Bariatric surgery reduces fasting total fatty acids and increases n-3 polyunsaturated fatty acids in morbidly obese individuals

Running title: **Bariatric surgery and fatty acids**

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ABSTRACT

Background.

Obesity is a global pandemic leading to increased mortality and increased risk of cardiovascular disease. Bariatric surgery is an established treatment of obesity leading to weight loss and reduction of mortality. To further elucidate how bariatric surgery improves metabolic control, we explored the fatty acid (FA) profiles in morbidly obese subjects treated with lifestyle intervention and subsequent bariatric surgery.

Methods.

The intervention group consisted of 34 morbidly obese patients scheduled for bariatric surgery and the control group of 17 non-obese patients scheduled for elective laparoscopic procedures. The intervention group had to undergo lifestyle changes preoperatively. Fasting blood samples were drawn at admission, after lifestyle intervention and one year after bariatric surgery.

Results.

At admission, the morbidly obese patients had significantly higher levels of monounsaturated FAs (MUFAs) and lower levels of n-6 polyunsaturated FAs (PUFAs) and n-3 PUFAs than healthy controls (all p-values<0.05). In the intervention group, there was a significantly lower level of total FAs after lifestyle intervention, and from admission to one year after surgical intervention (both, p<0.05), primarily reflecting a lower proportion of saturated FAs (SFAs). Following bariatric surgery, but not after lifestyle changes, there was an increase in the proportion of n-3 PUFA (p<0.05) reaching levels not significantly different from healthy controls.

Conclusions.

Our findings suggest that a reduced proportion of the proposed anti-atherogenic n-3 PUFAs characterizes morbidly obese individuals, and that this FA profile is reversed by bariatric surgery, but not by lifestyle intervention.

Keywords: Fatty acid; omega-3 fatty acid, omega-6 fatty acid; polyunsaturated fatty acid; bariatric surgery

INTRODUCTION

Obesity is a global pandemic leading to increased mortality and increased risk of cardiovascular disease [1-4]. Bariatric surgery is an established treatment of obesity causing weight loss, reversion of or better control of type 2 diabetes (T2D) and reduction of mortality [5-7]. Exactly how bariatric surgery mediates these effects is, however, not clear, and there are probably several mechanisms involved.

Fatty acids (FAs) are mainly transported and stored as triglycerides in the human body and are important in cell metabolism and function involving their participation in various intracellular signalling pathways [8], and several studies have suggested their involvement in cardiovascular and metabolic related diseases. Elevated levels of total (non-esterified) free FAs are associated with increased risk for adverse events in coronary artery disease [9,10]. Saturated FAs (SFAs) like lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0) increase low density lipoprotein (LDL) cholesterol, and trans-fatty acids, even if being unsaturated, also increase LDL-cholesterol. On the other hand, the polyunsaturated fatty acids (PUFAs) and especially the n-3 PUFAs including α-linolenic acid (ALA, C18:3), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) are thought to be protective of cardiovascular disease [11-13], although meta-analyses have not confirmed such a beneficiary effect so far [14,15]. n-6 PUFAs are precursors of potent inflammatory mediators such as certain prostaglandins and leukotrienes [16], but could also be the basis for certain resolving mediators like lipoxins [17], and the net effects of n-6 PUFAs are not clear.

To further elucidate how bariatric surgery improves metabolic control we explored the FA profiles in morbidly obese subjects treated with lifestyle intervention and subsequent bariatric surgery.

RESEARCH DESIGN AND METHODS

Patients and controls

The main study, including surgical techniques, has previously been described in detail [18], and the patients in the current substudy have recently been characterized [19]. Demographic data of the 51 participants are presented in Table 1.

The study was performed in accordance with the Helsinki Declaration, approved by the regional ethics committee, and written informed consent was obtained of all participants.

Life style intervention and diet

The study design is described in Figure 1. Thirty-four morbidly obese patients (BMI 44.8±6.9 kg/m^2, 17 diabetics and 17 non-diabetics) scheduled for bariatric surgery served as the intervention group. The control group consisted of 17 non-obese patients (BMI 25.3±2.1 kg/m^2) scheduled for elective laparoscopic procedures. All patients in the intervention group had to undergo lifestyle changes resulting in a minimum of 10% weight loss before bariatric surgery. Patients who did not achieve 10% preoperative weight loss were excluded from the study. In addition, the patients were free to contact the center for guidance if needed (according to Norwegian guidelines).

The patients were encouraged to perform physical activity according to the Norwegian guidelines for treatment of morbidly obese patients [20]. The intervention group was informed to have a total of three main meals daily, and three to four small meals in between, and was advised to an energy restricted diet of 2000 kcal for males and 1500 kcal for women. This diet included a fat energy content of less than 30%,

complex carbohydrates energy content of less than 50% and protein energy content up to 20%. Especially intake of saturated fats was discouraged, and the recommended proportion of total calories should be below 10% [21]. However, food intake and supplementation of different types of FAs including PUFAs were not registered. If the patients did not eat fatty fish products, they were advised to take n-3 PUFA supplementation. The average time for the patients to achieve 10% weight loss was 12 weeks. During the 12 weeks of lifestyle intervention, the advises from dietists were repeated every two weeks. Furthermore, the patients were counselled before leaving hospital after surgery, as well as two times after surgery the following year before the next evaluation one year after surgery.

Laboratory methods

Fasting blood samples were collected with standard venipuncture and transported on ice and centrifuged at 2500g for 15 minutes at 4˚C, then stored in aliquots at -70˚C for later analysis in batch. Samples were drawn at admission (when entering the study), after lifestyle intervention (average of 12 weeks) and finally one year after bariatric surgery (Figure 1).

Measurements of plasma total levels and composition of fatty acids

Measurement of total level and composition of FAs was performed after extracting lipids from plasma using a mixture of chloroform and methanol (2:1) [22]. The extracts were transesterified using BF_3/methanol [23]. Extracts of fatty acyl methyl esters (FAME) were heated in 0.5 mol L^{-1} KOH in ethanol/water solution (9:1), [24] to remove neutral sterols and nonsaponifiable material. Recovered FAs were re-

esterified using BF_3/methanol. The methyl esters were quantified by gas chromatography as previously described [25].

The FAME were analyzed on a GC8000 Top GC (Carlo Erba Instrument, Italy), equipped with a flame ionization detector (FID), and a capillary column coated with a highly polar SP2340 phase (Supelco, USA) (60m x 0.25mm x 0.20μm). Hydrogen constant flow 1.6ml/min was used as a carrier gas. PTV injector in split mode (split ratio 1:20) and FID were operated at 235°C. Column temperature was programmed from 130°C to 214°C (rate 1.4°C/min) and from 214°C to 220°C (rate 3.0°C/min). FAME were identified by comparison with known standards (Larodan Fine Chemicals, Sweden; Sigma, USA; Supelco, USA) and by GC/MS (GCQ Finnigan, USA) on the same column. Quantification of the fatty acids was made with Chrom-Card A/D 1.0 chromatography station (Carlo Erba Instruments, Italy) using heneicosanoic acid as the internal standard. Reference mixtures and individual FAME standards were used for calculation of response factors throughout the procedure. Standards of some minor fatty acids were not available on the market, and the response factors of these fatty acids were estimated according to the standards with the similar identity. The analytical reproducibility of whole procedure was regularly tested with intra assay coefficient of variation (CV) being below 1% for major fatty acids, about 2% for fatty acids proportion 0.1-1.0% of total fatty acids, and CV about 3% for fatty acid proportion below 0.1% of total fatty acids. The EPA CV=1.3% and eicosatetraenoic acid (ETA, C20:4n-3) CV=2.2%. Similar precision CV values were measured in the plasma concentration range of individual fatty acids with the high linearity of the calibration curves ($r>0.9999$).

Statistics

Numerical data are presented with mean and standard deviation (SD). An unpaired Student t-test was used to calculate the differences between the control group and the intervention group at admission. The D'Agostino-Pearson test was used to test for normality distribution. Non-parametric testing (Mann-Whitney) was performed if the samples were not normally distributed. A repeated measures one-way analysis of variance (RM one-way ANOVA) was used to calculate the longitudinally effect (admission/lifestyle intervention/one year postoperatively) of bariatric surgery on the different fatty acids. All tests were two-tailed and results with a $p<0.05$ were considered statistically significant. Correction for multiple testing was not performed due to the fact that the sample values were not independent. Analyses were done using PRISM 6 (Graph Pad Software Inc, La Jolla, CA).

RESULTS

Plasma FA profile in morbidly obese compared with controls (Table 2)

There was no difference between the controls and the intervention group at admission regarding the total amount of FAs, and there were no differences in the proportion of saturated FAs (SFAs) and trans-FAs. Interestingly, however, the plasma levels of myristic acid (C14:0) and palmitic acid (C16:0) were significantly higher in the morbidly obese. The level of MUFAs was higher in the morbidly obese group compared to the control group, and this was mainly due to higher levels of palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9). In contrast, the proportion of both n-3 and n-6 PUFAs were significantly lower in the morbidly obese compared to controls. The lower proportion of n-3 PUFAs seems primarily to reflect lower levels of DPA and DHA. The lower level of n-6 PUFAs seems primarily to reflect a lower level of linoleic acid (C18:2n-6). Interestingly, the precursor for prostaglandins, arachidonic acid (AA, C20:4n-6) showed no differences between morbidly obese and controls. The results seem to be driven mainly by a difference between diabetics and controls as there were statistically significant differences between these groups. However, there was a similar, non-significant trend also for non-diabetic obese vs. controls.

Plasma FA profiles during intervention in morbidly obese (Table 2)

The fasting total FA concentration was lower after lifestyle intervention than at admission, and even lower one year after bariatric surgery. This difference seemed due to lower levels of mainly SFAs, and to some extent trans-FAs. The lower level of SFAs one year after bariatric surgery was mainly due to reduced level of palmitic acid

(C16:0) and stearic acid (C18:0). Trans-FAs was lower after lifestyle intervention, driven by lower levels of *trans* vaccenic acid (C18:1t), while *trans* palmitoleic acid (C16:1n-7t) was unchanged. The reduction in C18:1t was not sustained one year after surgical intervention. The trans-FAs, however, had only 0.3 weighted % of the total FAs. For the reduction in total FAs, the same pattern with a significant decrease from admission to one year after surgery was seen in both diabetic (n=17) and non-diabetic (n=17) patients (data not shown).

It was of interest to note that the total MUFA level increased from admission through the lifestyle intervention, but decreased on year after bariatric surgery compared to admission. This change in FA profile was primarily due to oleic acid (C18:1n-9) and vaccenic acid (C18:1n-7).

Of note, whereas the proportion of n-3 PUFAs was unchanged from admission through the lifestyle intervention, there was a significant increase one year after bariatric surgery primarily reflecting an increase in DHA (C22:6n-3) and DPA (C22:5n-3). Moreover, while the proportion of eicosatetraenoic acid (ETA, C20:4n-3) and eicosapentaenoic acid (EPA, C20:5n-3) decreased during lifestyle intervention, it increased after bariatric surgery.

The proportion of n-6 PUFAs was unchanged throughout the study period. Notably, however, whereas the proportion of AA (C20:4n-6) increased during lifestyle intervention, it returned to baseline levels one year after bariatric surgery.

DISCUSSION

In the present study, we show that morbidly obese individuals are characterized by higher levels of MUFAs as well as significantly lower proportions of both n-6 and n-3 PUFAs compared to non-obese controls. Of note, following lifestyle intervention and bariatric surgery, there was a lower level of fasting total FAs, primarily reflecting a reduced level of SFAs. Furthermore, following bariatric surgery, but not after lifestyle intervention, there was an increase in the proportion of n-3 PUFA reaching levels comparable to healthy controls, primarily reflecting higher levels of DHA (C22:6n-3) and DPA (C22:5n-3). Our findings suggest that morbidly obese individuals are characterized by a decreased proportion of the proposed anti-atherogenic n-3 PUFAs, and that this disturbed FA profile is reversed by bariatric surgery, but not by lifestyle intervention. This increase in n-3 PUFA could potentially contribute to the beneficial effect of bariatric surgery on T2DM and mortality.

Furthermore, in the present study we also found that the proportion of the n-3 PUFAs was significantly lower in the morbidly obese than in controls, reflecting lower levels of DHA and DPA. Moreover, whereas we observed unchanged levels of n-3 PUFAs from admission throughout lifestyle intervention, one year after bariatric surgery there was a significant higher proportion of n-3 PUFA, and again, primarily reflecting changes in DHA and DPA. Based on the suggested beneficial role of n-3 PUFA in cardiovascular and metabolic related disorders, these findings could be of particular interest in relation to the beneficial effects of bariatric surgery in morbidly obese individuals. Previously, Chalut-Carpentier et al noted that most of the n-3 PUFAs except DHA were significantly lower six months after bariatric surgery [26]. Herein we extend these findings by showing that these changes in n-3 PUFA were not

obtained during dietary intervention, but were restricted to the period after bariatric surgery. The mechanisms for this effect are at present unclear, but could potentially reflect indirect or direct effects of loss of adipose tissue. It is unlikely that it was caused by changes in n-3 PUFA supplementation. Thus, the patients had close follow up by dietists in the lifestyle intervention period, and were advised to take n-3 supplementation if they did not eat fatty fish, and importantly, lifestyle intervention did not induce any changes in the proportion of n-3 PUFA. In fact, the proportion of EPA and ETA decreased during lifestyle intervention whereas it increased after bariatric surgery. However, we cannot draw firm conclusions as to the mechanism for these changes since we lack accurate registration of intake through meals and supplementations during the study. Nevertheless, it appears that weight loss through lifestyle intervention (reduced intake) affects lipid metabolism differently from a weight loss achieved through bariatric surgery (reduced absorption). Whatever the mechanisms, the increase in the proportion of n-3 PUFA following bariatric could be of importance for the beneficial effect of this procedure on cardiovascular and related metabolic disorders.

We demonstrate significantly lower levels of total FAs after lifestyle changes with a further decrease one year after bariatric surgery. The lower levels of total FAs were due to a reduction in the proportion of SFAs and to a lesser extent in trans-FAs. Our demonstration of a sustained reduction in SFAs can be important when keeping in mind the increasing awareness of the “gut-brain axis”, and the postulated effect that reducing saturated fats with bariatric surgery could affect hypothalamic neurons pivotal to human energy control [27]. Moreover, the reduction of SFAs one year after bariatric surgery was mainly due to reduced proportion of palmitic acid (C16:0) and

stearic acid (C18:0). Importantly, palmitate, a term for the salts and esters of palmitic acid, has recently been shown to be an important inducer of inflammation in metabolic related disorders like diabetes and obesity through activation of NLRP3 inflammasomes [28]. Previous studies have suggested an association between CRP and SFA in obese individuals [29,30], and we suggest that this could involve palmitate-NLRP3 inflammasome-related mechanisms.

The level of n-6 PUFAs was lower in the morbidly obese compared to controls at admission. This finding has previously been observed in a meta-analysis [31]. The finding of lower levels of n-6 PUFAs in morbidly obese could be of clinical interest since the n-6 PUFAs have been considered pro-inflammatory [32], but no clear relationship with risk of cardiovascular disease has been demonstrated [33]. In the current study the levels of n-6 PUFAs remained unchanged, in contrast to the findings of Chalut-Carpentier et al who demonstrated a reduction in n-6 PUFAs 6 months after bariatric surgery [26]. Interestingly, however, we found that while AA, the prototypical inflammatory n-6 PUFA (C20:4n-6) [34], increased following dietary intervention, it returned to baseline level following bariatric surgery.

In our study the proportion MUFAs was significantly higher in the morbidly obese than the controls, and the proportion of MUFAs was even higher after lifestyle intervention, though unchanged from baseline one year after bariatric surgery. Several national agencies recommend that the proportion of MUFAs is below 25 percent of the total energy intake, however, firm recommendations have not been made due to lack of evidence [35]. The increase in MUFA in our study was primarily due to an increased proportion of oleic acid (C18:1n-9) and vaccenic acid (C18:1n-7), and the

mechanism for these changes are uncertain. We have previously shown that high levels of vaccenic acid is associated with disease severity and mortality in patients with heart failure, potentially reflecting harmful effects on the myocardium [36], and this MUFA has also been implicated in the pathogenesis of steatohepatitis [37]. At present, however, the role of vaccenic acid in obesity related disorders is unknown.

Limitations

The sample size in the current study was relatively small, and the findings need confirmation in larger study group. Diet registration and registration of FA supplementation (e.g. n-3 fatty acid intake) should be accounted for in later studies. Extended follow-up and repeated measurements in the control group should also be performed in later studies.

Conclusion

Morbidly obese individuals are characterized by higher levels of MUFAs as well as significantly lower proportion of both n-3 and n-6 PUFAs compared to non-obese controls. Following lifestyle changes and after bariatric surgery, there was a lower level of fasting total FAs, primarily reflecting a reduced level of SFAs. Furthermore, following bariatric surgery, but not after lifestyle changes, there was an increase in the proportion of n-3 PUFA reaching levels comparable to healthy controls. Our findings suggest that a reduced proportion of the proposed anti-atherogenic n-3 PUFAs characterizes morbidly obese individuals, and that this FA profile is reversed by bariatric surgery, but not by lifestyle intervention. This increase in n-3 PUFA could potentially contribute to the beneficial effect of bariatric surgery on T2DM and mortality.

Author Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

TABLES

Table 1. Baseline characteristics

	Controls (n=17)	Intervention (n=34)
Males	5 (29%)	15 (44%)
Age (years)	48.1±14.9	43.2±9.0
Type 2 Diabetes	1 (6%)	17 (50%)
BMI (kg/m2)	25.3±2.1	44.8±6.9
Cholesterol (mmol/l)	5.5±0.9	5.18±1.1
Triglycerides (mmol/l)	1.1±0.3	1.8±0.9
HbA1c (mmol/mol)	38± 2.0	49±13

Data as mean±SD or numbers (%)

Table 2. Fatty acid groups in controls versus morbidly obese, and during intervention

	Controls (n=17)	Morbidly obese, admission (n=34)	Lifestyle intervention (n=34)	1 year after surgery (n=34)
Total FAs (µg/ml)	2973±539	3884±2457	3316±2304§	3053±1777$^{\$}$
Wt.% of total FAs				
Σ Saturated FAs	31.7±1.1	32.5±1.4	31.1±0.9§	30.8±1.2$^{\$}$
C12:0	0.09±0.07	0.09±0.04	0.06±0.07§	0.08±0.04
C14:0	0.8±0.2	0.9±0.2*	0.5±0.1§	0.8±0.3$^{\$\#}$
C16:0	20.7±1.3	21.5±1.4*	22.4±1.0§	20.6±1.3$^{\$\#}$
C18:0	7.5±0.8	7.4±0.6	6.1±0.7§	6.9±0.6$^{\$\#}$
C20:0	0.25±0.03	0.22±0.05	0.22±0.04	0.21±0.03
Σ Trans-FAs	0.3±0.1	0.3±0.1	0.25±0.07§	0.29±0.07$^{\#}$
C16:1n-7t	0.03±0.01	0.04±0.01	0.04±0.01	0.03±0.01
C18:1t	0.3±0.1	0.3±0.1	0.2±0.1§	0.3±0.1$^{\#}$
Σ MUFAs	22.6±2.6	25.8±3.4*	28.2±3.4§	26.9±3.9$^{\#}$
C16:1n-7	1.6±0.4	2.0±0.6*	1.8±0.5§	1.7±0.6$^{\$}$
C16:1n-9	0.20±0.06	0.25±0.05*	0.24±0.07	0.29±0.06$^{\$\#}$
C18:1n-7	1.5±0.2	1.6±0.2	1.9±0.3§	1.7±0.3$^{\$\#}$
C18:1n-9	17.6±2.4	20.3±3.1*	22.4±3.1§	21.2±3.2$^{\#}$
C24:1n-9	1.2±0.2	1.1±0.3	1.4±0.3§	1.4±0.3$^{\$}$
Σ n-3 PUFAs	6.6±2.4	5.5±1.2*	5.4±1.2	6.4±2.3$^{\#\$}$
C18:3n-3	0.6±0.2	0.7±0.2	0.6±0.1§	0.6±0.1
C18:4n-3	0.03±0.02	0.03±0.01	0.02±0.01§	0.03±0.03$^{\#}$
C20:5n-3	1.4±0.9	1.1±0.4	0.8±0.4§	1.3±1.0$^{\#}$
C20:4n-3	0.135±0.044	0.149±0.033	0.070±0.014§	0.146±0.073$^{\#}$
C22:5n-3	0.7±0.2	0.6±0.1*	0.6±0.1	0.8±0.2$^{\#\$}$
C22:6n-3	3.7±1.3	2.9±0.7*	3.3±0.8§	3.5±1.1$^{\$}$
Σ n-6 PUFAs	38.7±3.2	35.8±4.0*	35.0±3.3	35.5±3.9
C18:2n-6	29.2±3.4	26.0±4.4*	24.3±3.3§	25.5±3.5
C18:3n-6	0.36±0.21	0.44±0.15*	0.24±0.09§	0.33±0.13$^{\#\$}$
C20:3n-6	1.7±0.5	2.0±0.3*	1.2±0.2§	1.7±0.4$^{\#\$}$
C20:4n-6	6.9±1.2	6.9±1.4	8.7±1.9§	7.3±1.4$^{\#}$
C22:5n-6	0.130±0.054	0.119±0.043	0.127±0.042	0.154±0.053$^{\#\$}$
Σ n-9 PUFAs	**0.12±0.02**	**0.15±0.07**	**0.11±0.03§**	**0.15±0.05$^{\#}$**
C20:3n-9	0.125±0.023	0.150±0.065	0.108±0.034§	0.151±0.050$^{\#}$

Values are mean±standard deviation, *p<0.05 controls compared to morbidly obese,§p<0.05, morbidly obese at admission compared to lifestyle.

#p<0.05 lifestyle intervention compared to 1 year after surgery,$p<0.05 admission compared to 1 year after surgery

FAs:Fatty Acids, Wt%:Weighted%, MUFAs:Mono Unsaturated FAs, PUFAs:Poly Unsaturated FAs

FIGURE LEGENDS

Figure 1. Study design.

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