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Bariatric surgery improves lipoprotein profile in morbidly obese patients by reducing LDL-cholesterol and apoB, also reduces SAA/PON1 ratio, increases HDL-cholesterol, but has no effect on cholesterol efflux capacity

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ABSTRACT

Background

Bariatric surgery has been shown to reduce cardiovascular events and cause-specific mortality for coronary artery disease in obese patients. Lipoprotein biomarkers relating to LDLs, HDLs and their subfraction and macrophage cholesterol efflux have been hypothesized to be of value in CV risk assessment.

Objectives

The objective of this study was to examine the effect of a lifestyle intervention followed by bariatric surgery on the lipid profile of morbidly obese patients.

Methods

34 morbidly obese patients were evaluated before and after lifestyle changes and then one year after bariatric surgery. They were compared to 17 lean subjects. Several lipoprotein metrics, amyloid A (SAA), serum paraoxonase/arylesterase 1 (PON1) and macrophage cholesterol efflux capacity (CEC) were assessed.

Results

Average weight loss after the lifestyle intervention was 10.5% and one year after bariatric surgery 33.9%. The lifestyle intervention significantly decreased triglycerides (-28.7 mg/dL, $p<0.05$), LDL cholesterol (-32.3 mg/dL, $p<0.0001$) and had no effect on apolipoprotein apoB/apoA1 ratio. Bariatric surgery further reduced triglycerides (-36.7 mg/dL, $p<0.05$), reduced apoB/apoA1 ratio ($p<0.0001$), increased HDL cholesterol (+12mg/dL, $p<0.0001$) and reductions in LDL-C were sustained. Bariatric surgery reduced large, boyant (lb)LDL ($p<0.0001$), but had no effect on the small, dense (sd)LDL. The large HDL subfractions increased ($p<0.0001$), but there was no effect on the smaller HDL subfractions. The ratio for SAA/PON1 was reduced after the lifestyle intervention ($p<0.01$) and further reduced after bariatric surgery ($p<0.0001$). Neither the lifestyle intervention nor bariatric surgery had any effect on CEC.

Conclusions

Lifestyle intervention followed by bariatric surgery in 34 morbidly obese patients showed favourable effects on triglycerides, LDL-C and apoB. HDL-C, apoB/apoA1 ratio as well as SAA/PON1 ratio was reduced, but surgery did not influence CEC.

Keywords: Bariatric surgery; obesity; lipoprotein particle subclasses; paraoxonase/arylesterase 1 (PON1); serum amyloid A (SAA); cholesterol efflux capacity

INTRODUCTION

Obesity, defined as a body mass index (BMI) $>30 \text{ kg/m}^2$, is a growing health problem in large parts of the world. Worldwide obesity has doubled since 1980 and the prevalence of obesity in adults has passed 20% in most western countries [1]. Similar increases in childhood obesity predict that we have not yet seen the peak of this epidemic [2]. Obesity is associated with known cardiovascular risk factors such as hypertension, dyslipidaemia, type 2 diabetes and chronic inflammation[3]. An increased risk of morbidity and mortality from cardiovascular disease (CVD) is well documented in obese patients [4].

Bariatric surgery is considered a safe and effective treatment for obesity [3], usually recommended to patients with severe obesity, defined as a BMI $> 40 \text{ kg/m}^2$ or BMI $> 35 \text{ kg/m}^2$ in the presence of substantial co-morbidities such as hypertension, diabetes and obstructive sleep apnoea. Bariatric surgery results in significant and sustained weight loss, reduces all-cause mortality and cause-specific mortality from coronary artery disease (CAD) [5].

The typical dyslipidemia observed in obese patients consists of elevated fasting and postprandial triglycerides, reduced high-density lipoprotein cholesterol (HDL-C) and normal or just slightly elevated low-density lipoprotein cholesterol (LDL-C) [3]. These routine lipoprotein biomarkers fail to identify a significant proportion of patients at risk of cardiovascular events [6] and there has been intensive research into whether different advanced lipoprotein testing methods can improve cardiovascular risk prediction. LDL and HDL particles are heterogeneous with respect to size, density, composition and function. Several LDL and HDL metrics have been investigated, with the most common being total and subfraction particle numbers, sizes and lipid content. Small, dense LDL (sdLDL) has been associated with increased cardiovascular risk, obesity and diabetes. sdLDL biomarkers include LDL diameter or phenotype, particle number (sdLDL-P) or cholesterol content (sd-

LDL-C) [7-9]. Despite over three decades of research, the clinical utility of LDL sub-fractionation continues to be debated [6].

Cholesterol efflux capacity (CEC), the ability of HDL to accept cholesterol from macrophages, a step in reverse cholesterol transport, has been inversely associated with cardiovascular events [10]. The relevance of HDL subfractions and subclasses as markers for HDL-related risk is uncertain [11], but recent studies have shown that small, dense HDL particles seem to be more efficient in mediating ABCA1-mediated cholesterol efflux from macrophages [12]. Other aspects of HDL functionality include the ability to mitigate oxidative and inflammatory arterial wall responses. Paraoxonase-1 (PON1) is an HDL-associated enzyme capable of preventing LDL oxidation and reductions in PON1 activity is believed to lead to dysfunctional HDL [13]. Serum amyloid A (SAA) is an acute-phase protein that increases during inflammation and impairs the anti-inflammatory properties of HDL, possibly by replacing protective proteins in HDL [14]. An increased ratio of SAA abundance and PON1 activity has been proposed as a possible marker for dysfunctional and pro-inflammatory HDL [15].

The effect of bariatric surgery on lipoprotein composition and function is largely unknown. Furthermore, the mechanisms through which bariatric surgery affects morbidity and mortality are not fully understood. We hypothesized that lifestyle changes followed by bariatric surgery would induce a more favourable lipoprotein profile in morbidly obese patients and therefore examined the effects of bariatric surgery on triglycerides (TG), LDL-C and HDL-C, apoB, apoA1, HDL- and LDL-subfractions and HDL functions measured by SAA, PON1 activity and macrophage cholesterol efflux capacity (CEC).

MATERIAL AND METHODS

Study participants and experimental design

In this prospective study, we included 34 patients admitted to the Regional centre for treatment of morbid obesity, Nordland Hospital, Norway. The patient population has been described previously [16]. Briefly, inclusion criteria in the morbidly obese group were: >18 years of age, BMI >40 kg/m² or BMI >35 kg/m² with significant co-morbidity such as hypertension, type 2 diabetes mellitus (T2DM) or sleep apnoea. The patients underwent lifestyle changes for a mean period of 3 months prior to bariatric surgery. The control group consisted of 17 subjects with a BMI < 28 kg/m² and no established CVD, scheduled to undergo elective laparoscopic cholecystectomy or laparoscopic fundoplication.

Ethics

The study was approved by the Regional Ethics Committee of Northern Norway and by the Norwegian Data Protection Authority, and complied with the Helsinki II declaration. Written informed consent was obtained from all participants.

Blood sampling

Fasting blood samples were obtained by standard venipuncture on three occasions: At first admission, the day before surgery (after 3 months of lifestyle intervention), and 1 year after surgery. Routine blood analyses were performed on the day of sampling at the laboratory of Nordland Hospital. Serum, EDTA plasma, and citrate plasma were frozen in aliquots at -80°C and analyzed in batch at the end of the study.

Serum levels of TG, LDL-C, HDL-C, apoB and apoA1 were measured using an ADVIA®1800 system (Siemens Medical Solutions Diagnostics, Japan).

LDL and HDL subfractions

LDL and HDL subfractions were determined electrophoretically by the use of high-resolution 3% polyacrylamide gels and the Lipoprint® system (Lipoprint LDL system & Lipoprint HDL system, Quantimetrix Corporation, Redondo Beach, CA) according to the manufacturer's instructions. LDL subfractions were divided into LDL-1 and LDL-2 (large, buoyant LDL, lbLDL) and LDL-3 to LDL-7 (small, dense LDL, sdLDL). Based on these

results a lipoprotein profile is provided; Type A (predominance of lbLDL), intermediate and type B (predominance of sdLDL). Furthermore, the mean LDL particle size (in Å) was determined. HDL subfractions were divided into HDL 1-3 (large HDL), HDL 4-7 (intermediate HDL) and HDL 8-10 (small HDL).

PON1 arylesterase activity

PON1 arylesterase activity was measured in citrate plasma. Briefly, plasma was diluted 1:80 with a salt buffer (20mM Tris-HCl and 1.0 mM CaCl₂). A triplicate of 20 µl diluted plasma were added to the wells in an UV-transparent 96-well plate (Sigma-Aldrich). 200 µl of phenyl acetate solution, containing 3.26 mM phenyl acetate in salt buffer, was added to each well and the absorbance of produced phenol was measured at 270 nm with 250 nm as background in a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA). The initial period when the reaction was linear was used for calculation of activity, expressed as U/ml, using an extinction coefficient of phenol of 1310 M⁻¹ cm¹.

SAA ELISA

To investigate the acute phase response by SAA, plasma SAA1 levels were measured by an ELISA (DY3019-05, R&D systems, Minneapolis, MN) according to the manufacturers' instructions. In short, citrate plasma was added to the plate and incubated for 2 hours at room temperature. Following wash, a detection antibody was added and incubated for 2 hours. The plate was washed and streptavidin-horseradish peroxidase was added followed by incubation for 20 min. The plate was then washed a final time before a substrate solution was added before 20 min incubation. At the end of the incubation, stop solution was added and absorbance was measured at 450 nm with correction at 570 nm using a Spectramax 190 plate reader (Molecular Devices, Sunnyvale, CA).

Cholesterol efflux capacity

Cholesterol efflux was measured with a commercial kit from Sigma-Aldrich (MAK192) according to the manufacturers description. Briefly, a human monocyte cell line, THP-1, was differentiated into macrophages with 10 ng/ml phorbol myristate acetate (PMA) for 24 hours at 37° C and 5% CO₂ in a 96-well plate. The PMA containing medium was replaced with complete cultivation medium (RPMI1640 including 10% foetal bovine serum, 2 mM Glutamine) and incubated for another 30 hours. The serum containing medium was removed and then washed with serum free medium. A reaction mix, containing equilibration buffer and

fluorescence labeled LDL, was added to the cells and incubated for 16 hours. The reaction mix was removed and wells were washed with serum free medium. Patient serum samples were treated with reaction mix from the kit, and the apoB depleted, clear supernatant was added to the plate and incubated for 5 hours. After the incubation, supernatants were transferred to a new plate and the fluorescence was measured (482 ex/515 em). The cell layer was solubilized with a cell lysis buffer and incubated for 30 minutes on a shaker. The cell lysate was then transferred to the plate with supernatants and the fluorescence of the mixture was measured. Percent efflux was calculated as follows: $100 \times \text{fluorescence intensity of the medium} / \text{fluorescence intensity of the medium and cell lysate}$.

Lifestyle changes

Patients had to undergo lifestyle changes preoperatively and they were not accepted for surgery before they had achieved a 10% weight loss. It took patients on average 12 weeks to achieve this weight loss. The details of these lifestyle changes have been described previously [17].

Surgery

All operations were performed by two experienced bariatric surgeons at the Department of Surgery, Nordland Hospital, Bodø. Two surgical methods were used:

1. Laparoscopic Roux-en-Y gastric bypass for patients with a BMI $<50 \text{ kg/m}^2$, $n=27$ (79%).
2. Bileopancreatic diversion with duodenal switch or patients with a BMI $>50 \text{ kg/m}^2$, $n=7$ (21%).

Details of these methods of surgery have been described previously [17].

Among the controls 15 patients had laparoscopic cholecystectomy, while two patients had laparoscopic fundoplication performed

Statistics

Numerical data are presented with mean and standard deviation (SD). A repeated measures one-way analysis of variance (RM One-way ANOVA) was used to calculate the longitudinally effects of the lifestyle changes followed by surgery on the different lipids, LDL/HDL subfractions and parameters of HDL function and composition when appropriate. An unpaired t-test was used to calculate the differences between patients and controls. If the data was not normally distributed, non-parametric tests were applied; Friedman test for the longitudinally effects in the patients and Mann-Whitney test for testing differences between

patients and controls. All tests were two-tailed and results with a $p < 0.05$ were considered statistically significant. Analyses were performed using PRISM 6 (Graph Pad Software Inc, La Jolla, CA).

RESULTS

Anthropometric data

Anthropometric characteristics of the controls and the patients at baseline, after the lifestyle intervention and 12 months after bariatric surgery are presented in Table 1.

The patients were eligible for surgery when they reached a weight reduction of 10% from the lifestyle intervention. One year after the surgery the patients had a mean total weight loss of 44.4 kg, correspondingly a mean reduction in BMI of 15.5 kg/m².

17 patients had a diagnosis of diabetes mellitus type 2 at the inclusion of the study. Surgery was associated with better glycemic control as at 1-year follow-up only 5 patients still had HbA1c > 6.5% or had to remain on diabetes medication. Eight patients used statins at the time of inclusion; four patients used statins at one year after surgery (Table 1).

Lipoprotein profile

The patients had significantly higher baseline levels of TG (158.3 mg/dL vs 93.2 mg/dL, $p < 0.01$) (Fig.1), significantly lower baseline levels of HDL-C (41.9 mg/dL vs. 62.9 mg/dL, $p < 0.0001$) (Fig.1), and no difference in baseline levels of LDL-C compared to the controls (Fig 1).

After lifestyle intervention, the patients had significant reductions in TG (-28.7 mg/dL, $p < 0.05$), LDL-C (-32.3 mg/dL, $p < 0.0001$), and a small, but statistically significant reduction in HDL-C (-4 mg/dL, $p < 0.01$) (Fig.1). At the one year-follow-up after surgery the patients had maintained the significantly lower level of LDL-C (89.1 mg/dL vs. 117.9 mg/dL, $p < 0.001$) compared with the controls, but there was no additional effect compared to the lifestyle intervention (Fig.1). HDL-C had increased statistically significant (+12 mg/dL, $p < 0.0001$), but the levels of HDL-C were still lower than in the controls (53.9 mg/dL vs. 62.9mg/dL, $p < 0.05$). There was no difference in TG between the controls and the patients at this point (Fig.1).

ApoB, apoA1 and apoB/apoA1 ratio

There were no significant differences in baseline serum levels of apoB between patients and controls (331.8 µg/ml vs. 278 µg/ml, $p>0.05$, Fig.1), but the patients had lower baseline levels of apoA1 (860 µg/ml vs. 1061 µg/ml, $p<0.05$, data not shown). The lifestyle intervention reduced apoB (-62.9 µg/ml, $p<0.001$, Fig.1) and apoA1 (-90.6 µg/ml, $p<0.05$). At the one-year-follow up the patients had maintained significantly lower levels of apoB (-89.9 µg/ml, $p<0.0001$, Fig.1), but there was no additional effect compared to the lifestyle intervention. ApoA1 had at this point increased significantly (+137 µg/ml, $p<0.001$) and there were now no significant differences between controls and patients. The patients had higher apoB/apoA1 ratio compared to controls at inclusion ($p<0.001$, data not shown), after the surgery there was no difference between the groups. The lifestyle intervention had no effect on the apoB/apoA1 ratio, at the one year follow-up after surgery the patients had a significant decrease in apoB/apoA1 ratio ($p<0.0001$, data not shown).

LDL subfractions and LDL particle size

There were no significant differences in the baseline values of the lbLDL subfractions or the baseline values of the sdLDL subfractions between the patients and the controls (Fig.2). After lifestyle intervention the patients had a significant reduction in the lbLDL (-18.6 mg/dL, $p<0.0001$) and a small, but statistically significant reduction in the sdLDL (-1 mg/dL, $p<0.05$) (Fig.2). Compared to the controls, the patients had significantly lower lbLDL (41.0 mg/dL vs. 56.9 mg/dL, $p<0.01$), but there were no significant differences in sdLDL (Fig.2).

At the one year-follow-up after surgery the patients had significantly lower levels of lbLDL (-12.9 mg/dL, $p<0.001$) compared to baseline values, while the levels of sdLDL returned to baseline values (Fig.2). Compared to the controls the patients had lower levels of lbLDL (46.7mg/dL vs. 56.9 mg/dL, $p<0.05$), but there were no differences in sdLDL (Fig.2).

There were no significant differences in baseline values of mean LDL particle size (Å) or distribution of lipid pattern (A, B or Intermediate) between controls and patients (data not shown). After the lifestyle changes the patients had a small, but statistically significant increase in mean LDL particle size ($p<0.01$, data not shown). At the one year-follow after surgery up the mean particle size returned to baseline values.

HDL subfractions

The patients had a significantly lower concentration of the large ($p<0.0001$, Fig.2) and intermediate HDL ($p<0.0001$, data not shown) subfractions at baseline compared to the controls, but there were no significant differences in the small HDL subfractions (Fig.2). After the lifestyle intervention, there was a significant reduction in the intermediate ($p<0.05$, data not shown) and small HDL subfractions ($p<0.0001$, Fig.2), however, there was no change in the large HDL subfractions.

At the one year-follow-up after surgery the patients had a significant increase in the large HDL subfractions (+9.5 mg/dL, $p<0.0001$) and there was now no difference compared with the controls (Fig.2). There was also a significant increase in the intermediate HDL subfractions ($p<0.001$, data not shown), but the concentration was still significantly lower than compared to the controls. The small HDL subfractions returned to baseline values, with no significant difference compared with the controls (Fig.2).

PON1 arylesterase activity, SAA1 abundance and SAA/PON1 ratio

The patients had significantly lower baseline PON1 arylesterase activity compared with controls ($p<0.0001$, Fig.3). After the lifestyle changes, there was a significant reduction in PON1 activity ($p<0.01$) in the patients, bariatric surgery had no further effect on PON1 activity (Fig.3). The patients had higher levels of SAA at inclusion compared to controls (10.4 $\mu\text{g/mL}$ vs. 3.9 $\mu\text{g/mL}$, $p<0.01$, data not shown). After the lifestyle intervention, there was a significant reduction in SAA in the patients (-4.6 $\mu\text{g/mL}$, $p<0.01$, data not shown), bariatric surgery had no further effect. The patients had higher SAA/PON1 ratio at inclusion compared to controls ($p<0.001$, Fig.3) and there was a statistically significant decrease in SAA/PON1 ratio after the lifestyle intervention ($p<0.01$, Fig.3) and further improvement at one year after the surgery compared to inclusion ($p<0.0001$, Fig.3).

Cholesterol efflux capacity

We found no significant differences in baseline values of macrophage cholesterol efflux capacity between patients and controls. Neither lifestyle changes nor bariatric surgery had any significant effect of CEC in the patients (Fig.3)

DISCUSSION

In the present study where 34 morbidly obese patients underwent bariatric surgery, we demonstrate anticipated reductions in BMI and total weight loss, in line with systematic reviews and meta-analyses of bariatric surgery [18]. The patients achieved reductions in TG (41%), LDL-C (24%) and apoB (27%) at 12 months after surgery, results that are comparable with previously published literature [19-23]. The fact that bariatric surgery had no additional effect in reducing levels of LDL-C and apoB compared to the preoperative lifestyle intervention are probably due to the fact that the lifestyle intervention had already lowered them as low or even beyond what can be expected from bariatric surgery alone. A small study of pre-bariatric lifestyle changes showed a similar trend [24] and comparable improvements in LDL-C and TG from a similar lifestyle intervention has been published earlier [25]. It is also important to note that four of the eight patients using statins at inclusion of the study had discontinued this medication at the 12-month follow-up.

The patients in this study had low levels of HDL-C and apoA1 at admission, a hallmark of the obese dyslipidemia [26]. The reduction in HDL-C observed after the lifestyle intervention is expected, as reduced caloric intake during weight loss programs is associated with temporary declines in HDL-C, an effect that is reversed once a stable weight is achieved [26]. At 12 months after surgery the patients had a significant increase in HDL-C (29%) and apoA1 (16%) compared with baseline, results that are in line with previously published literature [19, 20, 22].

LDL and HDL subfractions

The patients in this study had a LDL profile at inclusion of the study dominated by pattern A (82%) and only 6% having a pattern B (data not shown), thus the measured levels of sdLDL were low. It is important to note that none of the included obese patients had known or symptomatic CVD. Another possible explanation for the low concentrations of sdLDL observed might be the fact that only half of the patients had diabetes and 41% were men, two important factors associated with elevated levels of sdLDL [27, 28]. The lifestyle changes induced a small, but statistically significant reduction of sdLDL. We did not find any significant improvements or changes in pattern, concentrations of sdLDL or mean LDL-particle size after bariatric surgery compared to inclusion.

As drug trials with niacin and cholesteryl ester transfer protein (CETP) inhibitors, both drugs that significantly increased HDL-C, failed to reduce the risk of CVD [29] and a Mendelian randomization study challenged the concept that raising HDL-C will lower CVD risk [30], serious doubts have been raised about using the cholesterol content of HDLs (HDL-C) as relevant to any aspect of HDL function related to protection of CHD. Instead hypotheses suggesting that the anti-atherogenic mechanisms of HDL are related to HDL function and/or composition, have gained momentum [31-33].

As for HDL composition, epidemiological and clinical studies are discordant regarding the prognostic value of measuring HDL subclasses and subfractions [34]. The reasons are suggested to be different assay methods used, subfraction heterogeneity or ethnic variation [35]. The pathophysiological aspect of HDL composition is still an evolving research field and a recent study indicates that the smaller HDL particles are more efficient mediators of ABCA1-mediated cholesterol efflux [12]. We did not observe any changes in macrophage cholesterol efflux capacity despite statistically highly significant changes in HDL composition and function, including the small HDL (Fig. 3).

The low levels of HDL-C observed in patients at admission compared to controls was due to significantly lower levels of the larger HDL-particles, while there was no significant difference in the concentration of smaller HDL-particles. Other studies of obese patients have observed a similar pattern [36, 37]. At 12 months after surgery the patients had a significant increase in HDL-C due to an increase in large HDL particles.

SAA, PON1 activity and macrophage cholesterol efflux capacity,

Reverse cholesterol transport (RCT), the transport of cholesterol from peripheral tissue to the liver for excretion, is one of many mechanisms by which HDL exerts its protective effect on the development of CVD [34, 38]. Efflux of cholesterol from macrophages in the arterial wall is suggested to be an important aspect of HDL function [39], and cholesterol efflux capacity is indeed inversely correlated with hard vascular endpoints [10]. We did not observe any significant differences in macrophage cholesterol efflux capacity between patients and controls, and no significant effects of the lifestyle intervention or bariatric surgery, despite significant changes in HDL-C concentration and HDL subfractions. However, it is important to keep in mind that using whole serum in an assay for cholesterol efflux capacity may not be a precise measurement of HDL-mediated efflux since cholesterol is transferred from

macrophages to serum via several mechanisms that contain other possible acceptors than HDL. In addition, different assays, as well as apoB depletion methods, that may be more suitable have been described [40, 41]

PON1 is an HDL-associated protein that may protect against CVD by reducing oxidative stress [42, 43]. Reduced PON1 enzyme activity has been observed in a number of diseases that increase risk of CVD, including diabetes and metabolic syndrome [43], and prospective studies have also established reduced PON1 activity as an independent risk factor for CVD [44, 45]. The morbidly obese patients in this study had significantly lower PON1 activity levels compared to controls at inclusion, and surprisingly we observed a significant reduction in PON1 activity after the lifestyle intervention and bariatric surgery. Previous studies have found an increase in PON1 activity after bariatric surgery [46, 47]. Differences in patient populations and methods of surgery, as well as other methodological factors such as time interval between intervention and analysis might explain these discordant findings but further investigation is warranted. There is a possibility that the reduction in PON1 activity after the lifestyle intervention may be related to the decrease in HDL and regarding the bariatric surgery intervention, it is well known that toxic compounds, that are substrates for PON1, are released at weight loss and thereby may influence the PON1 measurements [43, 48]. However, the acute phase protein SAA was significantly higher in patients at baseline compared to controls and SAA was also significantly reduced after the lifestyle intervention and further reduced after bariatric surgery.

Despite the observed reduction in PON1 activity, the recently proposed marker of HDL function, SAA/PON1 ratio [15], did show a significant difference between controls and patients at baseline, followed by a significant decrease after the lifestyle intervention and bariatric surgery.

STUDY LIMITATIONS

This study has several limitations. First the sample size was small, from a single center. Secondly the controls were patients hospitalized to undergo elective surgery (fundoplication or cholecystectomy), and they may not be representative of the healthy population. We also miss follow-up-data on the controls as they were only evaluated once. Measuring LDL- and HDL-subfractions with the Lipoprint system and macrophage cholesterol efflux capacity with the MAK192-kit from Sigma-Aldrich are only two

methods of a heterogeneous set of technologies. Other methodologies such as nuclear magnetic resonance spectroscopy (NMR) for measuring LDL and HDL subfractions [49], or a cholesterol efflux assay measuring ABCA1-mediated cholesterol efflux [40] may provide additional information.

CONCLUSION

Lifestyle intervention, followed by bariatric surgery improved the lipoprotein profile of 34 morbidly obese patients by significantly reducing serum levels of triglycerides, LDL cholesterol and apoB. The patients had a LDL profile at inclusion dominated by pattern A, with low concentrations of small, dense LDL-particles and the observed reductions in LDL cholesterol during the observation period were due to reduced concentrations of the large, buoyant LDL-particles. After bariatric surgery we observed a significant increase in HDL-C, due to increased concentrations of the large HDL particles, and a reduction in apoB/apoA1 ratio. Lifestyle intervention followed by bariatric surgery reduced PON1 activity, but the SAA/PON1 ratio was decreased. Neither the lifestyle intervention nor bariatric surgery had any effect on macrophage cholesterol efflux capacity, despite significant changes in serum levels of HDL-C and in HDL composition.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

CAK, AH and KTL had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: CAK, AH, KTL and TN. Acquisition or analysis: TN, MM, HK, SL, KC, MM-S.

Drafting of the manuscript: CAK, AH and KTL. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: CAK, AH, KTL

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Table 1.**Table 1. The effect of lifestyle changes and surgery on anthropometric characteristics compared to controls**

	Inclusion	Lifestyle intervention	12 months after surgery	Controls
n, No.	34	34	34	17
Age, median (range)	43 (30-58)			45 (27-70)
Female	20 (59%)			12 (71%)
Weight, mean, kg ($\pm$ SD)	130.4 ($\pm$ 24,7)	117.4 ($\pm$ 21.9)	86.0 ($\pm$ 17.3)	73.0 ($\pm$ 7.5)
BMI, mean, kg/m ² ($\pm$ SD)	44.8 ($\pm$ 6,9)	40.1 ($\pm$ 5.7)	29.3 ($\pm$ 4.2)	25.2 ($\pm$ 2.0)
TWL, mean, kg ($\pm$ SD)		13 ($\pm$ 5.7)	44.4 ($\pm$ 18.0)	
Diabetes, No. (%)	17 (50%)		5 (15%)	1 (6%)
Hypertension, No.	14 (41%)		n.d	3 (18%)
Statins, No. (%)	8 (24%)		4 (12%)	2 (11%)
Smoking, No. (%)	3 (9%)		3 (9%)	1 (6%)
HbA1c % ($\pm$ SD)	6.5 $\pm$ 1.1	n.d	5,7 $\pm$ 0.5	5,4 $\pm$ 0.2

BMI: Body mass index, TWL: Total weight loss. N.d: not determined. HbA1c: glycated hemoglobin.

Figures

Figure 1. Serum levels of **A)** LDL cholesterol, **B)** HDL cholesterol, **C)** triglycerides and **D)** apoB at inclusion, after a lifestyle intervention (~3 months) and one year after bariatric surgery in 34 morbidly obese patients, and at inclusion for controls. Values are mean with SD. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, ns = not statistically significant.

Figure 2. Serum levels of the **A)** large, boyant LDL subfractions, **B)** the small, dense LDL subfractions, **C)** large HDL subfractions **D)** small HDL subfractions at inclusion, after a lifestyle intervention (~3 months) and one year after bariatric surgery in 34 morbidly obese patients, and at inclusion for controls. Values are mean with SD. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, ns = not statistically significant.

Figure 3. **A)** Cholesterol efflux capacity, **B)** Serum paraoxonase/arylesterase 1 (PON1) activity and **C)** serum amyloid A1/PON1 (SAA/PON1) ratio determined at inclusion, after a lifestyle intervention (~3 months) and one year after bariatric surgery in 34 morbidly obese patients, and at inclusion for controls. Values are mean with SD. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$, ns = not statistically significant.