids (olive oil); sweets, cakes, sweetened drinks, alcohol and animal lipids were forbidden. Compliance of diet (and PA) was reviewed monthly by the multidisciplinary team.

Anthropometric measurements

After a 12-h overnight fast, all subjects underwent anthropometric evaluation. Anthropometric parameters of all the participants were measured according to standard methods (BW and height).[28] Subjects were instructed to take off their clothes and shoes before performing all the measurements. BW (kg) was measured to the nearest 0.1 kg, using a balance scale (Invernizzi, Rome, Italy). Height (cm) was measured using a stadiometry to the nearest 0.1 cm (Invernizzi). BMI was calculated using the formula: BMI = BW (kg)/height $(m)^2$.

DXA

Body composition was assessed by DXA (Lunar DPX-IQ; GE Medical Systems, Milwaukee, WI, USA), according to the previously described procedure.[29] The subjects were instructed not to exercise within 24 h from the test. The subjects were given complete instructions on the testing procedure. They wore a standard cotton t-shirt, shorts and socks. They laid supine on the DXA without moving, while the DXA scan recorded their results. The average measurement time was 20 min. The abdominal region of interest was selected by conventional whole body DXA from L2 to L5 vertebral disc space. Total, abdominal, trunk and legs indexes were calculated as previously reported.[29]

The effective radiation dose from this procedure is about 0.01 mSv. The coefficient of variation (CV% = 100 × s.d./mean) for intra- and intersubjects ranged from 1 to 5%. The coefficient of variation for bone mass measurements is $<1\%$; coefficient of variations on this instrument for five subjects scanned six times over a 9-month period were 2.2% for FM and 1.1% for lean mass (LM).

BIA

Resistance, reactance, impedance and phase angle at 50 kHz frequency were measured using a BIA phase sensitive system (BIA 101S, Akern/RJL Systems, Florence, Italy). Measurements were taken on left side of the body, with injection and sensor electrodes placed on the hand and foot in reference position. To calculate total body water (TBW) (%),the formula proposed by De Lorenzo *et al*.[30] was used.

RMR measurement

The calculation of RMR from oxygen consumption (VO_2) and carbon dioxide production (VCO_2) was made according

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to Weir's equation, after body composition analysis and LM evaluation.[31]

$VO_2 = LM\ (kg) \times 4.5\ (men)$
$VO_2 = LM\ (kg) \times 5.3\ (women)$
$VCO_2 = VO_2 \times 0.85$
$RMR\ (kcal/diet) = (3.941\ (VO_2) + 1.106\ (VCO_2)) \times 1.44$

Analysis of blood samples

Blood samples (10 ml) were collected into sterile tubes containing EDTA (evacuated tubes), via venipuncture early in the morning (0700–0900 hours) after an overnight fast (12 h). All materials were immediately placed in ice, and plasma was separated by centrifugation at $1600 \times g$ for 10 min at 4 °C. Plasma samples were stored at −70 °C in 1 ml aliquots until assayed for the cytokine measurements. For cardiometabolic evaluation, fasting glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride concentrations were measured. The cardiovascular risk indexes were calculated as previously performed.[14] Hypertension, dyslipidemia and metabolic syndrome were defined according to IDF criteria for metabolic syndrome definition.[32] Prevalence of diabetes mellitus was evaluated according to WHO/IDF Consultation report.[33]

Cytokine assay

Plasma concentration of IL-6 was measured in duplicate by using the multiplex sandwich enzyme-linked immunosorbent assay (SearchLight Human Inflammatory Cytokine Array 1; Endogen, Perbio, IL, USA). All assays were conducted according to the manufacturer's instructions. The lower limit of detection was $0.2\ pg\ ml^{-1}$. The intra- and interassay variations of the SearchLight Human Inflammatory Cytokine Array 1 were <12%.

DNA genotyping

Volunteers were genotyped for the −174G>C IL-6 gene promoter polymorphism. Genomic leukocyte DNA was extracted from peripheral blood according to the standard procedure. The genotyping of −174G>C IL-6 polymorphism was performed by polymerase chain reaction using primers previously published[34] and was followed by the single-strand conformation polymorphism analysis. The CC and the GC genotypes were grouped and indicated as C carriers (C+), and GG genotype was named as C no carriers (C−). Among Europeans, genotype frequencies are 50% of GG, 35% of GC and 15% of CC (www.SNPedia.com).

Statistical analysis

Data are presented as group means ± s.d. or percentage. Data were analyzed to check assumptions about the distribution of the measured variables. A χ^2 test was also used to evaluate the Hardy–Weinberg equilibrium of the observed genotype frequencies with respect to the general population. Three genotype groups were first considered to check differences in considered variables between groups. As a dominant or recessive effect existed, analysis was repeated comparing carrier (C+) vs non-carrier (C−) groups. Comparisons among genotype groups were performed using analysis of covariance, by using gender and baseline FM (kg, %) as covariates. Adjustment for baseline FM (kg, %) was performed only for the comparison of changes (%) across genotypes. A paired *t*-test was performed to evaluate differences before and after LAGB surgery. All tests were considered significant at $P \leqslant 0.05$. Statistical analysis was performed using a computer software package (SPSS for Windows, version 13.0; SPSS, Chicago, IL, USA).

Results

Table 1 shows the medical history of all 62 subjects screened for LAGB surgery according to bariatric international guidelines. In all 60% of the total population was at risk for high systolic BP (⩾130 mm Hg) and 25% for high diastolic BP (⩾85 mm Hg) and 30% of patients showed dyslipidemia (triglycerides ⩾150 mg dl^{-1}, HDL cholesterol ⩽40 mg dl^{-1} in males and ⩽50 mg dl^{-1} in females); 10% was affected by diabetes (fasting glucose: ⩾126 mg dl^{-1}); 50% was the prevalence of metabolic syndrome. According to the SNAQ score, the appetite was very good for 70% and regular for 30% of subjects. According to the Binge Eating Scale questionnaire, any patients showed binge eating disorders. The level of PA was classified into three categories (sedentary, moderate and vigorous) based on the time spent on life activity or programmed physical exercise. According to PA questionnaire, any subjects spent time for vigorous PA, the

Table 1 Medical history of total population

Variable (n = *62*)	*Women* (n = *47*)	*Men* (n = *15*)
Age[a]	43.7 ± 10.8	42.7 ± 10.6
Obesity of II degree[b]	25.6	40.0
Obesity of III degree[b]	74.4	60.0
Systolic BP (⩾130 mm Hg)[b]	59.6	60.0
Diastolic BP (⩾85 mm Hg)[b]	23.4	26.7
Triglycerides (⩾150 mg dl^{-1})[b]	19.1	60.0
HDL cholesterol (⩽40 mg dl^{-1} in males and ⩽50 mg dl^{-1} in females)[b]	14.9	80.0
Fasting glucose (⩾126 mg dl^{-1})[b]	10.6	13.3
Metabolic syndrome[b]	42.5	66.6
SNAQ[a]	17.1 ± 1.8	17.4 ± 2.1
BES[a]	2.5 ± 0.8	2.5 ± 0.9
Vigorous PA (time per week)[a]	—	—
Moderate PA (time per week)[a]	1.6 ± 0.4	1.9 ± 0.6
Sedentary behaviour (hours per day)[a]	10.9 ± 1.2	10.1 ± 1.4

Abbreviations: BES, Binge Eating Scale; BP, blood pressure; HDL, high-density lipoprotein; PA, physical activity; SNAQ, Simplified Nutritional Appetite Questionnaire. [a]Data are expressed as mean ± s.d. [b]Data are expressed as %.

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Table 2 Anthropometric, body composition, fluid distribution and cardiometabolic parameters at baseline and 6 months of follow-up after LAGB surgery

Variable (n = 40)	*T0*	*T1*
Weight (kg)[a]	120.77 ± 23.97	106.86 ± 24.11[d]
BMI (kg m^{-2})[a]	44.90 ± 7.81	39.53 ± 7.67[d]
FM (kg)[a]	55.15 ± 14.71	44.37 ± 14.10[d]
FM (%)[a]	47.43 ± 6.51	42.69 ± 6.30[d]
LM (kg)[a]	57.75 ± 14.13	55.73 ± 13.08
Total index[a]	1.09 ± 0.34	1.32 ± 0.35[d]
L2–L5 trunk FM (kg)[a]	6.74 ± 2.58	5.15 ± 2.13[c]
L2–L5 trunk FM (%)[a]	53.76 ± 5.39	48.52 ± 6.29[c]
L2–L5 trunk LM (kg)[a]	6.05 ± 2.42	5.40 ± 1.90[b]
Abdominal index[a]	0.89 ± 0.20	1.12 ± 0.25[c]
Trunk index[a]	0.97 ± 0.28	1.16 ± 0.31[c]
Legs index[a]	1.21 ± 0.44	1.54 ± 0.54[d]
BMD[a]	1.32 ± 0.14	1.24 ± 0.14[d]
T-score[a]	2.12 ± 1.01	1.26 ± 1.15[d]
BCM (kg)[a]	38.05 ± 10.70	36.65 ± 10.72
BCMI[a]	13.28 ± 3.28	12.97 ± 2.71
TBW (l)[a]	51.32 ± 13.12	48.83 ± 12.09[b]
ECW (l)[a]	21.75 ± 4.90	21.64 ± 5.43
ICW (l)[a]	29.58 ± 8.80	27.19 ± 7.59[b]
ECW/ICW ratio	0.76 ± 0.13	0.81 ± 0.15[b]
RMR (kcal per diet)[a]	2022.32 ± 377.28	1904.19 ± 309.85[d]
Fasting glucose (mg dl^{-1})[a]	95.25 ± 16.68	90.00 ± 18.56
Total cholesterol (mg dl^{-1})[a]	217.40 ± 48.44	211.41 ± 43.92
LDL cholesterol (mg dl^{-1})[a]	143.33 ± 54.04	135.67 ± 30.92
HDL cholesterol (mg dl^{-1})[a]	58.67 ± 19.53	54.95 ± 14.40
Triglycerides (mg dl^{-1})[a]	141.88 ± 52.39	117.50 ± 37.23
Total cholesterol/HDL cholesterol[a]	4.31 ± 1.13	4.28 ± 1.11
LDL cholesterol/HDL cholesterol[a]	2.44 ± 0.60	2.29 ± 0.50
Log triglycerides/HDL cholesterol[a]	0.41 ± 0.20	0.42 ± 0.35
Plasma IL-6 (pg ml^{-1})[a]	3.85 ± 0.40	2.93 ± 0.50

Abbreviations: Abdominal index, L2–L5 LM/L2–L5 FM; BCM, body cell mass; BCMI, body cell mass index; BMD, bone mineral density; ECW, extracellular body water; FM, Fat Mass; HDL, high-density lipoprotein; ICW, intracellular body water; LAGB, laparoscopic adjustable gastric banding; LDL, Low-density lipoprotein; Legs index, Legs LM/legs FM; LM, Free Fat Mass; L2–L5, L2 to L5 vertebral disc space; RMR, resting metabolic rate; TBW, total body water; Total index, total body LM/total body FM; Trunk index, Trunk LM/trunk FM; T0, Baseline; T1, 6-months follow-up. [a]Data are expressed as mean ± s.d. [b]$P \leqslant 0.05$. [c]$P \leqslant 0.01$ [d]$P \leqslant 0.001$ with paired *t*-test, T0 vs T1.

most spent 1–2 times per week for moderate PA, with a sedentary behavior ranging from 7 to 13 h per day.

Of all recruited subjects, 50 of them underwent to LAGB surgery and 40 (F = 28, M = 12) were selected for data analysis. No postoperative complications occurred in selected patients. After surgery, a significant reduction in food intake was observed at the repeated dietary assessments, and the composition of the diet fit quite well with dietary prescriptions, with a good nutritional compliance.

We evaluated the efficacy of LAGB surgery and the effects on anthropometry, body composition, fluid distribution, some cardiometabolic parameters and IL-6 plasma levels of selected patients after 6 months follow-up (Table 2).

Weight and BMI

LAGB surgery determined significant reductions in weight ($\Delta = -13.9 \pm 9.8$ kg; $\Delta\% = -11.66 \pm 7.78$, $P < 0.001$) and BMI

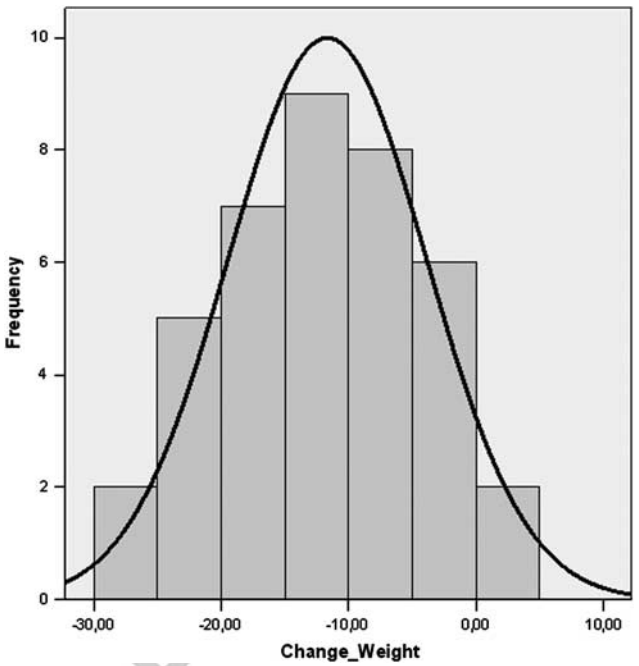

Figure 2 Variability in weight change after LAGB surgery.

($\Delta = -5.4 \pm 3.5$ kg; $\Delta\% = -11.95 \pm 7.37$, $P < 0.001$). As reported in Figure 2, we highlighted a strong variability in weight change after restrictive bariatric surgery, from −27 to +2%. In all, 20% of selected subjects showed a weight change <5% after the intervention.

Body composition and fluid distribution by DXA and BIA

FM-related variables measured by DXA were significantly reduced after the intervention. In particular, significant reductions in total FM (kg) ($\Delta = -12.7 \pm 8.2$ kg; $\Delta\% = -22.22 \pm 12.15$, $P < 0.01$), total FM (%) ($\Delta\% = -9.66 \pm 8.41$, $P < 0.001$), trunk FM (kg) ($\Delta\% = -21.96 \pm 22.14$, $P < 0.01$) and trunk FM (%) ($\Delta\% = -9.43 \pm 10.47$, $P < 0.01$) were observed. LM remained statistically unchanged, instead its trunk portion was significantly decreased with respect to baseline ($\Delta\% = -8.67 \pm 13.11$, $P < 0.05$). A significant increase in the ratio between total LM and FM was conversely obtained ($\Delta\% = 22.80 \pm 21.59$, $P < 0.001$), also in the trunk, abdominal and leg portions. A significant decrease of bone density, as revealed by bone mineral density and *T*-score parameters, was also observed. A significant decrease of TBW (l) ($\Delta\% = -4.60 \pm 6.80$, $P < 0.05$), accounting for intracellular water (ICW) (l) decrease ($\Delta\% = -7.10 \pm 10.34$, $P < 0.05$), after intervention was observed. On the other hand, extracellular water (ECW, l) remained unchanged after weight loss. As a consequence, a slight significant increase in ECW/ICW ratio was observed ($\Delta\% = 8.08 \pm 14.99$, $P < 0.05$). Moreover, RMR was significantly decreased ($\Delta\% = -5.21 \pm 6.78$, $P < 0.01$).

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Table 3 Anthropometric, body composition and fluid distribution parameters at baseline and after LAGB surgery, according to genotypes

Variable	*T0*		*Change (%)*	
	C− (n = 18)	*C+ (n = 22)*	*C− (n = 18)*	*C+ (n = 22)*
Sex (F/M)	11/7	17/5	11/7	17/5
Weight (kg)[a]	112.91 ± 20.08	116.17 ± 18.47	−13.10 ± 7.56	−17.41 ± 8.03
BMI (kg m^{-2})[a]	42.16 ± 8.47	44.37 ± 6.94	−13.10 ± 7.56	−17.41 ± 8.03
FM (kg)[a]	54.86 ± 16.30	58.12 ± 13.13	−18.06 ± 12.02	−25.19 ± 11.88[b]
FM (%)[a]	46.55 ± 7.89	50.60 ± 4.59[d]	−7.73 ± 8.11	−10.70 ± 7.52
LM (kg)[a]	59.05 ± 14.11	53.62 ± 10.61[d]	−3.72 ± 8.71	−8.51 ± 4.11
Total index[a]	1.15 ± 0.40	0.95 ± 0.18[d]	20.35 ± 24.04	24.66 ± 18.57
L2–L5 trunk FM (kg)[a]	6.68 ± 2.18	7.44 ± 2.05	−19.29 ± 17.84	−34.17 ± 15.18
L2–L5 trunk FM (%)[a]	53.44 ± 8.14	58.43 ± 4.23[c]	−6.74 ± 12.16	−9.67 ± 10.72
L2–L5 trunk LM (Kg)[a]	5.85 ± 1.75	5.21 ± 1.18	−6.60 ± 9.83	−19.26 ± 13.03
Abdominal index[a]	0.91 ± 0.31	0.72 ± 0.12[b]	24.70 ± 40.10	36.30 ± 22.90
Trunk index[a]	1.05 ± 0.36	0.82 ± 0.14[b]	14.80 ± 35.06	35.60 ± 21.12
Legs index[a]	1.23 ± 0.60	1.02 ± 0.41	37.01 ± 18.53	36.72 ± 16.12
BMD[a]	1.28 ± 0.14	1.28 ± 0.12	−8.07 ± 4.36	−4.94 ± 2.96
T-score[a]	1.96 ± 1.00	1.99 ± 1.29	−63.04 ± 20.71	−29.65 ± 16.00[b]
BCM (kg)[a]	36.89 ± 8.41	35.23 ± 8.03[d]	1.72 ± 12.88	−1.15 ± 20.69
BCMI[a]	13.25 ± 2.42	13.09 ± 2.98	3.66 ± 13.75	3.94 ± 27.09
TBW (l)[a]	48.91 ± 10.32	45.00 ± 8.56[d]	−2.53 ± 7.04	−5.08 ± 6.24
ECW (l)[a]	18.66 ± 2.86	19.79 ± 3.77[d]	−2.30 ± 6.42	−0.84 ± 4.95
ICW (l)[a]	27.73 ± 6.56	26.35 ± 6.24[d]	−2.52 ± 8.44	−7.97 ± 8.44
ECW/ICW ratio	0.77 ± 0.12	0.74 ± 0.14[d]	−0.48 ± 5.96	8.34 ± 8.59
RMR (kcal per diet)[a]	2023.66 ± 371.39	1945.78 ± 317.09[b]	−3.72 ± 8.71	−8.51 ± 4.11

Abbreviations: Abdominal index, L2–L5 LM/L2–-L5 FM; BCM, body cell mass; BCMI, body cell mass index; BMD, bone mineral density; C−, non carriers of IL-6 -174G/C polymorphism (GG); C+, carriers of IL-6 -174G/C polymorphism (GC/CC); ECW, extracellular body water; FM, Fat mass; ICW, intracellular body water; LAGB, laparoscopic adjustable gastric banding; Legs index, Legs LM/Legs FM; LM, free fat mass; L2–L5, L2 to L5 vertebral disc space; RMR, resting metabolic rate; TBW, total body water; Total index, total body LM/total body FM; Trunk index, Trunk LM/Trunk FM. [a]Data are expressed as mean ± s.d. [b]*P* value is calculated with analysis of covariance (ANCOVA), Gender and baseline FM are considered as covariates.

Q3

Cardiometabolic parameters and IL-6 plasma levels

After 6 months follow-up, no significant modifications of glucose and lipid levels were highlighted, but a trend of reduction of all assessed cardiometabolic parameters was observed. IL-6 plasma levels decreased but the difference was only borderline significant ($P = 0.080$).

Genotyping assessment. The genotype frequencies of −174G > C IL-6 polymorphism were 45%, 40% and 15% for GG, G/C and CC, respectively. Selected patients at baseline were analyzed according to −174G > C IL-6 polymorphism (Tables 3 and 4). The analysis of covariance showed an association between −174G > C IL-6 polymorphism and body composition, fluid distribution and cardiovascular risk indexes before surgery. C(−) subjects had a significantly lower total FM (%) ($P = 0.000$), compared with C(+) carriers, as well as trunk portion (%) ($P = 0.010$). Moreover, significantly higher LM (kg) ($P = 0.000$) and total LM/FM ratio ($P = 0.000$), even in trunk and abdominal portions, were observed in C(−) with respect to C(+) carriers. C(−) carriers had a significantly higher content of TBW (l), less ECW (l) and higher ICW (l) with respect to C(+) carriers. Conversely, ECW/ICW ratio was significantly higher in C(−) than C(+) carriers. Body cell mass (BCM) and RMR were significantly higher in C(−) than C(+) carriers. A trend of higher body cell mass index, a measure of metabolically active cell mass, was also observed in C(−) carriers ($P = 0.108$). All cardiovascular risk indexes were significantly higher in C(−) than C(+) carriers.

To investigate if genetic profile affects changes occurring after LAGB surgery, we compared anthropometric, body composition, fluid content, cardiometabolic and IL-6 concentration changes in patient subgroups according to genotypes. Tables 3 and 4 show the analysis of covariance of all variables assessed between C(−) vs C(+) carriers. The latter subjects had a significant stronger reduction of total FM (kg), but low bone density (*T*-score) compared with C(−) carriers, also after adjusting for gender and baseline FM. A trend of reduction for FM (%) ($P = 0.090$) and its trunk portion ($P = 0.071$), of increase for LM/FM ratio ($P = 0.071$), in C(+) than C(−) carriers, was also observed. No association between −174G > C IL-6 polymorphism and cardiometabolic parameters was highlighted. The −174G > C IL-6 polymorphism was not associated with modifications in the circulating plasma levels of IL-6, both before and after surgery.

Discussion

On the basis of the association between IL6 and obesity, we hypothesized that genetic variations in the IL-6 gene, such as the promoter −174G > C polymorphism, would in turn

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Table 4 Cardiometabolic parameters at baseline and after LAGB surgery, according to genotypes

Variable	*T0*		*Change (%)*	
	C− (n = 18)	*C+ (n = 22)*	*C− (n = 18)*	*C+ (n = 22)*
Sex (F/M)	11/7	17/5	11/7	17/5
Fasting glucose (mg dl^{-1})[a]	88.50 ± 19.31	95.30 ± 14.20	3.51 ± 21.66	−6.87 ± 20.64
Total cholesterol (mg dl^{-1})[a]	207.50 ± 30.78	217.40 ± 34.60	5.54 ± 17.07	−5.21 ± 17.21
LDL cholesterol (mg dl^{-1})[a]	135.70 ± 25.00	141.10 ± 28.79	6.79 ± 23.58	−4.12 ± 19.20
HDL cholesterol (mg dl^{-1})[a]	51.70 ± 11.24	61.00 ± 14.95	4.71 ± 16.80	−9.91 ± 15.69
Triglycerides (mg dl^{-1})[a]	154.50 ± 34.70	124.40 ± 59.41	−5.65 ± 19.50	−15.20 ± 19.46
Total cholesterol/HDL cholesterol[a]	4.62 ± 1.20	4.03 ± 0.82[c]	0.41 ± 10.27	−1.13 ± 11.29
LDL cholesterol/HDL cholesterol[a]	3.03 ± 0.93	2.62 ± 0.78[b]	−0.07 ± 13.88	−5.16 ± 15.23
Log triglycerides/HDL cholesterol[a]	0.48 ± 0.34	0.36 ± 0.27[b]	−0.07 ± 17.62	−13.88 ± 17.83
Plasma IL-6 (pg ml^{-1})[a]	3.10 ± 0.20	2.70 ± 0.35	−25.10 ± 10.68	−20.34 ± 8.96

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Abbreviations: HDL, high-density lipoprotein; LAGB, laparoscopic adjustable gastric banding; LDL, low-density lipoprotein; IL-6, interleukin-6. [a]Data are expressed as mean ± s.d. [b]*P* value is calculated with analysis of covariance (ANCOVA). Gender and baseline FM are considered as covariates.

influence the development of obesity and the response to weight loss. The aim of this study was to explore the relationships between BW, body composition, fluid distribution, cardiometabolic parameters and the −174G>C IL-6 polymorphism in carefully phenotyped obese subjects, before and after LAGB.

Among surgical techniques, LAGB is recognized as safe and reasonably effective in terms of weight loss.[35] In obese subjects, the weight losses induced by dietary or surgical treatment are more rapid in the first months and are associated with changes in body composition and fluid distribution. The desirable weight loss should consist mostly in a loss in FM with limited changes in LM and body fluids. Large losses in LM and fluid imbalance are detrimental because they are often associated with muscle weakness and disability.[36] Because weight loss following surgery is much greater than with non-surgical methods, it may be associated with a disproportionate decrease in lean body mass.[37] Therefore, an important therapeutic objective is to maintain the metabolic and physical capacities of obese patients, after bariatric surgery.

Most of the earlier studies following some form of bariatric surgery for obesity have reported weight change only, but recent studies have used more sophisticated methods of assessing body composition changes. Along with the increased acceptance of surgical procedures for weight loss in obesity, clinically useful baseline and follow-up measures of body composition and fluid distribution are critical to evaluate interventional outcomes. In the current study, DXA and BIA have been used to assess the body composition and the fluid content in 40 obese patients followed-up for 6 months after LAGB surgery.

Buchwald *et al*.[38] reported, in a bariatric surgery review and meta-analysis, weight-loss outcomes of gastric banding such as a decrease in BMI (mean (95% CI), 10.4 (11.5–9.3) in 1959 patients) and a decrease in absolute weight (mean (95% CI), 28.6 kg (32.7–24.5 kg) in 482 patients) upon a follow-up of <2 years. In our study, 6 months after LAGB, mean weight loss corresponded to 11.7% of initial BW (Δ = −13.9 ± 9.8 kg), and mean BMI (Δ = −5.4 ± 3.5 kg) loss was 11.9% of initial BMI (Table 1). Few studies have evaluated changes in body composition by DXA after LAGB. In our study, the weight reduction was mainly due to a significant loss of FM (Δ% = −22.2 of initial value on average), whereas LM was only slightly, although not significantly, reduced (Δ% = −5.21 of initial value on average) (Table 1). In other words, the decrease in FM was about four times greater than the decrease in LM. Our results are in agreement with mean weight, BMI, FM and LM loss after this surgical procedure with a comparable follow-up as reported in literature.[36–42] Moreover, the decrease of RMR is therefore a direct consequence of weight loss and FM changes, in terms of LM/FM proportion.

A loss in BCM with a concurrent increase in ECW would result in no detectable change in LM, evaluated by DXA.[43,44] On the other hand, the ICW reduction is believed to result from a loss of BCM or as a consequence of lean cell shrinking.[36,45] In our study, fluid distribution analysis by BIA demonstrated a normo-hydration status at baseline and changes in body hydration after surgery, not affecting BCM in a significant manner, as also suggested by the value of LM evaluated by DXA. During 6 months of follow-up after LAGB surgery, a significant decrease in TBW (l) accounting for a decrease in ICW (l), mismatching with a non-significant decrease of BCM and body cell mass index, was observed. The increased ECW/ICW ratio is related to the decline of ICW because ECW does not change after weight loss. ECW (l) did not change but the increase of ECW/ICW ratio was statistically significant.

Moreover, we observed that a significant proportion of patients (15%) had a relatively modest weight loss, similar to previous reported data,[21] and 5% of patients did not lose weight (Figure 2). A comparable variability in body composition changes after LAGB was also a characteristic of our study. It is unknown whether low compliance to dietary instructions or genetic factors, that have an important role in body composition, may account for the differences in the therapeutic effects of LAGB. It is important to note that

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previous studies have shown that during the first 6 months after LAGB, the patients experienced the most dramatic weight loss, because they were very compliant to the administered diet. Thus, in this clinical situation, it is possible to reveal the true impact of given genetic polymorphisms or their combination on weight loss after LAGB. In fact, a same standardized protocol for meal progression after LAGB was assigned to all patients, with a caloric intake of 850 kcal/diet in the first month and of 1200 kcal/diet after 1 month of LAGB. The repeated dietary assessments demonstrated a good nutritional compliance, suggesting a role of −174G>C IL-6 polymorphism in the variability of LAGB-induced BW and body composition changes, independently from caloric intake.

At baseline, we verified a gene interaction of −174G>C IL-6 polymorphism with anthropometry, body composition, fluid distribution, cardiometabolic parameters and IL-6 plasma levels. Tables 3 and 4 show the association between the −174G>C IL-6 genotype and LM, FM (%), as well as regional measures of FM, BCM, tissue hydration variables and cardiovascular risk indexes. To date, the relationship between −174G>C IL6 polymorphism and obesity has been investigated by several comparably small studies with inconsistent results.[16–18,21,46–48] Some authors reported that the C(+) carriers were associated with increased FM.[17,18] The −174G>C IL-6 genotype association with indirect indices of obesity, such as BMI, has been reported in several studies.[16,46–48] Two meta-analysis did not find an association between −174G>C IL6 polymorphism and BMI.[49,50] Data concerning the effects of this polymorphism has led to contradictory results, with both carriers, C(−) and C(+), of the single-nucleotide polymorphism being associated with obesity comorbidities. A relationship of −174G>C IL-6 polymorphism with diabetes, insulin resistance, metabolic syndrome, longevity and cardiovascular risk has been reported.[50–57] Huth *et al*.'s meta-analysis,[50] as the largest individual participants' data analysis, indicates that C(+) carriers have lower fasting glucose levels (association β coefficient = −0.32 (CI: −0.58 to −0.05)). No statistically significant association instead was found for quantitative BMI and IL-6 concentrations, in agreement with our study. Indeed, some studies showed that the C(+) carriers were associated with obesity traits,[52,56] whereas others reported the G allele, present in C(−) carriers, was a factor increasing the risk of developing type 2 diabetes mellitus,[51,53] metabolic disease and mortality.[54,55] Our data contribute to defining the C(−) carriers as risk subjects because, although they have a lower FM (%) and trunk FM (%), and higher LM (kg), ratio LM/FM, BCM (kg) and RMR, they show a significantly higher risk for cardiovascular diseases at baseline. With regard to fluid distribution, C(−) carriers showed a higher content of TBW (l) and ECW/ICW ratio than C(+) carriers. These data suggest that despite a significantly higher content of LM, C(−) carriers are characterized by a worst fluid distribution.

To the best of our knowledge, the effect of IL-6 (−174G>C) polymorphism on body composition and fluid distribution after LAGB has not been studied yet. Sesti *et al*.[21] reported that −174G>C IL-6 polymorphism is associated with increased weight loss in morbidly obese subjects at 6 months follow-up after LAGB. Poitou *et al*. (2005)[19] showed a relationship between −174G>C IL-6 polymorphism and circulating product in morbidly obese subjects, during weight loss after surgery. In our study, we observed genotype-dependent body composition changes that were significant for FM and *T*-score. We found that subjects who underwent LAGB lost more FM, but less bone mineral density, if C(+) carriers. The differences remained significant after adjusting for both baseline Fat Mass (kg) and Fat Mass (%), and gender.

Several mechanisms may explain the association of the −174G>C IL-6 polymorphism and body composition at baseline and changes after LAGB. IL-6 can regulate energy expenditure centrally, as it is expressed in hypothalamus, and adipose tissue homeostasis.[6–9,58] Both total lack and overabundance of IL-6 are detrimental in the control of BW. On the other hand, the effect of IL-6 −174G>C on circulating IL-6 is controversial.[16,19,59] IL-6 in circulation originates from a diversity of cell types and tissues, such as adipose tissue and immune cells, and there are also considerable irregular variations of circulating IL-6 dependent on the presence of immune challenge, age and BMI of subjects, as well as physiological and psychosocial stress, various metabolic factors and circadian rhythm.[56,58] With regard to BW regulation, tissue-specific expression may be more important, and thus, measurement of circulating IL-6 level may not reflect biological significance at tissue level.[59,60] According to previous papers,[16,19,59] in our study, no association between −174G>C IL-6 polymorphism and IL-6 plasma levels at baseline was observed. Moreover, IL-6 plasma levels showed a slight reduction after LAGB, borderline for statistical significance, even independent from IL-6 variant. Moreover, despite weight loss and body composition changes, our study revealed a quite small trend of cardiovascular risk reduction evaluated by cardiometabolic assessment, suggesting that weight and FM loss still have no sufficient healthy effects, at 6 months follow-up LAGB. The unsatisfying findings on IL-6 circulating amount and cardiometabolic parameters could be due to the modest or absent weight loss and body composition changes. It remains to be investigated if a longer time point of study will be necessary to assess cardiometabolic and IL-6 plasma level variations related to body composition changes. Moreover, it will be necessary to extend our study, in order to verify differences between ungrouped genotypes, and investigate other polymorphisms related to IL-6 production.

Because of the attention to gender-dependent body composition changes and −174G/C IL-6 polymorphism effects, we performed a multivariate linear regression analysis in a model including the −174G/C IL-6 polymorphism and gender as fixed factor and covariate, respectively (data not shown). Statistical analysis did not reveal any

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interaction between gender and the −174G/C IL-6 polymorphism, probably owing to the small subgroups.

To our knowledge, there is no published study overviewing the relationship between genetic variations, such as −174G>C IL-6 polymorphism, and FM in obese subjects screened for bariatric surgery, and gastric banding outcomes taking into account changes in body composition and fluid distribution. Previously, our data confirmed that the FM (%) is a major determinant of insulin resistance, and suggested that the FM-related increases in IL-6 production and insulin resistance are in part regulated by 174C>G IL-6 promoter polymorphism, proposing as well C(−) carriers as risk subjects.[29]

Our study shows that in obesity, LAGB seems to determine a weight loss sparing LM and causing only mild body fluid alterations. The loss of FM was significant, despite a slight proportional loss of LM. For the first time, this study provides evidence that the promoter polymorphism of IL-6 (−174G>C) gene is associated both with body composition and fluid distribution, in obese subjects, at baseline and at 6 months follow-up after LAGB. Despite lower adiposity and higher RMR, at baseline, C(−) obese carriers are at risk of cardiovascular disease and have still a higher content of FM with respect to the reference population. Moreover, they showed a lower capability to lose weight and FM after LAGB and a higher LAGB-induced detrimental effect on bone density. This implies that LAGB was less effective if the subjects were carrying risk genotypes (C-carriers) for obesity. Further studies will be needed to replicate these results on a larger scale and in populations with different genetic backgrounds.

In conclusion, this study suggests that genetic variations analysis would be an innovative tool for the bariatric surgery screening in order to predict therapeutic response of obese subjects, in terms of fat loss.

Conflict of interest

The authors declared no conflict of interest.

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