

# Intensive lifestyle intervention provides rapid reduction of serum fatty acid levels in women with severe obesity without lowering omega-3 to unhealthy levels

C. Lin<sup>1,2</sup>, J. R. Andersen<sup>3,4</sup>, V. Våge<sup>4,5</sup>, T. Rajalahti<sup>1</sup>, S. A. Mjøs<sup>2</sup> and O. M. Kvalheim<sup>2,3</sup>

## What is already known about this subject

- High levels of the marine omega-3 fatty acids (FAs) reduce obesity-induced inflammation and decrease the risk of developing conditions related to metabolic syndrome.
- The weight loss process after bariatric surgery is accompanied by a reduction of serum omega-3 levels.

## What this study adds

- Intensive lifestyle intervention rapidly reduces elevated total FA serum level and levels of saturated, monounsaturated and most omega-6 polyunsaturated FAs.
- Weight loss during lifestyle intervention is not significantly associated to change in FA levels and does not imply lowering of the omega-3 FAs to unhealthy levels.
- Lower rate of weight loss accompanying lifestyle intervention compared to bariatric surgery implies less input from fat deposits, which are poor in omega-3. This makes lifestyle patients less exposed to omega-3 shortage.

<sup>1</sup>Fjordomics, Førde Hospital Trust, Førde, Norway; <sup>2</sup>Department of Chemistry, University of Bergen, Bergen, Norway; <sup>3</sup>Faculty of Health Studies, Sogn og Fjordane University College, Førde, Norway; <sup>4</sup>Center of Health research, Førde Hospital Trust, Førde, Norway; <sup>5</sup>Department of Surgery, Voss Hospital, Bergen Health Trust, Voss, Norway

Received 3 January 2016; revised 24 April 2016; accepted 6 May 2016

Address for correspondence: OM Kvalheim, Department of Chemistry, University of Bergen, Realfagbygget, Allegt. 41, N-5007 Bergen, Norway. E-mail: olav.kvalheim@uib.no

## Summary

Serum fatty acid (FA) levels were monitored in women with severe obesity during intensive lifestyle intervention. At baseline, total FA levels and most individual FAs were elevated compared to a matching cohort of normal and overweight women (healthy controls). After 3 weeks of intensive lifestyle intervention, total level was only 11–12% higher than in the healthy controls and with almost all FAs being significantly lower than at baseline, but with levels of omega-3 being similar to the healthy controls. This is contrary to observations for patients subjected to bariatric surgery where omega-3 levels dropped to levels significantly lower than in the lifestyle patients and healthy controls. During the next 3 weeks of treatment, the FA levels in lifestyle patients were unchanged, while the weight loss continued at almost the same rate as in the first 3 weeks. Multivariate analysis revealed that weight loss and change of serum FA patterns were unrelated outcomes of the intervention for lifestyle patients. For bariatric patients, these processes were associated probably due to reduced dietary input and increased input from the patients' own fat deposits, causing a higher rate of weight loss and simultaneous reduction of the ratio of serum eicosapentaenoic to arachidonic acid.

**Keywords:** Bariatric surgery, intensive lifestyle intervention, multivariate analysis, serum omega-3 fatty acids.

## Introduction

Many fatty acids (FAs), particularly the polyunsaturated FAs (PUFAs), are crucial for metabolic health. After the discovery of the inverse correlation between levels of marine omega-3 FAs in plasma and incidence of cardiovascular diseases (CVDs) (1), numerous investigations have confirmed the importance of these PUFAs for reducing risk of CVD (2–4) by redistributing the lipoprotein particles towards a healthier pattern. The ratio of eicosapentaenoic acid (EPA) to arachidonic acid (AA), EPA/AA, is regarded as a marker of health, and the World Health Organization recommends a level of 0.1–0.2, while in Japan a ratio of 0.2–0.3 is recommended (5).

Obesity may lead to inflammation of adipose tissue and increased oxidative stress (6) accompanied by a reduction in PUFAs. Micallef *et al.* (7) compared relative levels of plasma PUFAs in adults with normal weight, overweight and obesity. They observed a statistically significant inverse relationship between weight status and percentage of EPA, docosahexaenoic acid (DHA) and total omega-3 FAs, i.e.  $\alpha$ -linolenic acid (ALA) and docosapentaenoic acid (DPA) in addition to EPA and DHA. However, the relative levels of omega-3 FAs in serum can be lower in persons with obesity even if the absolute levels are the same as in those without obesity. This may happen if the total concentration of FAs (TFAs) is significantly higher in subjects with obesity than those without obesity. Thus, comparison of serum FA levels should be based on absolute concentrations. Using absolute concentrations, Lin *et al.* (8) observed an inverse relationship between body mass index (BMI) and omega-3 between a group of adults with severe obesity ( $\text{BMI} \geq 40$  or  $35 \leq \text{BMI} \leq 40$  and presence of obesity-related disease) selected for bariatric surgery and a control group of healthy subjects with  $\text{BMI} \leq 30$ . Not only was the marine omega-3 FAs significantly lower in the subjects with obesity but also the plant-derived omega-3 ALA was lower. Furthermore, analysis of serum FAs 3 months after bariatric surgery showed a further 30% decline in serum levels of the omega-3 FAs and a 40% decline in EPA/AA from already low preoperative levels. One year after surgery, patients who had experienced laparoscopic sleeve gastrectomy (LSG) (9,10) had concentrations close to preoperative levels for EPA and EPA/AA, while patients who had been exposed to biliopancreatic diversion with duodenal switch (11) were still low on serum EPA and EPA/AA.

During weight loss, the FA levels in serum are influenced both by dietary intake and input from the patient's own fat deposits. The FAs stored in the fat deposits are not optimal from a nutritional point of view. ALA and DHA constitute approximately 0.7–1.1% and 0.1–0.2%, respectively, of adipose tissue, while EPA is only present in minute amounts (12,13). If the observed reduction in omega-3 levels after bariatric surgery is a response to the weight loss

process accompanying surgery, a similar reduction may also occur in subjects undergoing intensive lifestyle interventions. If this is the case, the patients may need omega-3 supplements during treatment. Thus, the primary aim of this work was to reveal possible changes in serum FA patterns during intensive lifestyle intervention. A secondary objective was to reveal possible differences in total FA level between the cohort of patients with obesity during lifestyle treatment and matching cohorts of healthy subjects and patients subjected to bariatric surgery.

## Methods

### Participants

Thirty-one women from the Fjord region of Western Norway were selected for an intensive lifestyle intervention focusing on a high-fibre low-fat reduced-energy Nordic diet, increased physical activity (10 h per week organized sessions plus individual sessions) and cognitive behaviour therapy (14). Inclusion criteria for lifestyle patients were age 18–60 years,  $\text{BMI} \geq 40$  or  $\text{BMI} \geq 35$  and the presence of obesity-related disease. Exclusion criteria were pregnancy, heart disease, drug or alcohol abuse, bariatric surgery and mental disorder or physical impairment. The treatment included four periods of enrolment at the Red Cross Haugland Rehabilitation Center (RCHRC), but the present work describes only the results during the first enrolment of 6 weeks as this was the period where significant changes occurred in FA profiles. A control group of 45 healthy women was included in our study design. Inclusion criteria were age 18–60 years and BMI in the interval 18.5–30. Exclusion criteria for healthy controls were pregnancy, smoking, drug abuse, use of lipid-lowering drugs and established CVD, type 2 diabetes and cancer. A second control group consisted of 18 adults measured 3 months after LSG. Before being discharged from hospital, the bariatric patients were recommended a diet of small protein-rich meals, to avoid sugar and sugar-containing products, to use a multivitamin supplement and 1 g of calcium on daily basis during the first year after surgery. Exclusion criteria for bariatric patients were alcohol or drug abuse and active psychosis. Three months after surgery, one of the 17 subjects was on lipid-lowering drugs and was excluded, but none showed signs of type 2 diabetes. The group included three men and 14 women. Men and women in the bariatric group had similar FA profiles, and the men were included in order to obtain a larger sample size. Written informed consent was obtained for each participant. The study was approved by the Regional Committee for Medical Research Ethics in southern Norway (reference numbers: 2009/2174 and 2010/3224) and was conducted in accordance with the WMA Declaration of Helsinki and its amendments.

Three lifestyle patients were excluded due to use of lipid-lowering drugs, and two were excluded because of type 2 diabetes. Blood samples were missing for two women at baseline and for one woman after intervention. For another patient, the weight was only available at baseline. This provided 24 lifestyle patients at baseline, 23 for comparing different sampling points during treatment and 25 for comparing the patients with the healthy controls and bariatric patients 3 and 6 weeks into the lifestyle intervention.

### Anthropometric and standard blood variables

Table 1 provides mean ± standard deviation at baseline for some commonly measured variables. BMI for lifestyle patients after 3 and 6 weeks of enrolment at RCHRC was 40.5 ± 5.9 and 39.7 ± 5.7 kg/m<sup>2</sup> (N = 23), respectively, and 37.5 ± 3.6 kg/m<sup>2</sup> (N = 17) for bariatric patients 3 months after surgery. Weight for lifestyle patients at baseline and after 3 and 6 weeks were 116.5 ± 16.5, 113.6 ± 16.0 and 111.3 ± 15.6 kg, respectively.

### Blood sampling and measurement of fatty acid profiles

Blood samples for lifestyle patients were collected at enrolment (baseline) and after 3 and 6 weeks of treatment. For bariatric patients, samples were taken just before and 3 months after surgery. All samples were taken in the morning after overnight fasting. Serum was obtained according to a standardized protocol (17) and stored at -80°C.

The serum samples were prepared and analysed by minor modifications of the procedure described in Lin *et al.* (17). Total amounts of 18 FAs in each sample were quantified as µg per gram sample.

### Univariate statistical analysis

Univariate comparisons of the FA profiles of the lifestyle patients at the three sampling points during their first enrolment at RCHRC were performed using the Wilcoxon sign-rank (WSR) test (15). The P-values were corrected for multiple testing by means of a false discovery rate (FDR) according to Benjamini and Hochberg (18). Univariate comparisons of lifestyle patients with either healthy controls or bariatric patients were performed by means of the Wilcoxon-Mann-Whitney (WMW) rank sum test (15,16) corrected for multiple testing by means of FDR. The univariate probabilities were calculated by means of Matlab R2013b (MathWorks, Natick, Massachusetts, USA).

### Multivariate data analysis

For the lifestyle patients, multivariate comparisons of serum FA profiles at baseline with profiles after 3 weeks of treatment were carried out using partial least squares discriminant analysis (19). BMI was included in the analysis in order to assess possible associations between changes in BMI and FA profiles. Post-processing by target projection (20) was performed to obtain the discriminatory FA pattern that could be quantitatively displayed as a selectivity ratio (SR) plot (21,22). This display shows an impact on individual FAs, with confidence limits calculated by repeated double cross validation (23) and if an FA increases or decreases from one sampling point to another. A similar analysis was carried out for bariatric patients to compare FA profiles before and after surgery. Due to large differences in amounts of the individual FAs, the variations in FAs were standardized to unit variance prior to the multivariate analysis. The analysis was carried out by means of the commercial software package Sirius (Pattern Recognition Systems AS, Bergen, Norway).

**Table 1** Mean and standard deviation for anthropometric variables and standard blood variables for healthy controls, lifestyle and bariatric patients at baseline. *p*<sub>WMW</sub> are the P-values calculated from the nonparametric Wilcoxon-Mann-Whitney (WMW) rank sum test (15,16) for comparing lifestyle and bariatric patients

Variable	Healthy controls (N = 45)	Lifestyle patients (N = 23)	Bariatric patients (N = 17)	Lifestyle vs. bariatric <i>p</i> <sub>WMW</sub>
Age	40.4 ± 10.6	43.1 ± 11.3	43.1 ± 12.4	0.95
Weight (kg)	65.6 ± 6.9	116.5 ± 16.5		
Body mass index (kg m <sup>-2</sup> )	23.2 ± 2.2	41.5 ± 6.1	45.1 ± 3.4	0.05
Fat mass (kg)	18.5 ± 5.2	58.2 ± 11.3		
Fat percent	27.7 ± 5.3	49.5 ± 3.3		
Total cholesterol (mmol L <sup>-1</sup> )	5.1 ± 1.1	5.2 ± 1.2 (N = 21)	5.0 ± 1.0	0.91
Low-density lipoprotein cholesterol (mmol L <sup>-1</sup> )	3.1 ± 1.0	3.7 ± 1.1 (N = 21)		
High-density lipoprotein cholesterol (mmol L <sup>-1</sup> )	1.6 ± 0.3	1.3 ± 0.3 (N = 21)	1.1 ± 0.3	0.12
Triglycerides (mmol L <sup>-1</sup> )	0.8 ± 0.3	1.2 ± 0.5 (N = 21)	1.5 ± 0.5 (N = 16)	0.08

## Results

### Fatty acid profiles in lifestyle patients vs. healthy controls

At baseline, the TFA serum level is approximately 45% higher in the lifestyle patients than in the healthy controls (Table 2). The WMW rank sum test, after correcting for multiple testing by FDR, shows that the differences in medians for the two groups are statistically significant for almost all FAs. Thus, at  $P = 0.05$ , only 14:0, EPA, DPA and the EPA/AA ratio obey the null hypothesis of equal medians, while at  $P = 0.01$ , the omega-3 FAs, ALA and DHA also have similar medians.

### Change in fatty acid profiles after intensive lifestyle intervention

Table 3 shows the medians of the individual FAs for the lifestyle patients at baseline (visit 1) and after 3 weeks of treatment (visit 2). The  $P$ -values from the WSR test, corrected for multiple testing by FDR, show significant changes in median at  $P = 0.01$  for all the saturated FAs. The omega-3 FAs show different behaviour after 3 weeks of lifestyle treatment. Thus, ALA and DPA are significantly reduced from baseline at  $P = 0.01$ , while EPA is reduced at

$P = 0.05$ , although borderline and DHA show no significant changes. The EPA/AA ratio is also not significantly changed from baseline. The levels of the monounsaturated C16 and C18 omega-7 and omega-9 FAs are significantly reduced from baseline except for 18:1 n-7. The essential omega-6 FA, linoleic acid (LA) shows reduction at  $P = 0.01$ . Actually, all the omega-6 FAs except AA show reduction from baseline at  $P = 0.01$  (Table 3).

The changes in each FA from baseline are displayed as SRs in an SR plot. The plot is obtained from a multivariate discriminant analysis with Visit No. as the dependent variable. The discriminating information of each FA is quantitatively displayed in the SR plot. A value of zero within the confidence limits, which correspond to  $P = 0.05$ , means that the FA carries no discriminating information. The higher the SR is for an FA, the larger the discriminating ability is for that FA and the larger the change is from visit 1 to 2. The FAs with a positive sign are increasing in concentration from baseline level, while a negative sign implies reduction from baseline level. Figure 1 shows that all the FAs are significantly reduced during lifestyle treatment except 18:1 n-7, EPA, 24:1 n-9 (nervonic acid), DHA and the EPA/AA ratio, which are unchanged. The largest change is observed for the long-chain saturated FAs with 24 and 22 carbons followed by the saturated FAs 14:0 and 18:0 and omega-6 FAs, LA and dihomo- $\gamma$ -linolenic acid

**Table 2** Univariate statistical measures calculated for women with severe obesity selected for intensive lifestyle intervention ( $BMI \geq 35 \text{ kg m}^{-2}$ ) and a matching cohort of healthy controls ( $BMI \leq 30 \text{ kg m}^{-2}$ ). Medians, minimum and maximum values are given in units of  $\mu\text{g}$  per g sample.  $p_{\text{WMW}}$  are the  $P$ -values calculated from the nonparametric WMW rank sum test (15,16). Asterisks (\* and \*\*) imply significance at  $P = 0.05$  ( $p_{\text{WMW}} = 0.04$ ) and  $P = 0.01$  ( $p_{\text{WMW}} = 0.007$ ), respectively, after correcting for multiple testing by false discovery rate (18)

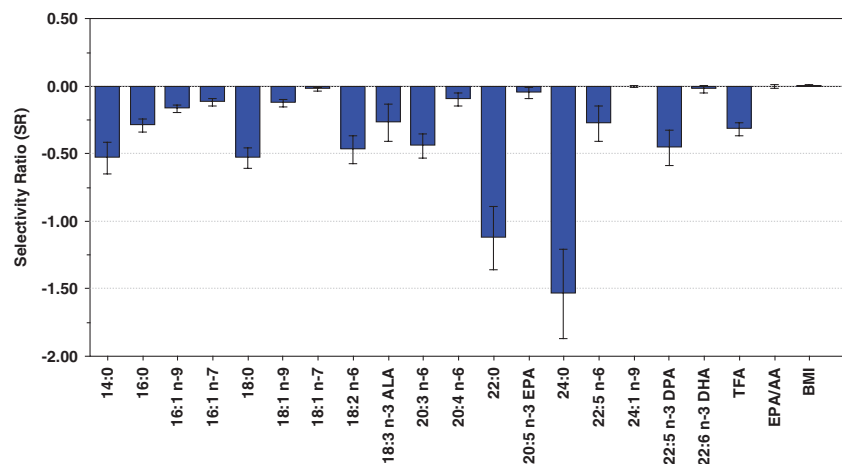
Fatty acid	Patients, $BMI \geq 35 \text{ kg m}^{-2}$ ( $N = 24$ )			Healthy controls, $BMI \leq 30 \text{ kg m}^{-2}$ ( $N = 45$ )			$p_{\text{WMW}}$
	Median	Minimum	Maximum	Median	Minimum	Maximum	
14:0	46.0	12.0	97.6	38.4	13.1	93.3	0.142
16:0	1252	545.5	2101	801.5	534.8	1343.5	$5.6 \times 10^{-6**}$
16:1 n-9	22.4	8.3	48.6	12.6	5.1	30.4	$1.1 \times 10^{-4**}$
16:1 n-7	141.1	43.6	292.3	69.9	28.7	196.9	$3.2 \times 10^{-6**}$
18:0	420.5	164.7	633.0	277.6	175.9	467.1	$4.6 \times 10^{-5**}$
18:1 n-9	1104	429.3	2192	674.5	481.9	1375.4	$1.3 \times 10^{-5**}$
18:1 n-7	80.4	37.7	177.5	48.9	31.3	117.0	$2.9 \times 10^{-7**}$
18:2 n-6 (LA)	1500	688.7	2327	1209.0	664.9	1777.3	$3.9 \times 10^{-4**}$
18:3 n-3 (ALA)	36.2	16.3	105.7	27.5	7.7	54.7	0.014*
20:3 n-6 (DGLA)	89.4	17.1	191.8	51.4	19.8	125.0	$2.2 \times 10^{-5**}$
20:4 n-6 (AA)	338.2	159.8	897.6	234.3	133.6	435.4	$1.2 \times 10^{-5**}$
22:0	50.5	19.0	88.7	34.3	20.1	65.0	$3.7 \times 10^{-5**}$
20:5 n-3 (EPA)	63.1	12.7	172.5	52.3	13.9	261.8	0.335
24:0	44.0	17.0	66.8	35.8	19.0	72.5	$3.3 \times 10^{-3**}$
22:5 n-6	6.0	3.0	15.7	3.9	0.0	18.3	$8.6 \times 10^{-4**}$
24:1 n-9	88.4	51.5	181.2	62.7	35.0	108.3	$2.0 \times 10^{-5**}$
22:5 n-3 (DPA)	38.2	18.5	63.7	32.0	21.6	56.4	0.064
22:6 n-3 (DHA)	130.4	81.1	277.6	111.1	58.9	319.1	0.035*
TFA	5531	2544.8	9261	3811.2	2403.7	6236.3	$9.5 \times 10^{-6**}$
EPA/AA	0.180	0.063	0.523	0.223	0.065	0.937	0.174

AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; BMI, body mass index; DGLA, dihomo- $\gamma$ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; TFA, total concentration of fatty acids; WMW, Wilcoxon-Mann-Whitney.

**Table 3** Medians, in units of  $\mu\text{g}$  per g sample, are provided for serum FAs from women with obesity ( $\text{BMI} \geq 35 \text{ kg m}^{-2}$ ) at baseline (visit 1) and after 3 weeks of intensive lifestyle intervention (visit 2). The  $P$ -values comparing the FA levels at visits 1 and 2 are calculated from the nonparametric WSR test (15). The nonparametric WMW rank sum test (15,16) is used to compare serum FA levels in the patients at visit 2 with levels in a cohort of healthy women ( $\text{BMI} \leq 30 \text{ kg m}^{-2}$ ) and levels 3 months after surgery in a cohort of patients that has experienced LSG (8). The omega-3 DPA and omega-6 22:5 n-6 were not quantifiable in the bariatric patients due to co-elution with major interferents. Asterisks (\* and \*\*) after a  $P$ -value imply significance at  $P = 0.05$  ( $p_{\text{WSR}} = 0.0375$ ,  $p_{\text{WMW}} = 0.0225$  and  $p_{\text{WMW}} = 0.011$  for comparison of lifestyle patients at visit 1 and 2 and lifestyle patients at visit 2 with healthy and LSG patients, respectively) and  $P = 0.01$  ( $p_{\text{WSR}} = 0.0065$ ,  $p_{\text{WMW}} = 0.004$  and  $p_{\text{WMW}} = 5.6 \times 10^{-4}$  for comparison with lifestyle patients at visit 1 and 2 and lifestyle patients at visit 2 with healthy and LSG patients, respectively), respectively, after correcting for multiple testing by means of false discovery rate (18)

FA	Lifestyle patients ( $N = 23$ )			Lifestyle patients at visit 2 ( $N = 25$ )			
	Medians			Healthy controls ( $N = 45$ )		LSG patients ( $N = 17$ )	
	Visit 1	Visit 2	$p_{\text{WSR}}$	Median	$p_{\text{WMW}}$	Median	$p_{\text{WMW}}$
14:0	43.6	30.9	$2.6 \times 10^{-4**}$	38.4	0.028	23.4	0.174
16:0	1226	989.6	$3.2 \times 10^{-3**}$	801.5	$1.7 \times 10^{-3**}$	890.1	0.249
16:1 n-9	21.6	17.6	$5.1 \times 10^{-3**}$	12.6	$3.5 \times 10^{-3**}$	13.1	0.061
16:1 n-7	137.9	122.1	$7.4 \times 10^{-3*}$	69.9	$4.7 \times 10^{-5**}$	85.5	0.271
18:0	418.9	288.4	$2.6 \times 10^{-4**}$	277.6	0.352	254.3	0.036
18:1 n-9	1084	869.0	$4.3 \times 10^{-3**}$	674.5	$7.2 \times 10^{-4**}$	838.4	0.608
18:1 n-7	79.3	81.2	0.201	48.9	$3.2 \times 10^{-7**}$	65.2	0.398
18:2 n-6 (LA)	1512	1195	$6.6 \times 10^{-4**}$	1209	0.902	955.2	$3.0 \times 10^{-3*}$
18:3 n-3 (ALA)	37.7	25.5	$2.4 \times 10^{-3**}$	27.5	0.835	13.2	$3.0 \times 10^{-4**}$
20:3 n-6 (DGLA)	89.1	54.6	$9.9 \times 10^{-5**}$	51.4	0.220	42.4	0.073
20:4 n-6 (AA)	343.7	320.6	0.068	234.3	$1.1 \times 10^{-4**}$	308.3	0.798
22:0	50.3	33.1	$4.6 \times 10^{-5**}$	34.3	0.096	32.0	0.838
20:5 n-3 (EPA)	62.3	51.5	0.033*	52.3	0.825	27.7	$1.6 \times 10^{-3*}$
24:0	43.1	29.3	$5.2 \times 10^{-5**}$	35.8	$2.5 \times 10^{-5**}$	31.4	0.573
22:5 n-6	6.0	4.7	$1.1 \times 10^{-3**}$	3.9	0.384	Not available	
24:1 n-9	90.0	81.5	0.738	62.7	$1.6 \times 10^{-7**}$	76.4	0.200
22:5 n-3 (DPA)	38.0	31.7	$3.5 \times 10^{-3**}$	32.0	0.286	Not available	
22:6 n-3 (DHA)	121.8	134.8	0.543	111.1	0.043	93.8	0.023
TFA	5514	4379	$1.6 \times 10^{-3**}$	3811	$8.4 \times 10^{-3*}$	3836	0.026
EPA/AA	0.179	0.172	0.068	0.223	0.062	0.084	$1.8 \times 10^{-3*}$

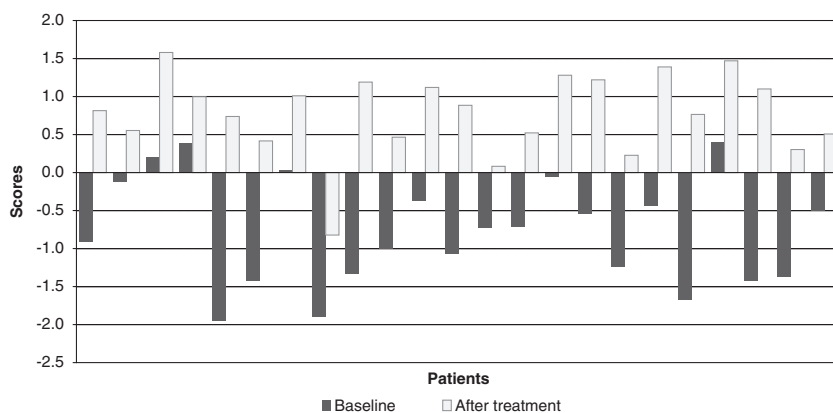
AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; BMI, body mass index; DGLA, dihomo- $\gamma$ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic acid; LSG, laparoscopic sleeve gastrectomy; TFA, total concentration of fatty acids; WMW, Wilcoxon-Mann-Whitney; WSR, Wilcoxon sign-rank.



**Figure 1** This figure shows the multivariate pattern of change caused by the first 3 weeks of lifestyle treatment. This selectivity ratio plot (21,22) quantifies impact of 3 weeks of lifestyle treatment on changes in absolute levels of serum fatty acids and body mass index. Confidence bounds shown on bars correspond to two standard deviations. Negative bar implies reduction, while positive bar implies increase of variable after treatment.

(DGLA). After these FAs, the FAs DPA, 22:5 n-6, 16:0 and TFA follow. Comparison of the SR values with the  $P$ -

values in Table 3 (visit 1 and visit 2) shows that the ranking of FAs according to discriminatory information from



**Figure 2** This figure shows the changes in fatty acid profiles on the multivariate pattern displayed in Fig. 1 as positions (scores) of lifestyle patients ( $N = 23$ ) at baseline (black bars) and after 3 weeks of lifestyle treatment (neighboring grey bars). The plot displays quantitatively the status for each subject at baseline (black bar), the change during 3 weeks of the treatment (difference between grey and black bar) and the status (grey) after 3 weeks.

these two measures, from multivariate and univariate methods, respectively, are in excellent agreement. However, some FAs are amplified in importance by the multivariate approach, e.g. 24:0, due to their correlation to the multivariate pattern of discrimination, while some are weakened, e.g. DGLA, compared to the ranking according to  $P$ -values from the univariate comparison of medians at baseline and after 3 weeks of lifestyle treatment. Note also that AA, which was on the borderline of being significant at  $P = 0.05$  in the univariate comparison, is still borderline but significant according to the multivariate analysis.

BMI was included in the multivariate analysis but shows no associations to the changes in FA patterns (Fig. 1). Thus, although the changes in BMI and FA pattern are both caused by the lifestyle intervention, they appear to be unrelated outcomes of the intervention (Fig. 1).

Black bars show the starting point (score) at baseline for patients on the multivariate scale described by the change in FA pattern from visit 1 to 2 (Fig. 2), while the neighbouring grey bar at the right shows the positions (score) for the same patients after 3 weeks of lifestyle change. The difference between grey and black bars for a patient quantifies the improvement on the multivariate FA profile (Fig. 1). We observe that all patients show improvements in their FA profiles during the first 3 weeks of intensive lifestyle intervention, implying a reduction in serum levels of most FAs, as displayed in Fig. 1. As expected, both the starting point and the response to the lifestyle intervention vary substantially from patient to patient.

#### Comparison of lifestyle patients with healthy controls after 3 weeks of treatment

Use of the WSR test revealed no statistically significant changes in FA levels from visit 2 (3 weeks into intervention) to visit 3 (6 weeks into intervention) for the lifestyle patients (not shown). We then compared levels in lifestyle patients at visit 2 with the healthy controls. Table 3 shows

the results of the WMW test. After correcting for multiple testing, the saturated FA 16:0 and the monounsaturated omega-7 and omega-9 FAs with 16 and 18 carbons in the chain are higher in the patients than the healthy subjects at  $P = 0.01$ . However, both the essential FAs, LA and ALA, possess the same serum levels in lifestyle patients as in healthy subjects, as do the omega-3 EPA, DPA and DHA and the EPA/AA ratio. Even if the TFA serum levels have decreased by approximately 20% on average from baseline to visit 2 for the patients, the levels are still higher than in healthy subjects at  $P = 0.05$ . Nervonic acid and AA are both higher in patients at  $P = 0.01$ . After 3 weeks of lifestyle treatment, the only FA that is significantly lower in concentration in patients compared to healthy controls is the saturated long-chain FA 24:0 ( $P < 0.01$ ).

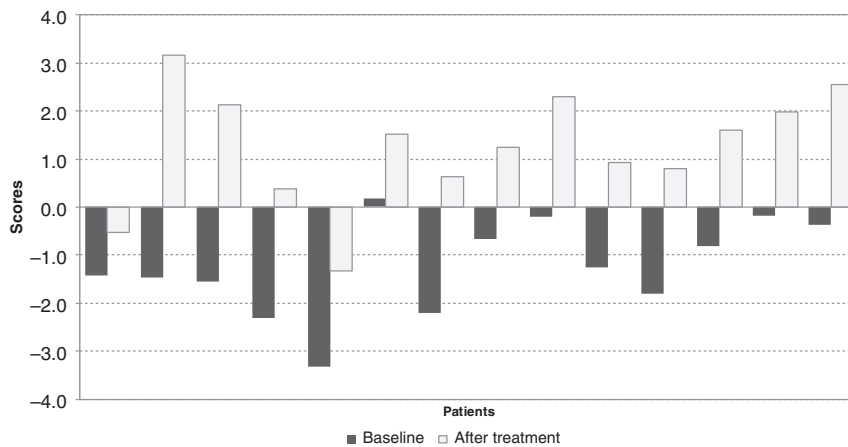
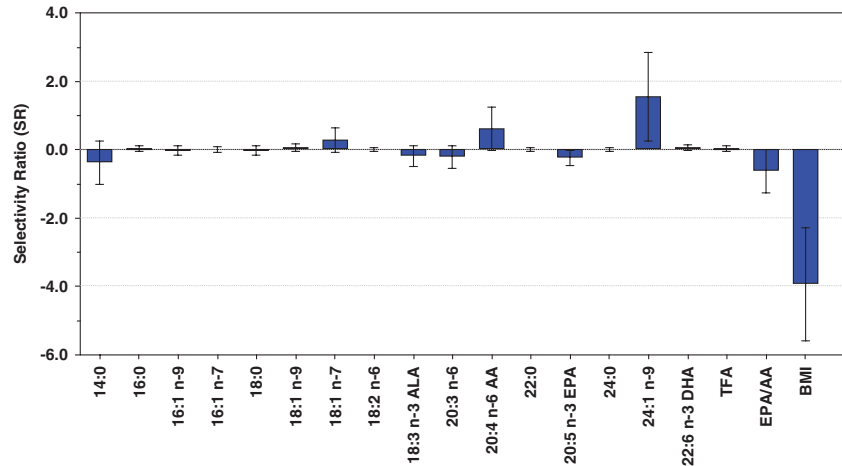
#### Comparison of lifestyle patients with bariatric patients

The lifestyle patients at visit 2 are also compared with a group of bariatric patients subjected to LSG surgery, which creates gastric restriction by reducing the gastric volume. Comparison of FA serum levels in lifestyle patients after 3 weeks of intensive lifestyle intervention with levels in a matching group of bariatric patients 3 months after surgery (Table 3, last column) reveals that only LA and the omega-3 FAs, ALA, EPA and EPA/AA are significantly different at  $P = 0.05$ , taking into account the effect of multiple testing on probability levels. These FAs are all lower in the bariatric patients.

To investigate a possible association between change in FA pattern and weight loss as measured by BMI, multivariate discriminant analysis was performed for bariatric patients at, before and 3 months after surgery (Fig. 3).

The difference from Fig. 1 is striking. After LSG surgery, reduction in BMI is the dominating discriminating feature, but this reduction is associated with a reduction of EPA and increase of AA, resulting in the concomitant decrease

**Figure 3** This figure shows the changes in fatty acid (FA) pattern and body mass index (BMI) after surgery. This selectivity ratio plot (21,22) quantifies impact of bariatric surgery on changes in absolute levels of serum FAs and BMI 3 months after surgery. Confidence bounds shown on bars correspond to two standard deviations. Negative bar implies reduction, while positive bar implies increase of variable after treatment.



**Figure 4** This figure shows the changes for the patients in the discriminating pattern displayed in Fig. 3, as positions (scores) of bariatric patients ( $N = 14$ ) just before (black bars) and 3 months after surgery (neighboring grey bars). The plot displays quantitatively the status for each subject at baseline (black bar), the change 3 months after surgery (difference between grey and black bar) and the status (grey) 3 months after surgery.

in BMI and EPA/AA. Thus, contrary to the lifestyle intervention, weight loss and changes in serum FAs after surgery appear connected (Fig. 3).

Although to different degrees, all the patients show the same pattern of reduced weight and EPA/AA after surgery.

### Discussion

At baseline, the lifestyle patients had significantly higher serum levels for all FAs compared to the matching group of healthy subjects, except for EPA, DPA and 14:0 (Table 2). This finding seems to contradict Ref. (7), where levels for omega-3 FAs were found to be lower in subjects with obesity than in healthy controls. However, that study compared relative levels, not absolute levels as we do here. Relative levels at baseline are lower in lifestyle patients for both EPA and DHA also in our study, 1.1% vs. 1.4% for EPA and 2.5% vs. 2.9% for DHA.

A large reduction in absolute serum levels of FAs is observed in patients after 3 weeks of lifestyle treatment at RCHRC (Table 3). Serum TFA was reduced from a median level at baseline, which was 45% higher to only 11–12% above the levels in healthy subjects after 3 weeks of treatment. A reduction is also observed in levels of EPA after 3 weeks but from a higher level in lifestyle patients than in healthy controls, and the relative level is increased to 1.3%, similar to the relative level in healthy subjects. The relative level of serum DHA increases to 3.1%, which is also similar to the level in healthy subjects. The main components that might impact FA levels in our study were change in diet and eating pattern and sessions of physical activity of a different kind and duration. Perez-Cornago *et al.* (24) carried out a weight loss intervention in a cohort of older adults ( $60 \pm 5$  years) with overweight and obesity ( $27 \leq \text{BMI} \leq 35$ ) by means of caloric restriction. The participants were provided a diet with 15% daily energy shortage over 8 weeks. The

participants experienced 0.9%TWL (percent total weight loss) per week, which is comparable to the lifestyle patients at RCHRC (see below). Similar to our study, they observed significant reduction in serum levels for the saturated FAs 14:0, 16:0, 18:0 and 24:0, the very abundant FAs 18:1 n-9 and LA and DGLA and no reduction in EPA. Contrary to us, they observed a significant reduction in AA and DHA. For AA, our analysis showed that changes in AA were on the borderline of being significant, but another reason for differences is that Perez-Cornago *et al.* did not correct their 31 univariate tests for multiple testing, providing very optimistic significance levels.

Table 3 further reveals that after 3 weeks of intensive lifestyle intervention, the serum levels of all the omega-3 FAs in patients are statistically equal to healthy controls at  $P = 0.05$ . This is contrary to the observations after bariatric surgery where serum ALA and EPA showed large reductions from preoperative levels during the weight loss process after surgery (8).

Comparison of FA levels in patients 3 weeks after lifestyle intervention with levels measured in bariatric patients 3 months after surgery using the WMW test are provided in Table 3. After correcting for multiple testing, significant differences ( $P < 0.05$ ) are observed for LA, ALA, EPA and the EPA/AA ratio. They are all lower in bariatric patients 3 months after surgery than in lifestyle patients after 3 weeks of intervention. The impact of different rates in the weight loss process may, at least partly, explain the lower levels of omega-3 FAs in bariatric patients. Thus, after LSG surgery, the average %TWL is 1.3% weekly during the first 3 months (8). The lifestyle patients experience 0.8%TWL per week during the first 3 weeks. The weight loss continues at almost the same rate during the following 3 weeks at RCHRC, i.e. 0.6–0.7%TWL per week. Thus, the lifestyle patients lose weight at approximately half the rate of the bariatric patients, although they are probably much more physically active with at least three training sessions 5 d a week (14). The faster weight reduction in the bariatric patients implies that they acquire a considerably larger input from their own fat deposits than do the lifestyle patients in order to compensate for larger energy shortage. The FA profile available from these deposits are poor in omega-3 FAs (12,13), which may lead to lower levels of omega-3 in bariatric patients compared to lifestyle patients. This can, however, be compensated by supplements that increase serum omega-3 levels without influencing the weight loss (25). Our analysis shows that supplements are not necessary for lifestyle patients as long as they follow the Nordic Nutrition Recommendations (26) or similar dietary advice. For diets low in marine omega-3, however, supplements may be necessary.

Thus, the differences in serum FA levels might be a reflection of how fast and by which means weight

reduction is obtained in the two groups. The multivariate analysis showed that the change in FA pattern and weight loss were unrelated in the lifestyle patients (Fig. 1), but highly associated in the bariatric patients (Fig. 3). Multivariate analysis of the changes in FA scores from baseline and 3 weeks into the lifestyle intervention showed a non-significant ( $P > 0.05$ ) correlation coefficient of 0.35 between change in scores and %TWL. Thus, change in diet and physical activity, and not weight loss, appears to be the factors influencing the FA profiles in lifestyle patients, while lowering of EPA/AA in bariatric patients may be caused by reduced dietary input of omega-3 FAs.

### Strengths and weaknesses

Comparison of lifestyle patients with both bariatric patients and healthy controls from the same region at different intervention points provides a possibility for a broad investigation. The determination of quantitative FA levels is important as the omega-3 FAs are present in small amounts, and relative levels may therefore be strongly influenced by variation in a few abundant FAs. Use of multivariate analysis provides patterns that can easily be visualized and assists the interpretation of intervention effects, both of variables and subjects, and offers a complementary picture to the univariate tests.

There are two weaknesses in our analysis. One is that the lifestyle and bariatric patients included a few smokers, while smokers were excluded in the healthy control. Recent work (27) showed a reduction of EPA and DHA in smokers due to less dietary input of fish. However, similar to our previous work about the effect of serum lipoproteins on the lifestyle patients (14), smoking showed no impact on omega-3 levels maybe because of the low number of smokers in our cohort. The lack of information on use of omega-3 supplements at baseline and during intervention for the patients may present a more serious weakness. For the bariatric patients, this information was available, and three patients were using omega-3 supplements. They all had levels of EPA and DHA similar to the other bariatric patients, probably because of small dosages of products bought in grocery stores. Our assumption is that this result is representative of the lifestyle patients and the healthy controls too.

### Conclusion

Intensive lifestyle intervention with a change in diet and eating pattern, and increased physical activity, provides slower rate of weight loss than does reduction of the gastric volume by bariatric surgery. Reduction of FA serum levels and weight loss are two almost uncorrelated effects of lifestyle intervention. This is in contrast to gastric reduction surgery, where changes in FA pattern and weight loss are



associated and imply a shortage of omega-3 FAs and a need for supplementation. Lifestyle patients can manage without supplementation as long as they follow the Nordic Nutrition Recommendations (26) or similar dietary advice. For diets low in marine omega-3, however, supplements may be needed.

### Conflict of Interest Statement

No conflict of interest was declared.

### Author contributions

The study was conceived by OMK and JRA; VV and OMK designed the study. CL and SAM did the FA analyses and CL, TR and OMK the data analyses. OMK drafted the manuscript. All authors read and approved the final manuscript.

### Acknowledgements

CL thanks Førde Hospital Trust for financial support to cover analysis costs. Anne Nilssen Magerøy and her team at Førde Central Hospital are thanked for collecting the blood samples of the healthy controls and the bariatric patients. Staff members at RCHRC are thanked for collecting the serum samples for the lifestyle patients.

### References

- Dyerberg J, Bang HO, Stoffersen E, Moncad S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 1978; 2: 117–119.
- Kagawa Y, Nishizawa M, Suzuki M *et al.* Eicosapolyenoic acids of serum lipids of Japanese islanders with low incidence of cardiovascular diseases. *J Nutr Sci Vitaminol (Tokyo)* 1982; 28: 441–453.
- Torres IC, Mira L, Ornelas CP, Melim A. Study of the effects of dietary fish intake on serum lipids and lipoproteins in two populations with different dietary habits. *Br J Nutr* 2000; 83: 371–379.
- Chowdhury R, Warnakula S, Kunutsor S *et al.* Association of dietary, circulating, and supplement fatty acids with coronary risk - a systematic review and meta-analysis. *Ann Intern Med* 2014; 160: 398–406.
- Kris-Etherton PM, Taylor DS, Yu-Poth S *et al.* Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr* 2000; 71(Suppl.): 179S–188S.
- Furukawa S, Fujita T, Shimabukuro M *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; 114: 1752–1761.
- Micallef M, Munro I, Phang M, Garg M. Plasma n-3 polyunsaturated fatty acids are negatively associated with obesity. *Br J Nutr* 2009; 102: 1370–1374.
- Lin C, Våge V, Mjøs SA, Kvalheim OM. Changes in serum fatty acid levels during the first year after bariatric surgery. *Obes Surg* 2016; 26. doi: 10.1007/s11695-015-1980-4.
- Cottam D, Qureshi FG, Mattar SG *et al.* Laparoscopic sleeve gastrectomy as an initial weight-loss procedure for high-risk patients with morbid obesity. *Surg Endosc* 2006; 20: 859–863.
- Våge V, Sande VA, Mellgren G *et al.* Changes in obesity-related diseases and biochemical variables after laparoscopic sleeve gastrectomy: a two-year follow-up study. *BMC Surg* 2014; 14: 8.
- Våge V, Gåsdal R, Laukeland C *et al.* The biliopancreatic diversion with a duodenal switch (BPDDS): how is it optimally performed? *Obes Surg* 2011; 21: 1864–1869.
- Geerling BJ, van Houwelingen AC, Badart-Smook A, Stockbrügger RW., Brummer R-JM. Fat intake and fatty acid profile in plasma phospholipids and adipose tissue in patients with Crohn's disease, compared with controls. *Am J Gastroenterol* 1999; 94: 410–417.
- Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr* 2006; 83(Suppl.): 1467S–1476S.
- Aadland E, Andersen JR, Anderssen SA, Kvalheim OM. Impact of physical activity and diet on lipoprotein particle concentrations in severely obese women participating in a 1-year lifestyle intervention. *Clin Obes* 2013; 3: 202–213.
- Wilcoxon F. Individual comparisons by ranking methods. *Biometrics Bull* 1945; 1: 80–83.
- Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Stat* 1947; 18: 50–60.
- Lin C, Rajalahti T, Mjøs SA, Kvalheim OM. Predictive associations between serum fatty acids and lipoprotein in healthy non-obese Norwegians: implications for cardiovascular health. *Metabolomics* 2016; 12: 6.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 1995; 57: 289–300.
- Sjöström M, Wold S, Söderström B. PLS discriminant plots. In: Gelsema ES, Kanal LN (eds). *Pattern Recognition in Practice II*. Amsterdam: Elsevier, 1986, pp. 461–470.
- Kvalheim OM, Karstang TV. Interpretation of latent-variable regression models. *Chemometr Intell Lab* 1989; 7: 39–51.
- Rajalahti T, Arneberg R, Berven FS *et al.* Biomarker discovery in mass spectral profiles by means of selectivity ratio plot. *Chemometr Intell Lab* 2009; 95: 35–48.
- Rajalahti T, Arneberg R, Kroksveen AC *et al.* Discriminating variables test and selectivity ratio plot – quantitative tools for interpretation and variable (biomarker) selection in complex spectral or chromatographic profiles. *Anal Chem* 2009; 81: 2581–2590.
- Westerhuis JA, Hoefsloot HCJ, Smit S *et al.* Assessment of PLS-DA cross validation. *Metabolomics* 2008; 4: 81–89.
- Perez-Cornago A, Brennan L, Ibero-Baraibar I *et al.* Metabolomics identifies changes in fatty acid and amino acid profiles in serum of overweight older adults following a weight loss intervention. *J Physiol Biochem* 2014; 70: 593–602.
- Munro IA, Garg ML. Dietary supplementation with long chain omega-3 polyunsaturated fatty acids and weight loss in obese adults. *Obes Res Clin Pract* 2013; 7: e173–e181.
- Becker W, Lyhne N, Pedersen AN *et al.* Nordic nutrition recommendations 2004 - integrating nutrition and physical activity. *Scand J Nutr* 2004; 48: 178–187.
- Scaglia N, Chatkin J, Chapman KR *et al.* The relationship between omega-3 and smoking habit: a cross-sectional study. *Lipids Health Dis* 2016; 15: 61.