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ORIGINAL CONTRIBUTION

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Effects of seafood consumption and weight loss on fasting leptin and ghrelin concentrations in overweight and obese European young adults

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Abstract *Background* Energy restriction affects circulating leptin and ghrelin concentrations. *The aim of this study* To investigate whether seafood consumption affects fasting leptin and ghrelin concentrations in addition to weight loss. *Methods* In this 8-week dietary intervention, subjects (324 Icelandic, Spanish and Irish subjects, 20–40 years, BMI 27.5–32.5 kg/m^2) were randomized to energy-restricted diets (−30%) of identical macronutrient composition but different amount of seafood: control (no seafood); lean fish (150 g cod, three times per week); fatty fish (150 g salmon, three times per week); EPA&DHA [daily docosahexaenoic (DHA)/eicosapentaenoic acid (EPA) capsules]. Anthropometric data, ghrelin, leptin, and insulin were measured at baseline and endpoint. Linear models investigated the effects of seafood on fasting leptin, ghrelin and insulin. *Results* Body weight (−5.2 ± 3.0 kg), leptin (−34.8%) and insulin (−13.5%) decreased, while ghrelin increased (5.6%) (all $P < 0.001$). According to linear models endpoint insulin was significantly lower in the EPA&DHA group (−16.4%, $P = 0.025$) compared to control, endpoint leptin in men was lower in the salmon group (−22.9%, $P = 0.026$), and the EPA&DHA group tended to have higher endpoint ghrelin (5.6%, $P = 0.060$), an effect seen only in women indicated by a significant gender × EPA&DHA interaction. Weight loss explained the effects of fatty seafood on leptin and ghrelin, but not insulin. *Conclusions* Consumption of fatty seafood can modulate fasting insulin, ghrelin and leptin during an 8-week intervention. Effects are partly gender specific and are partly explained by weight loss. Consumption of lean fish does not affect circulating hormones in comparison to control. The most consistent effect on circulating hormones is mediated by weight loss.

Key words leptin – ghrelin – insulin – weight loss – LC n-3 fatty acids

Introduction

Leptin and ghrelin are two hormones that have been recognized to affect energy balance. Leptin is a mediator of long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss [13]. Ghrelin functions as an appetite-stimulatory signal [13]. It has been shown that leptin levels are higher in subjects with a higher BMI and a higher

percent total body fat [29]. Ghrelin levels are lower in obese than in lean individuals [26, 34]. However, because there are large variations in leptin and ghrelin concentrations among individuals with similar body composition, it is likely that factors other than adipose mass influence plasma concentrations [10, 36].

Excess energy stored in adipose tissue has significant effects on circulating leptin and ghrelin levels [2, 7, 14, 17, 37]. Overfeeding results in an increase in leptin expression and circulating leptin in healthy human subjects [17]. Fasting has been shown to result in a decrease in plasma leptin concentration. This decline in plasma leptin is much greater than the change in adipose mass, indicating that the change in adipose mass is not solely responsible for the decrease in circulating leptin concentration [14, 37]. Short term fasting results in an increase in plasma ghrelin levels, with a nearly twofold increase [2, 7]. The composition of a meal is also a determinant of leptin and ghrelin levels [9, 12]. Dietary fatty acids may influence the expression of hormones like leptin or ghrelin directly by interaction with transcription factors, or indirectly possibly linked to fatty acid oxidation, synthesis, or storage [8, 27], although not all studies show an effect [18]. In vitro experiments show inconsistent results whether long chain n-3 polyunsaturated fatty acid (LC n-3 PUFA) reduce or increase the expression of leptin [21, 35]. A feeding study with rats showed that LC n-3 PUFA supplementation lowers plasma leptin concentration (−38%) and leptin mRNA expression (−66%) [35]. The long-term effects of dietary fatty acids on circulating fasting leptin and ghrelin in humans are not clearly identified. A cross sectional study showed that in men with diabetes, the serum leptin concentration correlates significantly with the intake of saturated fat, and negatively with the EPA and with the sum of the n-3 fatty acids from the plasma phospholipids [24]. However, two intervention studies did not find a significant effect of LC n-3 PUFA supplementation of leptin concentration [10, 23]. One study has shown that fat restriction avoids the increase in ghrelin levels caused by dietary energy restriction [38], but information on specific effects of LC n-3 PUFA on fasting ghrelin concentrations are not available.

It has been suggested that leptin and ghrelin have additional functions in humans, which go beyond the regulation of hunger, energy intake and body weight. Low plasma ghrelin and high leptin concentrations found in overweight and obese subjects may have a detrimental effect on vasculature and promote the development of cardiovascular disease [4, 11, 19, 30, 41]. Although evidence for these two hormones being of importance for cardiovascular problems is still very limited, affecting circulating ghrelin and leptin concentrations in obese or overweight individuals by diet composition or single nutrients in addition to weight loss might be beneficial. Recently we showed that including seafood in an energy restricted diet increases weight loss [33]. Even though the effects found were not solely attributable to LC n-3 PUFA, but possibly also, e.g., to fish protein [1] or taurine [44], it indicates that food of seafood origin is a feasible choice for overweight people.

In order to elucidate the effects of regular seafood consumption (lean fish, fatty fish or fish oil) on circulating fasting leptin and ghrelin levels, we analysed data from the of SEAFOOplus YOUNG study, a randomized controlled dietary intervention trial in overweight and obese young adults from three European countries. The primary goal of SEAFOOplus YOUNG study was to investigate the effects of seafood on weight loss during energy restriction. These results were published previously [33].

The main goal of the present analysis was to investigate whether seafood consumption improves circulating fasting leptin and ghrelin levels in addition to weight loss. In this analysis, we also paid attention to insulin, which has been shown to be a powerful regulator of both leptin and ghrelin [5, 18, 28] and to be affected by LC n-3 PUFA consumption [22].

Methods

Subjects

A total of 324 overweight individuals (138 men and 186 women) were recruited to our study SEAFOODplus YOUNG (http://www.seafoodplus.org) through advertisements, 140 from Iceland, 120 from Spain and 64 from Ireland. All subjects were screened for inclusion and exclusion criteria. The inclusion criteria were body mass index (BMI) 27.5–32.5 kg/m^2, age 20-40 years, and a waist circumference of ≥94 and ≥80 cm for men and women, respectively. Exclusion criteria were weight change (±3 kg) due to a weight loss diet within three months before the start of the study, use of supplements containing n-3 fatty acids, calcium or vitamin D during the last three months, allergy for fish, drug treatment of diabetes mellitus, hypertension or hyperlipidemia and pregnancy or lactation. About 86% (n = 278) of the subjects completed the intervention. The study was approved by the National Bioethical Committee in Iceland (04-031), the Ethical Committee of the University of Navarra in Spain (24/2004) and the Clinical Research Ethics Committee of the Cork University Hospital in Ireland. The study followed the Helsinki guidelines and all subjects participating gave their written consent.

Study design

This study was a randomized controlled dietary intervention trial and took place in the period of April 2004 to November 2005. It was conducted at the Landspitali-University Hospital in Reykjavik, Iceland, the University College of Cork, Ireland, and the University of Navarra in Pamplona, Spain. The intervention lasted for eight consecutive weeks during which the subjects were instructed to follow a energy-restricted diet (1,461 ± 185 kcal/day), 30% (range 502–838 kcal) from estimated energy expenditure by Harris–Benedict equations and physical activity level. All participants were randomly assigned to one of four diets which varied in respect to the amount of LC n-3 PUFA:

Diet 1: no seafood (control, 6 placebo capsules/day, protein source was lean meat),
Diet 2: lean fish (150 g cod 3 times/week),
Diet 3: fatty fish (150 g salmon 3 times /week), or
Diet 4: fish oil capsules (6 capsules/day, protein source was lean meat).

The diets provided different amounts of EPA and DHA: The placebo capsules in diet group 1 provided 0 g EPA and DHA/day, cod in diet 2 gave 0.26 g/day, salmon in diet group 3 provided 2.1 g EPA and DHA/day, and fish oil capsules gave 1.3 g/day. Originally it was planed to provide the same amount of n-3 fatty acids with salmon and capsules. According to available nutrition databases 450 g farmed salmon per week (3 × 150 g) was estimated to equal about 1.3 g EPA and DHA per day, which is the amount the capsules provide. However, according to chemical analysis (fatty acid content) of the salmon used in the present study it turned out to provide considerably more EPA and DHA than estimated by the nutrition databases.

In order to minimize unwanted differences between diets, which could affect outcomes [16], the diets prescribed identical macronutrient composition: total fat (~30% of total energy), carbohydrate (~50% of total energy), protein (~20% of total energy) and dietary fiber (~20–25 g). Each subject got a detailed meal plan to follow, as well as recipe booklets and instructions in order to synchronize sources of fat (other than LC n-3 PUFA), fruit and vegetable consumption and meal frequency. The dietary interventions, i.e., salmon fillet, cod fillet, fish oil capsule and placebo capsule differed in energy, fat content, protein content and volume. However, the menu plans for each individual were very specific and aimed to even out these differences. Intended dietary differences between groups during the intervention were in n-3 fatty acids consumption (but not overall amount of fat) and protein quality (fish protein vs. meat protein).

During the intervention the absolute and relative intakes of fat, saturated fatty acids, monounsaturated fatty acids and absolute polyunsaturated fatty acid intake were not significantly different between groups (corrected for gender and country), however, relative intake of polyunsaturated fatty acids was significantly higher in the EPA&DHA group than in the control (1.0% difference, $P = 0.001$), cod group (1.5% difference, $P < 0.001$) or salmon group (0.9% difference, $P = 0.0031$).

Physical activity level of the participants was unchanged during the intervention. Dietary intake before baseline (habitual diet) and compliance with the intervention diets was assessed by 2-day weighed food records (baseline and end of intervention), and by a validated FFQ for seafood (baseline, middle, and end of intervention) [42]. The compliance was also assessed by analyzing fatty acids in erythrocyte phospholipids in fasting blood samples, as earlier reported [33]. Results showed good compliance with the intervention diets [43].

Anthropometric measurements

All measurements were done using standard measurement procedures as outlined in a research protocol approved and used by all countries participating in the study. Anthropometrical measurements were performed at baseline and endpoint of the study. Body weight was measured in light underwear on a calibrated scale (SECA 708, Hamburg, Germany). Subject's height was measured with a calibrated stadiometer, and waist circumference was measured using a tape measure following accepted procedures. Fat mass and fat-free mass were assessed by bioelectrical impedance analysis (BIA) (Bodystat 1500, Bodystat Ltd, Douglas, Isle of Man, UK).

Biochemical measurements

Subjects were told to avoid strenuous exercise and alcohol consumption the day before the blood samples were drawn at baseline and endpoint, which were then analyzed for fasting concentrations of plasma leptin, ghrelin and insulin. The analyses were performed centrally. Ghrelin was measured with a radioimmunoassay kit developed by Linco Research (St Charles, MO, USA). The analysis is linear over the range of 93–6,000 pg/ml, intra-assay precision is between 4.4 and 10.0%. Leptin was measured by a radioimmunoassay kit from DPC Co. (Los Angeles,

CA, USA). The analysis is linear over the range of 0.10–120 ng/ml, intra-assay precision is between 2.6 and 4.9%. Insulin was measured with a electrochemiluminescence immunoassay (ECLIA) on a Modular Analytics E170 system from Roche Diagnostics (Manheim, Germany). The analysis is linear over the range of 0.2–1,000 mU/l, intra-assay precision is between 1.5%.

Statistical analysis

The data were entered into the SPSS statistical package 11.0. Wilcoxon test was used to calculate whether there were significant changes in the variables between baseline and endpoint. Distributions of the investigated variables were estimated using the Kolmogorov–Smirnov test. Insulin, leptin, and ghrelin values were log transformed for the analyses. Baseline characteristics of the groups were compared using linear models with fixed effects (country, gender, and diet group) and covariate (age). In order to find out whether diet groups predict endpoint fasting insulin, ghrelin, and leptin after 8 weeks, linear models with fixed effects (country, gender, and diet group) and covariates (age, baseline value of the relevant outcome variable) were constructed. In order to find out whether changes in body weight can explain possible effects of diet groups on outcomes, this anthropometrical variable was entered as additional covariate in separate linear models. Results from the linear models in Table 3 are shown as parameter estimates where the cod-, salmon- and fish oil group were each compared to the control group. Variances were checked using Levene's test of homogeneity and residuals of the linear model were checked for normality using Kolmogorov–Smirnov test. The numbers in the parameter estimates (B, lower confidence limit, higher confidence limit) were backtransformed and are shown as $1 - B$, $1 -$ lower confidence limit, $1 -$ higher confidence limit, respectively, thus giving percentual differences between groups in endpoint variables. Parameter estimates given in the Results describe percentual changes of the outcome variables due to changes of the dependent variables by one unit, e.g., 1 kg weight loss reduces endpoint fasting insulin by 3.6%. $P < 0.05$ was regarded as statistically significant.

Results

Baseline anthropometric variables and hormone concentrations can be seen in Table 1. There were no significant difference between the groups (corrected for age, gender, country, and age) with the exception

Table 1 Baseline data of the participants

	Control	Cod	Salmon	Fish oil
Body weight (kg)				
Mean	87.7	89.5	90.4	85.0
SD	10.1	9.4	11.4	9.4
Minimum	70.1	70.5	67.3	66.7
25th percentile	79.9	83.5	82.7	79.2
Median	86.7	88.6	88.3	84.8
75th percentile	95.4	95.9	98.0	91.6
Maximum	120.2	117.8	122.3	109.7
BMI (kg/m^2)				
Mean	30.0	30.2	30.4	29.9
SD	1.5	1.4	1.4	1.5
Minimum	27.3	27.5	27.7	27.2
25th percentile	28.6	29.1	29.4	28.5
Median	30.0	30.2	30.6	29.9
75th percentile	31.1	31.4	31.6	31.4
Maximum	33.0	33.0	32.7	32.7
Waist circumference (cm)				
Mean	95.2	96.8	96.9	94.0
SD	7.4	6.7	7.9	6.7
Minimum	78.0	83.0	76.0	80.0
25th percentile	90.0	92.0	91.0	89.0
Median	95.0	97.0	98.0	94.0
75th percentile	100.8	102.0	102.8	98.0
Maximum	116.0	116.0	116.0	114.0
Leptin (ng/ml)				
Mean	29.7	26.4	25.6	28.6
SD	17.4	18.7	19.5	19.3
Minimum	5.9	4.6	5.1	3.5
25th percentile	16.9	12.5	12.4	14.6
Median	25.3	18.7	19.0	21.7
75th percentile	42.6	37.6	36.3	36.6
Maximum	70.3	79.1	98.7	82.5
Insulin (mU/l)				
Mean	10.5	10.1	10.8	10.1
SD	5.4	4.1	5.2	4.6
Minimum	3.9	3.0	3.8	2.6
25th percentile	6.9	6.8	7.3	7.0
Median	9.5	8.8	9.9	9.0
75th percentile	12.5	12.6	13.5	12.1
Maximum	40.2	22.2	36.6	26.6
Ghrelin (pg/ml)				
Mean	1,242	1,073	1,094	1,137
SD	376	297	263	321
Minimum	700	419	540	607
25th percentile	931	876	883	918
Median	1,231	1,005	1,077	1,110
75th percentile	1,475	1,221	1,305	1,294
Maximum	2,786	1,907	1,510	2,300

og ghrelin, which was significantly lower in the cod group (−133 ± 47 pg/ml, $P = 0.006$) and in the salmon group (−107 ± 47 pg/ml, $P = 0.024$) compared to the control group. Mean energy intake at baseline was 2,322 ± 760 kcal/day without significant differences between groups. During the intervention energy intake decreased to 1,448 ± 356 kcal/day, which was in good agreement with the prescribed energy intake of 1,463 ± 188 kcal/day, also without significant differences between groups. Body weight (−5.2 ± 3.2 kg), waist circumference (−4.9 ± 3.0 cm), leptin (−9.6 ± 10.7 ng/ml) and insulin concentrations

(−2.0 ± 4.1 mU/l) of the subjects decreased significantly (all $P < 0.001$), plasma ghrelin concentration increased significantly (53 ± 168 pg/ml, $P < 0.001$) (Table 2). LC n-3 PUFA in erythrocyte phospholipids changed in good agreement with the amount of LC n-3 PUFA in the different diets (Table 2).

In the linear model (Table 3) endpoint insulin was significantly lower in the EPA&DHA group (−16.4%, $P = 0.025$) compared to control. A smaller, but not significant effect was seen in the salmon group (−7.7%, $P = 0.327$). After inclusion of weight loss (−3.6%, $P < 0.001$) as additional covariate in the model, EPA&DHA group remained significant (−14.4%, $P = 0.045$).

Due to unequal variances of leptin between sexes (Levene's test of homogeneity > 0.05), separate models for men and women were constructed to predict endpoint leptin. Endpoint leptin in men was significantly lower in the salmon group (−22.9%, $P = 0.026$) compared to control. A smaller, borderline significant effect was seen in the EPA&DHA group (−19.5%, $P = 0.084$). After inclusion of weight loss (−8.3%, $P < 0.001$), salmon group remained no longer significant. No significant effect of diet groups was observed in women, but each kg weight loss reduced leptin by 9.9% ($P < 0.001$). Reduction of fasting insulin during the 8 weeks was associated with reduced endpoint leptin in male (−2.7%, $P = 0.001$) and female participants (−2.5%, $P < 0.001$) .

The EPA&DHA group tended to have higher endpoint ghrelin levels (5.6%, $P = 0.060$) compared to control, however a significant gender × EPA&DHA group interaction was observed, indicating that this increase is only in women but not in men. After inclusion of weight loss (+1.2%, $P < 0.001$) as additional covariate the effect of EPA&DHA group disappeared but the gender × EPA&DHA group interaction remained significant. Reduction of fasting insulin during the 8 weeks was associated with increased endpoint ghrelin concentrations (+0.8%, $P < 0.001$).

Table 2 Anthropometrical, hormonal, and erythrocyte membrane changes (unadjusted) during the 8 weeks intervention and hormonal endpoint values

	Control	Cod	Salmon	Fish oil
Weight loss (kg)				
Mean	4.4	5.4	5.5	5.4
SD	2.8	2.7	3.3	3.2
Minimum	−2.2	−1.4	−0.7	−0.7
25th percentile	2.6	3.5	3.1	2.9
Median	4.5	5.4	5.6	5.2
75th percentile	5.9	7.2	7.4	7.8
Maximum	11.6	12.0	15.3	12.7
Waist circumference reduction (cm)				
Mean	4.0	5.0	5.4	5.1
SD	2.4	2.9	3.3	3.1
Minimum	−1.5	−0.5	−1.7	−2.0
25th percentile	2.4	2.9	3.1	2.6
Median	3.9	5.2	5.5	5.2
75th percentile	5.8	6.9	7.9	7.0
Maximum	9.5	12.4	15.5	11.0
Leptin at endpoint (ng/ml)				
Mean	20.3	17.4	16.6	17.9
SD	14.6	12.8	13.9	14.7
Minimum	1.2	2.3	1.4	1.8
25th percentile	8.8	6.8	5.7	7.3
Median	17.8	14.7	11.9	14.2
75th percentile	28.7	25.2	23.7	25.0
Maximum	75.1	49.9	67.8	66.1
Leptin reduction (ng/ml)				
Mean	9.4	9.4	9.0	10.7
SD	10.7	11.3	8.8	12.1
Minimum	−15.3	−8.4	−3.6	−8.2
25th percentile	3.5	2.6	3.6	2.4
Median	7.4	6.8	6.9	8.1
75th percentile	16.1	12.4	11.7	15.2
Maximum	46.3	53.0	51.0	54.9
Insulin at endpoint (mU/l)				
Mean	8.60	8.88	8.41	7.71
SD	4.34	4.06	3.98	3.78
Minimum	3.00	3.60	2.40	2.40
25th percentile	5.30	5.85	5.50	5.13
Median	8.05	7.50	7.55	6.85
75th percentile	10.18	11.35	10.48	9.43
Maximum	24.70	24.40	22.40	19.60
Insulin reduction (mU/l)				
Mean	1.79	1.10	2.73	2.38
SD	4.65	4.10	3.78	3.92
Minimum	−7.40	−14.30	−7.40	−9.10
25th percentile	−1.35	−0.50	0.58	0.80
Median	1.35	1.40	2.35	2.30
75th percentile	4.53	3.30	4.70	4.00
Maximum	16.20	10.10	17.80	13.60
Ghrelin at endpoint (pg/ml)				
Mean	1,276	1,123	1,156	1,207
SD	337	266	293	426
Minimum	700	601	516	640
25th percentile	1,044	934	909	930
Median	1,313	1,073	1,161	1,111
75th percentile	1497	1313	1440	1388
Maximum	2451	1815	1701	2,898
Ghrelin increase (pg/ml)				
Mean	27.5	49.7	62.5	70.6
SD	158.9	133.2	163.4	209.9
Minimum	−378.0	−260.0	−324.0	−265.6
25th percentile	−51.0	−35.0	−34.2	−37.7
Median	32.8	35.1	44.5	41.6
75th percentile	128.3	146.8	181.0	126.4
Maximum	378.4	339.4	552.8	1056.6
Changes in LC n-3 PUFA in erythrocyte membrane (in % of total lipids)				
Mean	−0.81	0.55	2.34	1.76
SD	2.60	1.81	2.36	2.50
Minimum	−10.04	−3.32	−3.58	−7.82
25th percentile	−1.64	−0.49	1.11	0.67
Median	−0.64	0.73	2.58	2.00
75th percentile	0.55	1.54	3.45	2.71
Maximum	8.95	5.82	9.59	8.15

Table 3 Estimated changes in endpoint insulin, leptin and ghrelin of the intervention groups relative to the control group

Parameter	B[a]	95% CI[a]		*P* value
Endpoint insulin (mU/l)				
Cod	0.032	−0.115	0.202	0.689
Salmon	−0.075	−0.208	0.081	0.324
Fish oil	−0.144	−0.264	−0.004	0.045
Weight loss (kg)	−0.036	−0.051	−0.021	<0.001
Endpoint leptin, men (ng/ml)				
Cod	−0.026	−0.167	0.140	0.744
Salmon	−0.070	−0.200	0.080	0.336
Fish oil	−0.083	−0.218	0.075	0.282
Weight loss (kg)	−0.083	−0.098	−0.068	<0.001
Endpoint leptin, women (ng/ml)				
Cod	0.103	−0.022	0.243	0.108
Salmon	0.070	−0.050	0.207	0.263
Fish oil	0.053	−0.065	0.184	0.392
Weight loss (kg)	−0.099	−0.115	−0.082	<0.001
Endpoint ghrelin (pg/ml)				
Cod	0.006	−0.049	0.064	0.836
Salmon	−0.003	−0.058	0.054	0.912
Fish oil	0.044	−0.012	0.103	0.123
Cod × male	−0.026	−0.108	0.064	0.561
Salmon × male	−0.011	−0.093	0.078	0.797
Fish oil × male	−0.115	−0.190	−0.032	0.008
Weight loss (kg)	0.012	0.006	0.018	<0.001

Linear models included dietary group, weight loss, age, gender, and country and baseline concentrations of the relevant hormone. Gender × intervention interactions are only shown when significant

[a] *B*s and 95% confidence intervals are presented as 1 minus back transformed *B*, e.g., endpoint insulin are 14.4% lower in the fish-oil group than in the control group

Discussion

In this randomized dietary intervention trial we investigated the effects of seafood consumption and weight loss on fasting hormone levels in young overweight and obese European adults from three different countries. Subjects received different amounts of seafood but otherwise consumed diets of similar macronutrient composition and percentual energy restriction. During these 8-week diets, there was a mean weight loss of 5.2 kg. Fasting leptin and insulin blood concentrations decreased significantly and ghrelin concentrations increased in the participants.

The most important finding of our study is that consumption of fatty seafood (salmon and EPA&DHA) can modulate fasting levels of circulating hormones insulin, ghrelin and leptin during an eight weeks intervention. The effects of fatty seafood are sometimes gender specific and are partly explained by weight loss. Consumption of lean fish (cod group) does not affect circulating hormones in comparison to a control without seafood. The most consistent effect on circulating hormones is mediated by weight loss.

Our results show fatty seafood to decrease circulating leptin and insulin, as well as to increase ghrelin. Because no effects of cod (lean fish) consumption on circulating hormones were observed in our study, LC n-3 PUFA can be considered as the principal bioactive components of fatty seafood in this regards. In our male participants salmon consumption was associated with lower endpoint leptin levels. No significant effect was observed in women. In vitro experiments have shown that EPA and DHA reduce the expression of leptin, and a feeding study with rats showed that LC n-3 PUFA supplementation lowers plasma leptin concentration and leptin mRNA expression [35]. In a randomised, double-blinded trial without energy restriction plasma leptin concentration were not affected in male subjects by LC n-3 PUFA supplementation of 5 g daily [23]. Possibly, altered leptin expression does not automatically translate into lower plasma concentrations [23]. In our male participants, the effects of salmon consumption on circulating leptin were mediated by the greater weight loss of the salmon group.

There is a lack of research on n-3 fatty acids and ghrelin, and only few studies have investigated the long term effects of diet composition or nutrients on fasting ghrelin [38, 39]. Human studies showed that fat restriction avoids the increase in ghrelin levels caused by dietary energy restriction [38] and that ghrelin concentrations increase after several weeks of ad libitum high-protein intake [39]. In acute studies the response of ghrelin to changes in macronutrient composition of meals are related to insulin [3, 31, 32]. Our study indicates that ghrelin concentrations in women of the EPA&DHA group are higher compared to control. No effect of EPA&DHA or salmon consumption was observed in male participants. Considering that ghrelin is an orexigenic hormone, higher ghrelin concentrations would put women in the EPA&DHA group into higher risk to increase their food intake [40] and thus for weight gain. This seems contrary to studies reporting increased fat oxidation after fish oil consumption [6] and implying positive effects on weight loss of n-3 fatty acids [15, 43]. Interestingly, in the SEAFOODplus YOUNG study [43] significant weight loss effects of EPA&DHA were only observed in men, not women. Also, in the study by Kunesová et al [15] weight loss difference between the n-3 group and the control group was strictly spoken not significant, although this study was limited by its power ($N = 20$), and the P value was still below 0.1. However, n-3 fatty acid consumption seems to decrease hunger sensations rather than to increase them [20]. Also important, the significant effects of EPA&DHA disappear after weight loss is included the statistical analysis, indicating that higher ghrelin concentrations are rather a consequence of weight loss in this group and not LC n-3 PUFA consumption.

Our results show that regular EPA&DHA consumption decreases fasting insulin independently from weight loss by 14.4%, which is equivalent to a weight loss of ~4 kg according to the statistical model. Insulin has been reported to be an important regulator of both leptin and ghrelin levels. According to several study groups using hyperinsulinaemic–euglycaemic clamps in human subjects, insulin decreases the circulating ghrelin concentration and increases serum leptin levels dose-dependently [5, 28]. In our study reductions of fasting insulin during the eight weeks were significantly associated with increased ghrelin and reduced leptin concentrations.

It has been known that energy intake and weight loss can have significant effects on circulating leptin and ghrelin levels [2, 7, 14, 25, 37]. In our study weight loss is the most consistent predictor of hormonal changes. The mean weight loss of 5.2 kg corresponds to a percentual weight loss of 5.9%. The decrease in body weight does not directly translate into similar percentual changes of circulating hormones, which ranged from 5.6% (ghrelin) to 34.8% (leptin) in our investigation.

Limitations

A limitation of each dietary intervention trial is the uncertainty whether dietary intakes of subjects during the study period were as reported or prescribed. As there was an intense support of the study participants by our staff, frequent contact via phone and in personal, and as compliance was tested during the intervention trial using a FFQ, validated for assessing frequency of seafood consumption [42], and red blood cell fatty acid composition, this risk was minimized. Changes in n-3 red blood cell fatty acids and results from the FFQ confirmed good compliance [43].

Conclusion

Consumption of fatty seafood (salmon group or EPA&DHA) can modulate fasting levels of circulating hormones insulin, ghrelin and leptin during an 8 weeks intervention. However, these effects are partly gender specific and are partly explained by weight loss. Consumption of lean fish does not affect circulating hormones in comparison to a control without seafood. The most consistent effect on circulating hormones is mediated by weight loss.

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