Clinical Biochemistry 63 (2019) 46–53

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Weight loss achieved by bariatric surgery modifies high-density lipoprotein subfractions and low-density lipoprotein oxidation towards atheroprotection

Susana Coimbra[a,⁎], Flávio Reis[b], Cátia Ferreira[c], Sara Nunes[b], Sofia Viana[b,d], Alice Catarino[e], Petronila Rocha-Pereira[f,g], Luís Belo[f], Luís Monteiro[e], Cristina Catarino[f], Alice Santos-Silva[f,⁎]

[a] UCIBIO\REQUIMTE, Porto; CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, Gandra-Paredes, Portugal
[b] iCBR, Instituto de Investigação Clínica e Biomédica de Coimbra, Faculdade de Medicina, Universidade de Coimbra, Coimbra, Portugal
[c] Departamento de Ciências Biológicas, Laboratório de Bioquímica, Faculdade de Farmácia da Universidade do Porto (FFUP), Porto, Portugal
[d] Instituto Politécnico de Coimbra, Escola Superior de Tecnologia da Saúde de Coimbra, Coimbra, Portugal
[e] Hospital da Prelada-Dr. Domingos Braga da Cruz, Porto, Portugal
[f] UCIBIO\REQUIMTE, Departamento de Ciências Biológicas, Laboratório de Bioquímica, Faculdade de Farmácia da Universidade do Porto (FFUP), Porto, Portugal
[g] Centro de Investigação em Ciências Saúde (CICS), Universidade da Beira Interior, Covilhã, Portugal

ARTICLE INFO

Keywords:
HDL subfractions
LDL subfractions
Oxidized LDL
Weight loss
Cardiovascular risk
Bariatic surgery

ABSTRACT

Objectives: Weight loss achieved by laparoscopic adjustable gastric banding (LAGB) induces an increase in high-density lipoprotein cholesterol (HDLc) but a small effect on low-density lipoprotein (LDL), although changes in their quality (size and composition) are uncertain. Our aim was to study the impact of weight loss, achieved 13-months after LAGB, on inflammation and dyslipidemia, focusing on HDL and LDL subfractions, and oxidized LDL (oxLDL).

Design & methods: We evaluated standard lipid profile, HDL and LDL subfractions, oxLDL, interleukin (IL)-6 and C-reactive protein (CRP), in twenty obese patients, before (T0) and 13-months after LAGB (T1), and in seventeen healthy controls.

Results: At T1, patients showed lower body weight (12% median weight loss) and anthropometric indices; reduction in TG, atherogenic indices, oxLDL, oxLDL/LDL ratio, CRP and IL-6, and enhancement in HDLc; an increase in large HDL and intermediate HDL subfractions, and a decrease in small HDL subfraction; LDL subfractions were not modified. Percentual change (%Δ) of oxLDL, from T0 to T1, correlated significantly and positively with %Δ of small HDL subfraction and with %Δ of body mass index.

Conclusions: Weight loss induced atheroprotective changes on inflammation, and lipid profile, enhancing larger HDL, the more atheroprotective subfraction, reducing the less protective subclass, small HDL, and reducing oxLDL and oxLDL/LDL ratio. Quality of lipoproteins appears useful cardiovascular risk biomarkers, deserving further studies.

1. Introduction

Obesity is increasing worldwide, representing an important public health concern. Dyslipidemia, oxidative stress and chronic inflammation are common in obesity, increasing the risk for CV diseases (CVD). Triglyceride (TG) levels are usually increased, whereas high-density lipoprotein cholesterol (HDLc) values are often decreased. A low-grade chronic inflammation, with enhanced levels of pro-inflammatory cytokines and acute phase reactants, as interleukin (IL)-6 and C-reactive protein (CRP), are common features in obesity [1,2]. There is a close relationship between inflammation and dyslipidemia in obesity. Chronic inflammation induces a decrease in HDLc that compromises reverse cholesterol transport, favouring cholesterol accumulation in the cells [3]. Pro-inflammatory cytokines stimulate lipolysis of TG, increasing free fatty acid flux to the liver and, therefore, may contribute to enhance TG production [4]; by supressing the adipocyte synthesis of lipoprotein lipase, they also contribute to increase triglycerides and reduce HDLc levels [5]. During inflammation, the uptake of cholesterol by cells is reduced and cell metabolism increases which may also favor the decrease in HDLc values [3]. In addition, increased oxidation of low-density lipoprotein (oxLDL), crucial for the initiation and progression of atherosclerosis, has been associated with overweight and

⁎ Corresponding authors at: UCIBIO\REQUIMTE, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, R. Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.
E-mail addresses: ssn.coimbra@gmail.com, carla.coimbra@ipsn.cespu.pt (S. Coimbra), assilva@ff.up.pt (A. Santos-Silva).

https://doi.org/10.1016/j.clinbiochem.2018.10.007
Received 4 June 2018; Received in revised form 11 October 2018; Accepted 16 October 2018
Available online 17 October 2018

obesity in different pathological conditions [6–8].

Weight loss strategies to reduce the prevalence of overweight and obesity became an important health goal. These strategies may include lifestyle changes, as diet and physical exercise programs, medical treatment and surgical interventions. Bariatric surgery is a surgical intervention strategy that may be recommended for subjects with a body mass index (BMI) > 40 kg/m^2 and for those with 35–40 kg/m^2, when presenting obesity-related comorbidities, such as hypertension, diabetes or dyslipidemia [9,10]. Laparascopic adjustable gastric banding (LAGB) is a minimally-invasive surgical intervention and is associated with low rates of associated complications, lower hospital readmission and mortality rates [11,12]. Roux-en-Y gastric bypass and sleeve gastrectomy are two of the most commonly performed bariatric interventions in the present, both associating with good outcomes of weight loss [13].

A recent study by our group showed that body weight loss, achieved 13 months after LAGB, was associated with a significant reduction in inflammation [14]. Another study reported that body weight loss (7.5–12.5%) achieved by LAGB was associated with improvements in TG, HDLc and total cholesterol (TC) levels and in TC/HDLc ratio; for a total body weight loss above 25%, the patients achieved normal values [15]; however, even when substantial weight loss occurred (> 25%), no significant alterations were observed for LDLc [15]. Few studies showed a reduction in LDLc, at different periods after LAGB (2-weeks, 3 and 6 months) that did not persist for long periods [16–18]. Most data, reported no alterations in LDLc concentrations after different periods of follow-up, following LAGB surgery [15,19–21]. As pointed [15], unlike other more invasive bariatric surgeries, LAGB induces weight loss through caloric restriction, without significant interference in gastrointestinal hormones and gut anatomy, which could affect lipid absorption and metabolism. A meta-analysis performed by Heffron et al. [22] showed that LDLc response (decrease) at 1-year after surgery, either in patients submitted to adjustable gastric banding or in patients submitted to sleeve gastrectomy, was not different from that observed in nonsurgical control patients. However, the unaltered LDL levels may be linked to an improvement in the profile of LDL subfractions, or even in HDL particles. The LDL and HDL lipoproteins are heterogeneous populations of particles that may present different sizes and composition. It has been proposed that these characteristics are more important determinants for the atheroprotective functions of HDL and LDL lipoproteins than their total circulating levels. Thus, HDLc and LDLc concentrations *per se* may not entirely reflect a beneficial or a risk profile for CVD. Indeed, current knowledge regarding the biology of lipoproteins points to different atherogenic/atheroprotective properties of different lipoprotein subfractions.

Several patients' populations of increased CV risk appear to have impaired ratios of HDL subfractions, showing a lower amount of larger subfractions (the more functional, atheroprotective) and a higher amount of small (less functional, atherogenic) subfractions [23,24]. Large HDL subfractions were found to be reduced in dyslipidemic conditions, and increased values of small dense HDL particles were found in coronary artery disease patients [25]. Most data suggest that large buoyant HDL subfractions present a more protective effect than small HDL, however this is not consensual [26]. Small dense LDL subfractions are more atherogenic than large LDL subfractions, as they present a higher degree of penetration into the arterial wall, lower affinity for LDL receptors, lower resistance to oxidative stress and a longer circulating half-life [26,27].

Data in literature, regarding the impact of weight loss achieved through bariatric surgical intervention on the profile of the subfractions of HDL and LDL are scarce and controversial [28,29]. The aim of our work was to contribute to clarify the impact of weight loss, achieved 13-months after LAGB surgical intervention, on dyslipidemia, with especial focus on HDL and LDL subfractions, and on the interplay between lipoprotein subclasses, LDL oxidation and inflammation.

2. Methods

2.1. Subjects

The study protocol was approved by the Committee on Ethics of "Hospital da Prelada-Dr. Domingos Braga da Cruz" (Ref: DC 64/2013). This study was carried out in accordance with the principles from the Declaration of Helsinki for experiments involving humans. Subjects were invited to participate and enrolled in the study after informed and written consent, respecting their privacy rights. Adult patients with > 18 years old, 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 dyslipidemia, were included in the study; one patient, with a BMI of 34.2 kg/m^2 and presenting two comorbidities, arterial hypertension and hypercholesterolemia, was also selected.

Twenty obese patients were clinically and analytically evaluated before (T0) and 13-months after LAGB (T1; LAP-BAND®, Allergan, Inc., Irvine, CA, USA), when the adaptive mechanisms to lower weight are expected to be achieved and stabilized. During this period, patients were regularly scheduled for medical follow-up, received nutritional recommendations and were encouraged to adopt lifestyle changes, namely to increase physical activity. The control group included 17 healthy volunteers, selected based on normal hematological and biochemical values and no history of cardiovascular diseases.

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

2.2. Collection and preparation of blood samples

Blood was collected, at T0 and T1, after overnight fasting, by venepuncture, into tubes with (ethylenediaminetetraacetic acid) and without anticoagulant, in order to obtain plasma and serum, respectively. Samples were processed within 2 h of collection; aliquots of plasma and serum were prepared and immediately stored at −80 °C until assayed.

2.3. Analytical assays

Lipid profile was evaluated by routine procedures, using an autoanalyser (Cobas Integra 400 Plus, Roche Diagnostics, Basel, Switzerland) and commercially available kits; TC, TG and HDLc concentrations were determined by enzymatic colorimetric tests (Roche Diagnostics, Basel, Switzerland). LDLc was calculated according to Friedewald formula [30], as our samples did not present TG > 400 mg/dL (4.52 mmol/L), dysbetalipoproteinemia, and were collected on a fasting basis. The ratios TC/HDLc, TG/HDLc and LDLc/HDLc were also determined.

HDL and LDL subpopulations were separated and quantified using a Lipoprint® kit from Quantimetrix Corp. (Redondo Beach, CA, USA), which is a commercially available Lipoprotein Subfractions Testing System approved by Food and Drug Administration (FDA) as a diagnostic tool [31]. This system separates all lipoprotein fractions and subfractions (large, intermediate and small) in fasting serum or plasma by using a non-denaturing, linear, polyacrylamide gel electrophoresis, followed by a complete data acquisition and quantification of lipoprotein subpopulations. The bands are scanned, identified by their mobility and the relative area under the curve is used to quantify the concentration of LDL or HDL subfractions, using respectively total cholesterol or HDL cholesterol concentrations as reference.

HDL is separated into 10 subfractions that are classified as large HDL particles (1–3 subfractions), intermediate HDL particles (4–7 subfractions), and small HDL particles (8–10 subfractions). The relative area for each band of HDL subpopulation is multiplied by the total HDLc concentration of the sample to yield the amount of cholesterol for each band in mg/dL.

LDL particles are divided into 7 subfractions the LDL1 and LDL2 subfractions corresponding to large LDL particles, and LDL3 to LDL7 subfractions corresponding to small LDL particles. Besides the LDL subfractions, the LDL Lipoprint profile also includes one band of very-low-density lipoprotein (VLDL), 3 Midbands corresponding to intermediate-density lipoproteins (IDL) and one HDL band. The mean LDL particle size is determined. A lipoprotein profile Pattern A, presenting predominant larger LDL subfractions, is atheroprotective, while a lipoprotein profile Pattern B, presenting predominant smaller and denser LDL subfractions (LDL 3 to LDL 7), is atherogenic.

Plasma levels of oxLDL and IL-6 were evaluated by commercially available enzyme-linked immunosorbent assays (Oxidized LDL ELISA, Mercodia, Uppsala, Sweden; Quantikine ELISA Human IL-6 Immunoassay, R&D Systems Inc., Minneapolis, USA; respectively); ELISAs were carried out as described in the manufacturer's instructions. The ratio oxLDL/LDL was calculated to measure the value of LDL oxidation within LDL particles. CRP values were measured by immunoturbidimetry (CRP (latex) High-Sensitivity, Roche Diagnostics, Basel, Switzerland).

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 22.0, Chicago, IL, USA) for Windows. The parametric variables are presented as mean ± standard deviation and the non-parametric variables are presented as median [interquartile range]. For comparisons between controls and patients, we used the Mann-Whitney *U* test and the unpaired Student's *t*-test. To evaluate the differences before and after LAGB intervention, we used the 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 performed to evaluate relationships between sets of data. A P value lower than 0.05 was considered as statistically significant.

3. Results

Patients and controls were matched for age (52.0 [40.8–55.5] *vs* 55.0 [49.5–56.0] years old, respectively; P = 0.133) and gender (18 female/2 male *vs* 14 female/3 male, respectively; P = 0.511).

Before LAGB surgical intervention, the patients presented higher weight, BMI, WC, HC, WC:Ht and WC:HC ratios, compared to controls (Table 1). Thirteen months after LAGB intervention, weight, BMI, WC, HC, WC:Ht, and WC:HC ratios decreased significantly; these values persisted significantly higher than control values, except for WC:HC (Table 1). Before surgical intervention, 5% of patients presented a BMI between 30.0 and 34.9 kg/m^2, 60% between 35.0 and 39.9 kg/m^2 and 35% presented 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%). At T1, patients presented a mean weight reduction of 13.5 kg, and a mean BMI reduction of 5.2 kg/m^2; 15% of 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 only one patient (5%) had a BMI > 40 kg/m^2.

Regarding the lipid profile (Table 2), at T0, patients presented significantly higher values of TG, TC/HDLc, LDLc/HDLc and TG/HDLc ratios and significantly lower HDLc levels, as compared to controls. At T1, patients showed a significant decrease in TG, TC/HDLc, LDLc/HDLc and TG/HDLc ratios; a significant increase in HDLc was also observed. The TG, TG/HDLc and HDLc persisted significantly different from those of control group, at T1, while TC/HDLc and LDLc/HDLc showed values that were already similar to those of the control.

The analysis of HDL subfractions (Table 2), at T0, as compared to controls, showed that patients presented significantly lower percentage of large HDL particles and cholesterol content; intermediate HDL and small HDL showed a similar percentage, but a significantly lower cholesterol content. Thirteen-months after LAGB, we found a significant increase in the percentage of large HDL and in cholesterol content, reaching values that were similar to those of the control group (Fig. 1); the percentage of intermediate HDL persisted, but the cholesterol content showed a significant increase, though significantly lower than the control value; small HDL presented a significant decrease in their percentage and a significant increase in cholesterol content, reaching values that were similar to those of the control group.

In the analysis of LDL subfractions (Table 3), at T0, patients, as compared to controls, presented significantly lower values of LDL1, the large LDL particles, and significantly higher values of LDL3–7, the small LDL particles; the size of total LDL particles was significantly lower. After 13-months of LAGB intervention, no significant changes were observed, in the relative profile of LDL subfractions. In accordance, the differences observed at T0, between patients and controls, persisted at T1 (Fig. 1).

Concerning LDL pattern, we found that patients presented a more atherogenic patterns than the control, and that, at T1, they showed a slight improvement in LDL pattern (Table 3).

After LAGB, we found a significant decrease in VLDL levels that were significantly higher in patients at T0, reaching the control values. No significant changes were observed in Midbands (T0 *vs* T1), excepting Mid-B that showed a significant percentual decrease. Midband A was lower in patients, as compared to controls, at both times of evaluation.

The inflammatory markers, IL-6 and CRP, presented higher levels in patients, at T0, when compared to controls; both biomarkers presented a significant decrease at T1; however, only IL-6 reduced for values that were similar to control values; in spite of the reduction (> 50%), CRP persisted with significantly higher values, as compared to control (Table 2).

Considering the median value of weight loss, 12%, and comparing patients that lost > 12% of weight with those who lost a lower percentage, the first presented lower oxLDL (44.0 (37.8–64.5) *vs* 62.0 (54.8–75.5), respectively; P = 0.027) and higher VLDL values (14.6 [13.5–17.1] *vs* 12.6 [10.8–14.1], respectively; P = 0.009), as well as a trend towards lower values of LDLc (115 ± 32 *vs* 143 ± 34, respectively; P = 0.068) and small LDL subfractions (1.4 [0.0–9.5] *vs* 14.4

Table 1
Anthropometric data for controls and obese patients before (T0) and 13-months after (T1) laparoscopic adjustable gastric banding.

	Control (n = 17)	Patients T0 (n = 20)	Patients T1 (n = 20)	P_{T0vsT1}	P_{CvsT0}	P_{CvsT1}
Height (m)	1.64 ± 0.09	1.60 ± 0.07	–	–	0.093	–
Weight (kg)	65.7 ± 11.3	100.6 ± 11.0	87.1 ± 11.3	<0.001	<0.001	<0.001
BMI (kg/m^2)	24.8 [22.1–26.7]	39.1 [37.6–40.8]	34.1 [31.2–37.7]	<0.001	<0.001	<0.001
WC (cm)	82.0 [71.5–85.5]	115.0 [111.0–120.0]	101.0 [98.0–105.0]	<0.001	<0.001	<0.001
W:H	0.49 [0.43–0.53]	0.72 [0.70–0.75]	0.64 [0.59–0.66]	0.001	<0.001	<0.001
HC (cm)	91.9 ± 7.2	125.2 ± 11.6	115.5 ± 9.8	0.001	<0.001	<0.001
WC:HC	0.86 [0.81–0.89]	0.93 [0.89–0.96]	0.89 [0.84–0.93]	0.009	0.004	0.259

BMI, body mass index; WC, waist circumference; W:H, waist to height ratio; HC, hip circumference; WC:HC, waist circumference to hip circumference ratio. Values are presented as mean ± standard deviation or as median [interquartile range].

Table 2
Lipid profile, HDL subfractions, oxidized LDL and markers of inflammation for controls and obese patients before (T0) and 13-months after (T1) laparascopic adjustable gastric banding.

	Control (n = 17)	Patients T0 (n = 20)	Patients T1 (n = 20)	P_{T0vsT1}	P_{CvsT0}	P_{CvsT1}
TC (mg/dL)	214.5 ± 29.9	198.2 ± 38.3	201.5 ± 39.2	0.717	0.164	0.270
TG (mg/dL)	85.0 [72.5–104.5]	159.5 [115.3–201.0]	117.5 [84.3–138.3]	< 0.001	< 0.001	0.022
LDLc (mg/dL)	139.3 ± 25.3	129.7 ± 34.0	129.1 ± 35.4	0.935	0.346	0.315
HDLc (mg/dL)	56.9 ± 12.8	35.0 ± 8.2	49.0 ± 9.5	< 0.001	< 0.001	0.037
Large HDL (%)	26.1 ± 5.7	20.6 ± 8.5	26.9 ± 9.4	< 0.001	0.030	0.763
Large HDL (mg/dL)	15.4 ± 6.5	7.5 ± 4.1	13.6 ± 6.8	< 0.001	< 0.001	0.429
Intermediate HDL (%)	48.3 ± 4.2	49.4 ± 5.3	48.0 ± 4.5	0.356	0.485	0.837
Intermediate HDL (mg/dL)	27.6 ± 6.3	17.2 ± 4.2	23.5 ± 4.7	< 0.001	< 0.001	0.028
Small HDL (%)	25.6 ± 7.7	29.6 ± 11.5	25.1 ± 8.6	0.032	0.224	0.868
Small HDL (mg/dL)	14.0 ± 3.2	10.1 ± 3.7	12.0 ± 3.3	0.037	0.002	0.064
TC/HDLc	3.77 [3.42–4.56]	5.89 [4.77–6.86]	3.81 [3.42–5.30]	< 0.001	< 0.001	0.775
TG/HDLc	1.57 [1.12–2.16]	4.90 (2.95–7.08)	2.50 (1.76–3.03)	< 0.001	< 0.001	0.013
LDLc/HDLc	2.49 [2.12–3.15]	3.73 [3.02–4.38]	2.30 [1.95–3.66]	0.001	< 0.001	0.845
oxLDL (U/L)	68.0 [56.0–73.5]	81.5 [61.5–88.0]	57.0 [44.0–72.8]	< 0.001	0.042	0.110
oxLDL/LDL (U/mg)	0.048 [0.042–0.053]	0.061 [0.049–0.076]	0.044 [0.040–00.51]	< 0.001	0.005	0.357
CRP (mg/L)	1.50 [0.65–2.50]	6.55 [3.18–10.20]	2.95 [1.20–4.83]	0.001	< 0.001	0.048
IL-6 (pg/mL)	1.18 [0.83–2.14]	2.71 [1.69–4.68]	1.60 [1.00–2.72]	0.001	< 0.001	0.270

TC, total cholesterol; TG, triglycerides; LDLc, low-density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; oxLDL, oxidized LDL; CRP, C-reactive protein; IL, interleukin. Values are presented as mean ± standard deviation or as median [interquartile range].

[1.2–20.7], respectively; P = 0.068).

Small LDL subfraction of patients at T0 were correlated significantly and positively with CRP (r = 0.448, P = 0.047). The oxLDL was correlated positively and significantly with TG (r = 0.511, P = 0.021) and total cholesterol (r = 0.452, P = 0.045), and inversely with cholesterol of intermediate HDL subfractions (r = −0.525, P = 0.017). The oxLDL/LDL ratio correlated significantly with % and cholesterol of intermediate HDL subfractions (r = −0.483, P = 0.031; r = −0.525, P = 0.017; respectively). TG were correlated inversely with HDLc (r = −0.583, P = 0.007), with % and cholesterol of intermediate HDL (r = −0.570, P = 0.009; r = −0.696, P = 0.001; respectively) and with cholesterol of large HDL (r = −0.460, P = 0.041); a significant positive correlation between TG and small HDL % (r = 0.470, P = 0.034) was also found.

At T1, we found that TG related with percentual and cholesterol of small HDL (r = 0.519, P = 0.019; r = 0.555, P = 0.011; respectively).

The percentual change (%Δ) of oxLDL, from baseline to 13-months after LAGB intervention, correlated significantly and positively with %Δ of percentual and cholesterol of small HDL subfraction (Fig. 2), and with %Δ of BMI (r = 0.463, P = 0.040).

4. Discussion

Obesity has been associated to a low-grade chronic inflammatory state [1], and, in accordance, we found significantly increased levels of the inflammatory biomarkers, CRP and IL-6, in obese patients before LAGB intervention, as compared to controls. Our results are also in accordance with reported data linking chronic inflammation and obesity with decreased values in HDLc, hypertriglyceridemia, high values of circulating oxLDL and in the proportion of oxidized LDL particles [3,32,33].

The size and composition of lipoprotein subfractions, rather than the total concentration of lipoprotein's cholesterol, seems to be a more adequate marker of CV risk. Actually, atherogenic changes in lipoprotein subfractions, with less functional HDL and LDL particles, have been reported in several conditions associated with CVD risk, as coronary artery disease, acute coronary syndrome, incident diabetes and hypertension [25,34–37]. In obese patients (before LAGB) we found decreased (% and cholesterol) levels of large HDL subfractions, which seem to be more protective, and increased values of small HDL. Thus, severe obese patients seem to present low HDLc levels and less protective HDL particles.

An association between TG levels and HDL quality has been reported, suggesting that increasing values of TG favor smaller HDL subclasses [38]. We found in obese patients (before LAGB) a positive correlation between TG and small HDL subfractions, and inverse correlations of TG with large and intermediate HDL subfractions, strengthening the hypothesis that the increase in TG may interfere with HDL maturation and with reverse cholesterol transport [38].

Our data suggest that, obese patients (before LAGB) present a more atherogenic LDL subfraction profile, as they presented a lower LDL size, due to a different profile, with lower levels of large LDL subfraction, and higher levels of small LDL, the more atherogenic LDL subpopulation [26,27]; 70% of obese subjects presented B or intermediate LDL pattern. This profile towards smaller LDL particles is in accordance with a higher degree of LDL oxidation (higher levels of oxLDL and oxLDL/LDL ratio). We also found a significant and positive correlation between small LDL subfraction and CRP, strengthening the relationship between inflammation and atherogenic dyslipidemia. Actually, smaller and denser LDL particles are more prone to oxidation, and oxLDL particles are known to present pro-inflammatory properties.

LAGB involves the placement of an adjustable gastric band around the upper stomach, by laparoscopy. The gastric band is connected to an inflatable balloon that can be adjusted by adding or removing saline *via* a small subcutaneous access port. Data showed that LAGB surgery in morbidly obese patients lead to a significant weight loss [39,40], with reductions in BMI, total fat mass, trunk fat mass and in the ratio trunk fat mass/legs fat mass [20,41]. Concerning abdominal adipose tissue, the loss in visceral adipose tissue area seems to be greater than in subcutaneous depots [42]. In accordance, thirteen-months after bariatric surgery, studied patients improved their body weight, as showed by the significant decrease in anthropometric indices (Table 1); the changes in BMI profile (after weight loss) also strengthen this improvement. In fact, the obesity profile before LAGB surgery - grade I, II and III (5, 60 and 35%, respectively) - showed an improved profile - pre-obesity; grade I, grade II, and grade III (15, 45, 35 and 5%, respectively). All patients lost weight, however, 15% of them were still overweight and 85% obese. Besides, the HDLc and large LDL values remained lower, small LDL and TG were still higher; the positive correlation between TG and small HDL was still observed.

The inflammatory status reduced with weight loss, for about half of T0 values, however a residual inflammation still persisted, as shown by the high CRP levels, when compared to control, probably reflecting the adiposity that subsisted.

Some beneficial changes in the traditional lipid profile were also achieved with weight loss, namely a reduction in TG and an increase in

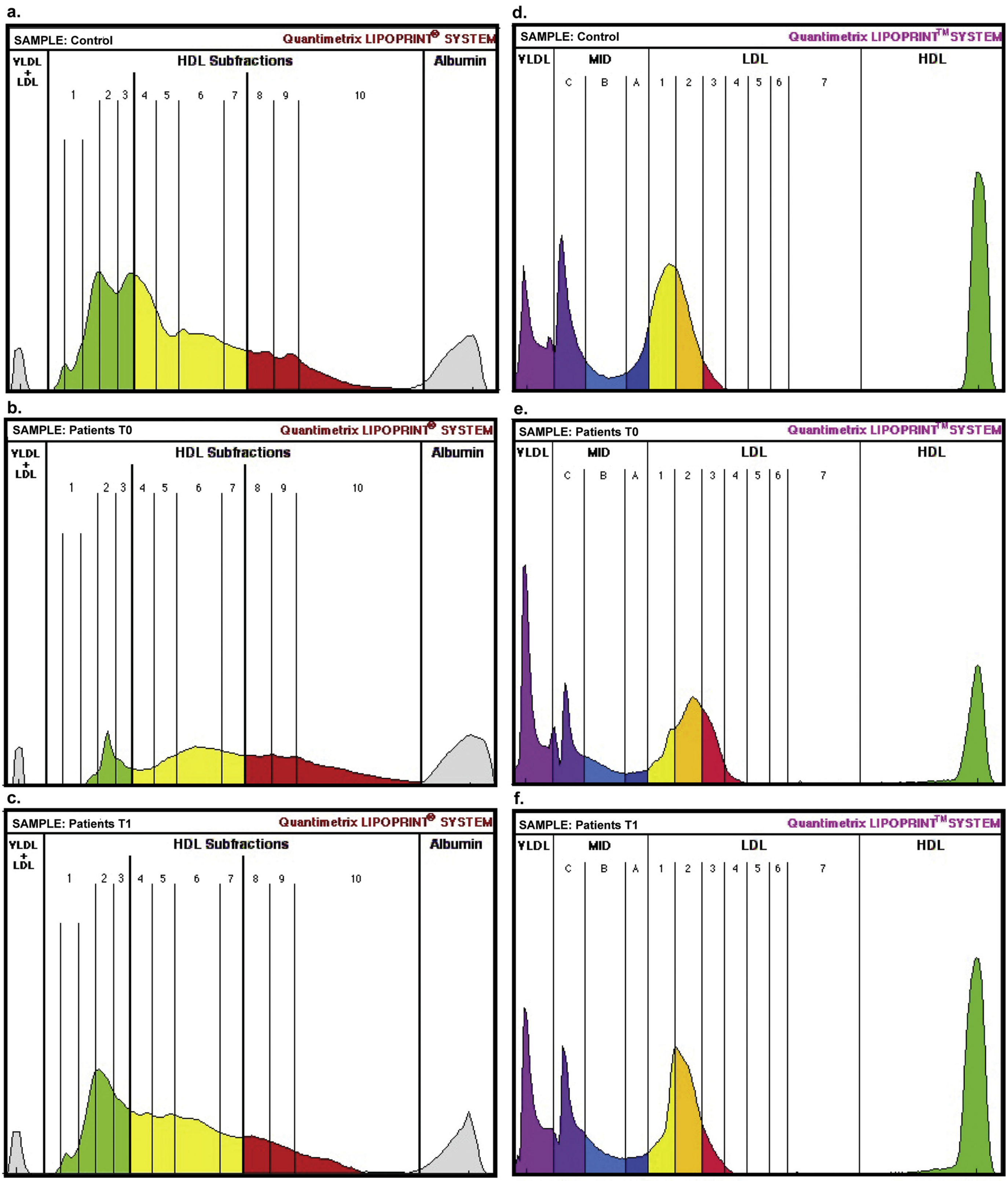

Fig. 1. - Ilustration of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) separation into subfractions of a studied control (a. and d., respectively) and a patient before (T0; b. and e., respectively) and 13-months after (T1; c. and f., respectively) laparascopic adjustable gastric banding.
(HDL is separated into 10 subfractions that are classified as large HDL particles (1–3 subfractions – green color), intermediate HDL particles (4–7 subfractions – yellow color), and small HDL particles (8–10 subfractions – red color). LDL particles are divided into 7 subfractions, the LDL1 (yellow color) and LDL2 (orange color) subfractions correspond to larger LDL particles; LDL3 to LDL7 subfractions (red color) correspond to small LDL particles; very-low-density lipoprotein (VLDL) correspond to the purple subfraction; the 3 Midbands (MID C, MID B and MID A), corresponding to intermediate-density lipoproteins (IDL), are presented as blue subfractions, and HDL as a green band.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Lipoprotein fractions and subfractions for controls and obese patients before (T0) and 13-months after (T1) laparascopic adjustable gastric banding.

	Control (n = 17)	Patients T0 (n = 20)	Patients T1 (n = 20)	P_{T0vsT1}	P_{CvsT0}	P_{CvsT1}
VLDL (%)	13.4 [12.8–15.2]	17.2 [15.6–18.8]	13.9 [12.3–15.4]	< 0.001	0.003	0.916
MID-C (%)	13.6 [13.2–16.0]	13.0 [11.7–15.5]	12.8 [11.1–13.6]	0.076	0.149	0.024
MID-B (%)	6.6 [5.8–7.8]	7.1 [6.6–7.9]	6.5 [5.5–7.6]	0.002	0.390	0.641
MID-A (%)	6.7 [5.3–9.0]	4.6 [3.6–6.1]	4.6 [3.8–6.9]	0.191	0.004	0.010
LDL 1 (%)	18.9 [17.5–20.5]	11.8 [9.4–18.5]	10.3 [9.0–19.3]	0.351	0.004	0.010
LDL 2 (%)	10.5 [8.0–14.0]	13.6 [10.7–17.1]	11.8 [9.7–18.2]	0.204	0.074	0.341
LDL 3–7 (%)	0.8 [0.3–1.7]	8.6 [1.1–13.7]	3.0 [1.0–17.2]	0.527	0.002	0.009
HDL (%)	25.9 [23.9–28.1]	22.1 [19.7–22.9]	27.6 [22.9–31.0]	< 0.001	< 0.001	0.729
LDL size	271.0 [269.5–271.5]	262.5 [258.3–269.8]	264.5 [256.0–271.0]	0.186	0.002	0.033
LDL Pattern	14 A/2 Interm/1 B	6 A/3 Interm/11 B	8 A/2 Interm/10 B	–	–	–

VLDL, very-low-density lipoprotein; MID, midbands (these comprise intermediate-density lipoprotein – IDL); LDL, low-density lipoprotein; HDL, high-density lipoprotein. LDL 3–7 corresponds to small LDL subfractions. Values are presented as median [interquartile range].

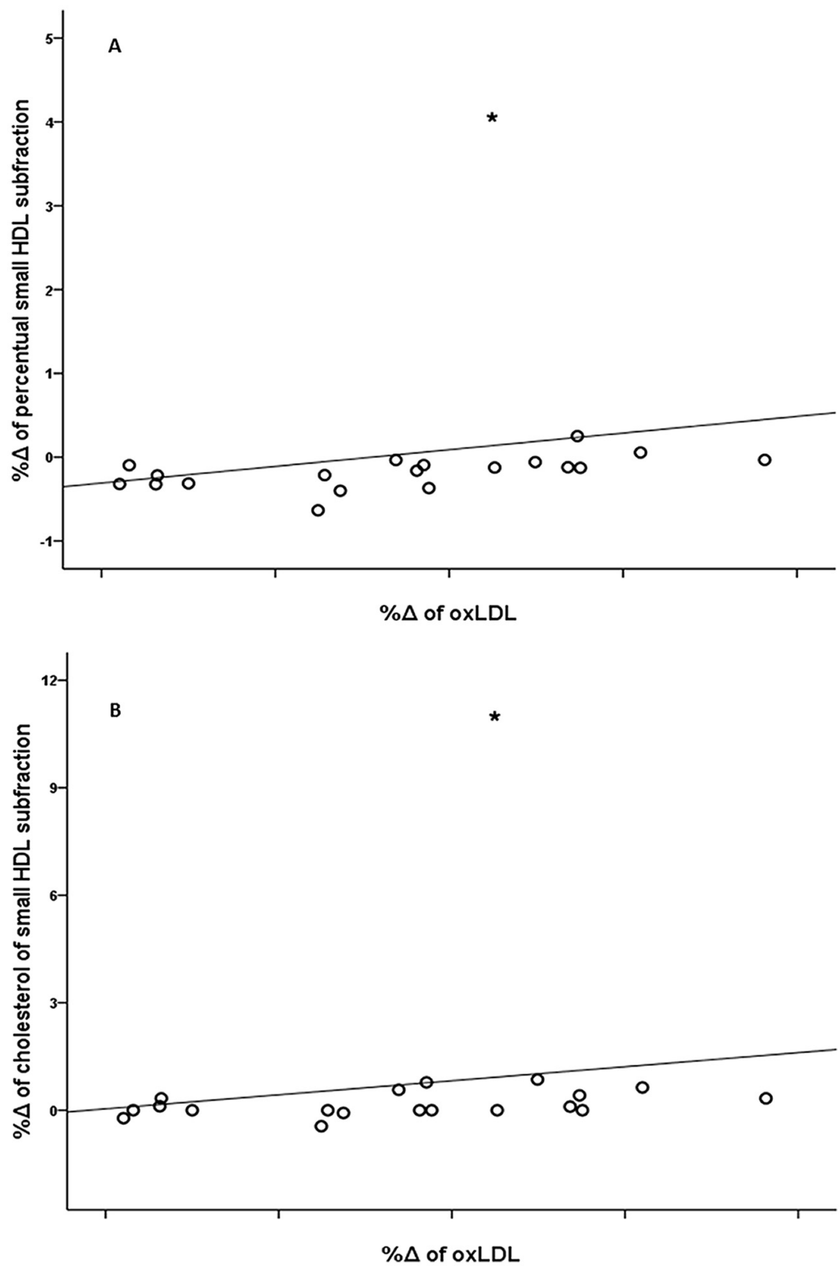

Fig. 2. – Correlations observed in patients, from baseline to 13-months after laparascopic adjustable gastric banding intervention, between percentual change (%Δ) of oxidized low-density lipoprotein (oxLDL) and %Δ of percentual (A) and cholesterol (B) of small high-density lipoprotein (HDL) subfraction (r = 0.623, P = 0.003; r = 0.467, P = 0.038; respectively. When removing the outlier, marked as *, the correlation significance still persisted (r = 0.626, P = 0.004; r = 0.467, P = 0.044; respectively).

HDLc; no significant changes were found for LDLc.

Analyzing HDL subfractions of obese patients after weight loss, we found some significant beneficial changes, namely an increase in large HDL particles and a decrease in % of small HDL particles. Our data suggest that weight loss leads to higher HDLc levels and more protective HDL particles. However, the relationship between size and functionality is not consensual, being suggested that the composition of HDL varies in different metabolic states, affecting its functionality. Considering that we evaluated only the alterations in HDL size, we cannot rule out the possibility that the changes, both in composition and size, may influence HDL functionality.

As referred, we did not find changes in LDLc with weight loss. Still, in agreement with the reduction in TG levels, we found a decrease in VLDL, the TG rich lipoprotein band.

The oxidative changes in LDL were reduced with weight loss and with the reduction in inflammation. Indeed, it seems that the reduction in LDL oxidation is closely related to weight loss, as showed by the positive correlation between %Δ of BMI and %Δ of oxLDL. The decrease found in atherogenic indices, in oxidation of LDL within LDL particles and the more protective profile of HDL particles show a beneficial impact of weigh reduction in CVD risk.

Besides the important function of HDL in reverse cholesterol transport, HDL has also anti-inflammatory and antioxidant properties [43]. We found a significant inverse correlation between oxLDL and intermediate HDL, which is in agreement with antioxidant protection of HDL. We also observed a positive correlation between the %Δ of oxLDL and the %Δ in small HDL subfraction (Fig. 2), suggesting that small HDL is less efficient as antioxidant and strengthens the hypothesis that HDL subfractions have different roles in CVD protection.

As far as we know, Lipoprint® Lipoprotein Subfractions Testing System was used in only one study evaluating the impact of bariatric surgery on weight loss and on the profile of HDL and LDL subfractions [29]. In a lifestyle intervention program, followed by bariatric surgery (laparoscopic Roux-en-Y gastric bypass or biliopancreatic diversion with duodenal switch, for patients with BMI > 50 kg/m^2), Kjellmo et al. [29] reported that one year after surgical intervention, a reduction in LDLc and in large LDL subpopulation, as well as an increase in HDLc and large HDL subfraction, were observed, but no effect was observed on small LDL and on smaller LDL subclasses. The results found for HDLc and larger HDL are in agreement with our data, however, we did not find significant changes in LDLc and on LDL subclasses. A study conducted in metabolic syndrome patients submitted to a combined program of physical exercise and diet, showed significant improvements in lipoprotein subfractions, after 3 weeks; however, after a 12-months follow-up period, as compared to the 3-weeks follow-up period, a decrease in LDL size and an increase in small LDL and small HDL, were observed and these values persisted significantly different from control; nonetheless, large HDL increased throughout the follow-up period [44]. This study in obese patients suggests that the impact of weight loss intervention on lipoprotein subfractions may be higher at a short period of time after intervention. In another study, morbidly obese patients who underwent Roux-en-Y gastric bypass bariatric surgery, presented, one year after intervention, a reduction in oxLDL and an increase in large HDL subfraction, HDL-2; no significant alterations were found in LDL subfractions [28]; it should be pointed that HDL and LDL subclasses analysis was performed by a different technique than Lipoprint® System. More invasive bariatric surgeries have been associated with reductions in LDLc [45,46], which does not seem to occur with lifestyle changes or with pure restrictive bariatric surgery interventions [15,47,48]. It seems that weight loss *per se*, as that achieved after LAGB, may not be sufficient to reduce LDL levels and its subfractions, but the reduction in the oxidation of LDL particles is certainly important to reduce CVD risk. A more significant loss of weight, with no residual inflammation remaining, may be needed to observe reduction in LDLc and a more protective LDL subpopulation profile; nonetheless, the weight loss achieved through LAGB seems to be sufficient enough to enhance HDLc levels and ameliorate HDL subpopulations profile towards atheroprotection.

LAGB is a restrictive surgery that reduces stomach's capacity, as well as, the hunger sensation; a gradual, continuous and well-programmed weight loss can be achieved. Different studies reported significant weight loss after surgery - 11.5% after 3-years [49], 16%, after 1 to 11 years [50], 18.3 ± 7.9% after 2-years [15], 15,8% after 1-year [51]. A systematic review reported that through lifestyle modification programs, one-year after intervention, 30% of participants presented a weight loss ≥10%, 25% between 5% and 9.9%, and 40% ≤4.9% [52]. In our study, the median value of weight loss registered, 13-months after LAGB, was 12%, which is in line with previous reports of similar studies. We found that obese patients achieving > 12% of weight loss presented, actually, lower oxLDL, higher VLDL values and trends towards lower values of LDLc and small LDL subfractions, when compared to obese patients achieving lower weight loss. It appears that weight loss is especially important for LDL quality and, therefore, for CVD risk.

The controversy of data on the changes of lipoprotein subfractions associated to weight loss may result from the use of different techniques to evaluate HDL and LDL subclasses and, for similar methods, the use of different characterization and nomenclature of lipoprotein subfractions [53]; different types of bariatric surgeries or intervention programs; enrollment of patients with different degrees of obesity, achieving different weight loss, and the coexistence of different comorbidities.

We are aware that our study presents some limitations. The small number of participants in both groups may have reduced the capacity to detect significant alterations in other variables, or in their correlations; thus, further studies are needed in order to strength our data, as well as to evaluate the impact of weight loss, not only in lipoprotein size and cholesterol content, but also in lipids and protein composition of lipoprotein subclasses, and its functionality. Patients were encouraged to adopt a healthier lifestyle, not only involving a balanced diet, but to combine it with regular practice of exercise; however, it was not possible to assess with precision if physical exercise recommendations were fully taken in account.

Our data shows that 13-months after LAGB, the loss of weight favored a reduction in LDL oxidation that was closely correlated with the decrease found in small HDL subfraction. Moreover, weight loss had a beneficial impact on inflammation and on lipid risk profile, inducing an enhancement in larger HDL, the more atheroprotective subfraction, and a reduction in the less protective subclass, small HDL. Quality of lipoproteins appears useful cardiovascular risk biomarkers, deserving further studies.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

This work received financial support from FCT/MEC through national funds and was co-financed by FEDER, under the Partnership Agreement PT2020 from UCIBIO (UID/MULTI/04378/2013 - POCI/01/0145/FEDER/007728), CESPU (PI4AC; FULAGB/LPB-CESPU-2017), CNC.IBILI (UID/NEU/04539/2013) and Centro 2020 (CENTRO-01-0145-FEDER-000012-HealthyAging2020).

References

[1] K.E. Wellen, G.S. Hotamisligil, Obesity-induced inflammatory changes in adipose tissue, J. Clin. Invest. 112 (2003) 1785–1788.

[2] D.B. Panagiotakos, C. Pitsavos, M. Yannakoulia, C. Chrysohoou, C. Stefanadis, The implication of obesity and central fat on markers of chronic inflammation: the ATTICA study, Atherosclerosis 183 (2005) 308–315.

[3] E. Esteve, W. Ricart, J.M. Fernandez-Real, Dyslipidemia and inflammation: an evolutionary conserved mechanism, Clin. Nutr. 24 (2005) 16–31.

[4] K.R. Feingold, W. Doerrler, C.A. Dinarello, W. Fiers, C. Grunfeld, Stimulation of lipolysis in cultured fat cells by tumor necrosis factor, interleukin-1, and the interferons is blocked by inhibition of prostaglandin synthesis, Endocrinology 130 (1992) 10–16.
[5] M. Kawakami, A. Cerami, Studies of endotoxin-induced decrease in lipoprotein lipase activity, J. Exp. Med. 154 (1981) 631–639.
[6] S. Coimbra, H. Oliveira, F. Reis, L. Belo, S. Rocha, A. Quintanilha, A. Figueiredo, F. Teixeira, E. Castro, P. Rocha-Pereira, A. Santos-Silva, Circulating levels of adiponectin, oxidized LDL and C-reactive protein in Portuguese patients with psoriasis vulgaris, according to body mass index, severity and duration of the disease, J. Dermatol. Sci. 55 (2009) 202–204.
[7] P. Holvoet, D.H. Lee, M. Steffes, M. Gross, D.R. Jacobs Jr., Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome, JAMA 299 (2008) 2287–2293.
[8] H. Nascimento, A.I. Alves, S. Coimbra, C. Catarino, D. Gomes, E. Bronze-Da-Rocha, E. Costa, P. Rocha-Pereira, L. Aires, J. Mota, H. Ferreira Mansilha, C. Rego, A. Santos-Silva, L. Belo, Bilirubin is independently associated with oxidized LDL levels in young obese patients, Diabetol. Metab. Syndr. 7 (2015) 4.
[9] L. Sjostrom, A.K. Lindroos, M. Peltonen, J. Torgerson, C. Bouchard, B. Carlsson, S. Dahlgren, B. Larsson, K. Narbro, C.D. Sjostrom, M. Sullivan, H. Wedel, Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery, N. Engl. J. Med. 351 (2004) 2683–2693.
[10] D. Kulick, L. Hark, D. Deen, The bariatric surgery patient: a growing role for registered dietitians, J. Am. Diet. Assoc. 110 (2010) 593–599.
[11] M.S. Parikh, S. Laker, M. Weiner, O. Hajiseyedjavadi, C.J. Ren, Objective comparison of complications resulting from laparoscopic bariatric procedures, J. Am. Coll. Surg. 202 (2006) 252–261.
[12] D.R. Flum, S.H. Belle, W.C. King, A.S. Wahed, P. Berk, W. Chapman, W. Pories, A. Courcoulas, C. McCloskey, J. Mitchell, E. Patterson, A. Pomp, M.A. Staten, S.Z. Yanovski, R. Thirlby, B. Wolfe, Perioperative safety in the longitudinal assessment of bariatric surgery, N. Engl. J. Med. 361 (2009) 445–454.
[13] J.H. Kang, Q.A. Le, Effectiveness of bariatric surgical procedures: a systematic review and network meta-analysis of randomized controlled trials, Medicine (Baltimore) 96 (2017) e8632.
[14] S. Coimbra, C. Ferreira, L. Belo, P. Rocha-Pereira, A. Catarino, L. Monteiro, C. Catarino, A. Santos-Silva, Impact of weight loss on inflammation and red blood cell biomarkers after laparoscopic gastric banding surgery, J. Investig. Med. 66 (2018) 304–308.
[15] G.J. Ooi, A. Earnest, L. Doyle, C. Laurie, J.M. Wentworth, K. Sikaris, C.W. le Roux, P.R. Burton, P.E. O'Brien, W.A. Brown, Detailed Description of Change in Serum Cholesterol Profile with Incremental Weight loss after Restrictive Bariatric Surgery, Obes. Surg. 28 (2018) 1351–1362.
[16] K. Samaras, A. Viardot, N.K. Botelho, A. Jenkins, R.V. Lord, Immune cell-mediated inflammation and the early improvements in glucose metabolism after gastric banding surgery, Diabetologia 56 (2013) 2564–2572.
[17] H.R. Hady, J. Dadan, P. Golaszewski, 100 obese patients after laparoscopic adjustable gastric banding - the influence on BMI, gherlin and insulin concentration, parameters of lipid balance and co-morbidities, Adv. Med. Sci. 57 (2012) 58–64.
[18] R. Ramalho, C. Guimaraes, C. Gil, C. Neves, J.T. Guimaraes, L. Delgado, Morbid obesity and inflammation: a prospective study after adjustable gastric banding surgery, Obes. Surg. 19 (2009) 915–920.
[19] G.A. Woodard, J. Peraza, S. Bravo, L. Toplosky, T. Hernandez-Boussard, J.M. Morton, One year improvements in cardiovascular risk factors: a comparative trial of laparoscopic Roux-en-Y gastric bypass vs. adjustable gastric banding, Obes. Surg. 20 (2010) 578–582.
[20] C. Lubrano, S. Mariani, M. Badiali, M. Cuzzolaro, G. Barbaro, S. Migliaccio, G. Genovesi, F. Rossi, M. Celanetti, D. Fiore, M.M. Pandolfo, P. Specchia, G. Spera, Metabolic or bariatric surgery? Long-term effects of malabsorptive vs restrictive bariatric techniques on body composition and cardiometabolic risk factors, Int. J. Obes. 34 (2010) 1404–1414.
[21] J. Santos, P. Salgado, C. Santos, P. Mendes, J. Saavedra, P. Baldaque, L. Monteiro, E. Costa, Effect of bariatric surgery on weight loss, inflammation, iron metabolism, and lipid profile, Scand. J. Surg. 103 (2014) 21–25.
[22] S.P. Heffron, A. Parikh, A. Volodarskiy, C. Ren-Fielding, A. Schwartzbard, J. Nicholson, S. Bangalore, Changes in lipid profile of obese patients following contemporary bariatric surgery: a meta-analysis, Am. J. Med. 129 (2016) 952–959.
[23] L. Camont, M.J. Chapman, A. Kontush, Biological activities of HDL subpopulations and their relevance to cardiovascular disease, Trends Mol. Med. 17 (2011) 594–603.
[24] F. Mascarenhas-Melo, J. Sereno, E. Teixeira-Lemos, S. Ribeiro, P. Rocha-Pereira, E. Cotterill, F. Teixeira, F. Reis, Markers of increased cardiovascular risk in postmenopausal women: focus on oxidized-LDL and HDL subpopulations, Dis. Markers 35 (2013) 85–96.
[25] A. Pirillo, G.D. Norata, A.L. Catapano, High-density lipoprotein subfractions–what the clinicians need to know, Cardiology 124 (2013) 116–125.
[26] S. Maeda, S. Nakanishi, M. Yoneda, T. Awaya, K. Yamane, T. Hirano, N. Kohno, Associations between small dense LDL, HDL subfractions (HDL2, HDL3) and risk of atherosclerosis in Japanese-Americans, J. Atheroscler. Thromb. 19 (2012) 444–452.
[27] K.K. Berneis, R.M. Krauss, Metabolic origins and clinical significance of LDL heterogeneity, J. Lipid Res. 43 (2002) 1363–1379.
[28] J. Julve, E. Pardina, M. Perez-Cuellar, R. Ferrer, J. Rossell, J.A. Baena-Fustegueras, J.M. Fort, A. Lecube, F. Blanco-Vaca, J.L. Sanchez-Quesada, J. Peinado-Onsurbe, Bariatric surgery in morbidly obese patients improves the atherogenic qualitative properties of the plasma lipoproteins, Atherosclerosis 234 (2014) 200–205.
[29] C.A. Kjellmo, H. Karlsson, T.K. Nestvold, S. Ljunggren, K. Cederbrant, M. Marcusson-Stahl, M. Mathisen, K.T. Lappegard, A. Hovland, Bariatric surgery improves lipoprotein profile in morbidly obese patients by reducing LDL cholesterol, apoB, and SAA/PON1 ratio, increasing HDL cholesterol, but has no effect on cholesterol efflux capacity, J. Clin. Lipidol. 12 (2018) 193–202, https://doi.org/10.1016/j.jacl.2017.
[30] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.
[31] A. Dukat, S. Oravec, M. Wawruch, P. Gavornik, P. Sabaka, The incidence of dyslipidemia in a sample of asymptomatic probands established by the means of Lipoprint system, Vnitr. Lek. 57 (2011) 258–260.
[32] G. Ruotolo, B.V. Howard, Dyslipidemia of the metabolic syndrome, Curr. Cardiol. Rep. 4 (2002) 494–500.
[33] S. Furukawa, T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda, I. Shimomura, Increased oxidative stress in obesity and its impact on metabolic syndrome, J. Clin. Invest. 114 (2004) 1752–1761.
[34] M.G. Carnuta, C.S. Stancu, L. Toma, G.M. Sanda, L.S. Niculescu, M. Deleanu, A.C. Popescu, M.R. Popescu, A. Vlad, D.R. Dimulescu, M. Simionescu, A.V. Sima, Dysfunctional high-density lipoproteins have distinct composition, diminished anti-inflammatory potential and discriminate acute coronary syndrome from stable coronary artery disease patients, Sci. Rep. 7 (2017) 7295.
[35] Y. Zhang, C.G. Zhu, R.X. Xu, S. Li, X.L. Li, Y.L. Guo, N.Q. Wu, Y. Gao, P. Qing, C.J. Cui, J. Sun, J.J. Li, HDL subfractions and very early CAD: novel findings from untreated patients in a Chinese cohort, Sci. Rep. 6 (2016) 30741.
[36] R.H. Mackey, S. Mora, A.G. Bertoni, C.L. Wassel, M.R. Carnethon, C.T. Sibley, D.C. Goff Jr., Lipoprotein particles and incident type 2 diabetes in the multi-ethnic study of atherosclerosis, Diabetes Care 38 (2015) 628–636.
[37] Y. Zhang, S. Li, R.X. Xu, Y.L. Guo, N.Q. Wu, C.G. Zhu, Y. Gao, Q. Dong, G. Liu, J. Sun, J.J. Li, Distribution of high-density lipoprotein subfractions and hypertensive status: a cross-sectional study, Medicine (Baltimore) 94 (2015) e1912.
[38] L. Tian, Y. Xu, M. Fu, T. Peng, Y. Liu, S. Long, The impact of plasma triglyceride and apolipoproteins concentrations on high-density lipoprotein subclasses distribution, Lipids Health Dis. 10 (2011) 17.
[39] P. Cigdem Arica, A. Kocael, O. Tabak, M. Taskin, K. Zengin, H. Uzun, Plasma ghrelin, leptin, and orexin-A levels and insulin resistance after laparoscopic gastric band applications in morbidly obese patients, Minerva Med. 104 (2013) 309–316.
[40] R. Gelisgen, K. Zengin, A. Kocael, B. Baysal, P. Kocael, H. Erman, M. Taskin, H. Uzun, Effects of laparoscopic gastric band applications on plasma and fundic acylated ghrelin levels in morbidly obese patients, Obes. Surg. 22 (2012) 299–305.
[41] Z. Visockiene, G. Brimas, T. Abaliksta, L. Siauliene, V. Liakina, K. Strupas, Metabolic changes one year after laparoscopic adjustable gastric banding operation in morbidly obese subjects, Wideochir. Inne Tech. Malo Inwazyjne 8 (2013) 13–21.
[42] M.L. Heath, L. Kow, J.P. Slavotinek, R. Valentine, J. Toouli, C.H. Thompson, Abdominal adiposity and liver fat content 3 and 12 months after gastric banding surgery, Metabolism 58 (2009) 753–758.
[43] P.J. Barter, S. Nicholls, K.A. Rye, G.M. Anantharamaiah, M. Navab, A.M. Fogelman, Antiinflammatory properties of HDL, Circ. Res. 95 (2004) 764–772.
[44] F. Dutheil, G. Walther, R. Chapier, G. Mnatzaganian, B. Lesourd, G. Naughton, J. Verney, A. Fogli, V. Sapin, M. Duclos, A. Vinet, P. Obert, D. Courteix, G. Lac, Atherogenic subfractions of lipoproteins in the treatment of metabolic syndrome by physical activity and diet - the RESOLVE trial, Lipids Health Dis. 13 (2014) 112.
[45] N.T. Nguyen, E. Varela, A. Sabio, C.L. Tran, M. Stamos, S.E. Wilson, Resolution of hyperlipidemia after laparoscopic Roux-en-Y gastric bypass, J. Am. Coll. Surg. 203 (2006) 24–29.
[46] D. Benaiges, J.A. Flores-Le-Roux, J. Pedro-Botet, J.M. Ramon, A. Parri, M. Villatoro, M.J. Carrera, M. Pera, E. Sagarra, L. Grande, A. Goday, Impact of restrictive (sleeve gastrectomy) vs hybrid bariatric surgery (Roux-en-Y gastric bypass) on lipid profile, Obes. Surg. 22 (2012) 1268–1275.
[47] M.J. Franz, J.L. Boucher, S. Rutten-Ramos, J.J. Van Wormer, Lifestyle weight-loss intervention outcomes in overweight and obese adults with type 2 diabetes: a systematic review and meta-analysis of randomized clinical trials, J. Acad. Nutr. Diet. 115 (2015) 1447–1463.
[48] To VT, T.P. Huttl, R. Lang, K. Piotrowski, K.G. Parhofer, Changes in body weight, glucose homeostasis, lipid profiles, and metabolic syndrome after restrictive bariatric surgery, Exp. Clin. Endocrinol. Diabetes 120 (2012) 547–552.
[49] N. Al Sharqawi, S. Al Sabah, A. Al Mulla, K. Al Anezi, T. Jumaa, Conversional surgery: single-step conversion of laparoscopic adjustable gastric band to laparoscopic sleeve gastrectomy, Obes. Surg. 24 (2014) 1808–1811.
[50] A.P. Courcoulas, N.J. Christian, R.W. O'Rourke, G. Dakin, E. Patchen Dellinger, D.R. Flum, P.D. Melissa Kalarchian, J.E. Mitchell, E. Patterson, A. Pomp, W.J. Pories, K. Spaniolas, K. Steffen, B.M. Wolfe, S.H. Belle, Preoperative factors and 3-year weight change in the Longitudinal Assessment of Bariatric Surgery (LABS) consortium, Surg. Obes. Relat. Dis. 11 (2015) 1109–1118.
[51] C.J. Chiu, D.W. Birch, X. Shi, S. Karmali, Outcomes of the adjustable gastric band in a publicly funded obesity program, Can. J. Surg. 56 (2013) 233–236.
[52] J.G. Christian, A.G. Tsai, D.H. Bessesen, Interpreting weight losses from lifestyle modification trials: using categorical data, Int. J. Obes. 34 (2010) 207–209.
[53] A. Hafiane, J. Genest, High density lipoproteins: measurement techniques and potential biomarkers of cardiovascular risk, BBA Clin. 3 (2015) 175–188.