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ORIGINAL ARTICLE

Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content

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Objective: To investigate the effect of including seafood and fish oils, as part of an energy-restricted diet, on weight loss in young overweight adults.
Design: Randomized controlled trial of energy-restricted diet varying in fish and fish oil content was followed for 8 weeks. Subjects were randomized to one of four groups: (1) control (sunflower oil capsules, no seafood); (2) lean fish (3 × 150 g portions of cod/week); (3) fatty fish (3 × 150 g portions of salmon/week); (4) fish oil (DHA/EPA capsules, no seafood). The macronutrient composition of the diets was similar between the groups and the capsule groups, were single-blinded.
Subjects: A total of 324 men and women aged 20–40 years, BMI 27.5–32.5 kg/m^2 from Iceland, Spain and Ireland.
Measurements: Anthropometric data were collected at baseline, midpoint and endpoint. Confounding factors were accounted for, with linear models, for repeated measures with two-way interactions. The most important interactions for weight loss were (diet × energy intake), (gender × diet) and (gender × initial-weight).
Results: An average man in the study (95 kg at baseline receiving 1600 kcal/day) was estimated to lose 3.55 kg (95% CI, 3.14–3.97) (1); 4.35 kg (95% CI, 3.94–4.75) (2); 4.50 kg (95% CI, 4.13–4.87) (3) and 4.96 kg (95% CI, 4.53–5.40) on diet (4) in 4 weeks, from baseline to midpoint. The weight-loss from midpoint to endpoint was 0.45 (0.41–0.49) times the observed weight loss from baseline to midpoint. The diets did not differ in their effect on weight loss in women. Changes in measures of body composition were in line with changes in body weight.
Conclusion: In young, overweight men, the inclusion of either lean or fatty fish, or fish oil as part of an energy-restricted diet resulted in ∼1 kg more weight loss after 4 weeks, than did a similar diet without seafood or supplement of marine origin. The addition of seafood to a nutritionally balanced energy-restricted diet may boost weight loss.
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Keywords: fish proteins; n-3 fatty acids; weight-loss-diet; overweight; humans

Introduction

Obesity has become a serious worldwide healthcare problem, which is becoming increasingly prevalent among young adults and children.[1,2] It is therefore of great importance to help young overweight adults to lose weight. Inclusion of fish in a weight-loss-diet has been shown to have positive effects on several health-related variables,[3,4] which could be due to n-3 fatty acids or other seafood constituents, such as fish proteins as reported in animal studies.[5–7] Beneficial effects of fish consumption in relation to cardiovascular health have been thoroughly described, and mainly attributed to n-3 fatty acids.[8–11] Studies in rodents have demonstrated that marine n-3 fatty-acid-enriched diet decrease adipose growth and increase β-oxidation.[12,13] Additionally, taurine, an amino acid abundant in fish protein, has been suggested to decrease body weight.[14] The impact of including fish or a supplement of marine origin, in a weight loss diet, on the efficacy of such diets in humans has not been investigated to date.

The aim of the present study was to investigate the specific effects of seafood consumption on weight loss in young overweight adults, by comparing energy-restricted diets

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which were similar in terms of their energy content and macronutrient composition and contained either, lean fish, fatty fish, supplemental fish oil capsules or supplemental placebo capsules. We conducted a randomized controlled trial in three European countries, with weight loss as the main outcome.

Subjects and methods

The study was a randomized 8-week intervention trial of four isocaloric diets in three European countries: Iceland, Spain and Ireland. The study is part of a large multicenter study, funded by the European Union: SEAFOODplus – a better life with seafood (see www.seafoodplus.org). SEAFOODplus-YOUNG is a Research and Technology Development (RTD) project in Pillar 1 (Seafood and Human Nutrition) of SEAFOODplus.

Participants

Subjects were recruited through advertisements. Recruitment of 320 subjects (140 from Iceland, 120 from Spain and 60 from Ireland) was planned; it was estimated that a 70–80% participation allowed a detection of approximately 1 kg difference in weight loss between the four diet groups, assuming a standard deviation (s.d.) of 3 kg, a significance *P*-value of 0.05 and a statistical power of 0.8. All potential subjects that answered the call were screened for inclusion and exclusion criteria, first over the phone and then in person. Initially 324 subjects (138 men and 186 women) were included. The inclusion criteria were body mass index (BMI) 27.5–32.5 kg/m^2, age 20–40 years, and a waist circumference of $\geqslant$94 cm and $\geqslant$80 cm for men and women, respectively. Exclusion criteria were weight change due to weight-loss diet within 3 months before the start of the study, use of supplements containing n-3 fatty acids, calcium or vitamin-D during the last 3 months, drug treatment of diabetes mellitus, hypertension or hyperlipidemia and women's pregnancy or lactation. Recruitment was undertaken during 2004 and 2005. 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.

Protocol

The subjects had at least three visits to the clinic during the 8-week trial, at baseline, mid- and endpoint. Additionally, they were contacted on at least two other occasions by phone and/or email, in weeks 2 and 6. The subjects were also encouraged to contact the clinic on their own initiative if they had any questions regarding the research during the 8-week intervention period. At baseline, mid- and endpoint visits they met a nutritionist, anthropometric measurements were performed and seafood intake was assessed by a validated food frequency questionnaire (FFQ).[15] Dietary intake was assessed by 2-day weighed food records before baseline (habitual diet) and in week 6 or later during the last 2 weeks of the intervention trial. At baseline, information on physical activity patterns during the previous year, smoking habits and alcohol consumption was collected using a questionnaire.[16] Subjects were instructed not to change their physical activity level during the 8-week intervention period and to keep their alcohol consumption to a minimum (max 1 drink of wine or beer per week).

The dietary records were analyzed using the food composition database in each country. Compliance with the intervention diets was assessed by the 2-day weighed food records, by the validated FFQ and also by analyzing n-3 and n-6 fatty acids in erythrocyte phospholipids in fasting blood samples from the subjects. Results showed good compliance with the intervention diets.

Dietary intervention

Basal metabolic rate was estimated by applying Harris–Benedict equations,[17] and a correction factor due to the overweight status of the subjects.[18] To estimate total energy expenditure, the physical activity level was set to 1.3, as a relatively low physical activity level was reported by all subjects.[19] Each subject was instructed to follow a diet, energy restricted by 30% from estimated energy expenditure (approximately 600 kcal/day), for eight consecutive weeks. The subjects were randomly assigned to four diets (see Table 1) varying in types of dietary protein and amount of n-3 fatty acids (especially very long-chain n-3 fatty acids): diet (1) no seafood (control, including 6 placebo capsules/day Loders Croklaan (Lipid Nutrition), Wormerveer, The Netherlands, encapsulated by Banner Pharmacaps, Tilburg, The Netherlands); diet (2) lean fish (150 g cod × 3/week, Samherji, Iceland); diet (3) fatty fish (150 g salmon × 3/week, Marine Harvest, Nutreco, Norway); or diet (4) fish oil capsules (6 capsules/day, Loders Croklaan (Lipid Nutrition), Wormerveer, The Netherlands, encapsulated by Banner Pharmacaps, Tilburg, The Netherlands). The diets were similar in total fat (30% of total energy), carbohydrate (50% of total energy), protein (20% of total energy) and dietary fiber (20–25 g).

Table 1 Fatty acids (%) in erythrocyte phospholipids of subjects at endpoint in each diet group (*n* = 278)

Diets	*Erythrocyte phospholipids n-3 fatty acids (%)*	*Erythrocyte phospholipids n-6 fatty acids (%)*
Control capsules, 6 per day	9.9 (2.5)	29.5 (4.1)
Cod 150 g three times per week	10.8 (2.4)	29.7 (4.0)
Farmed salmon 150 g three times per week	12.2 (2.3)	28.3 (3.6)
Fish oil capsules, 6 per day	12.7 (2.9)	29.5 (3.3)

The values are mean (s.d.).

Each subject got a detailed meal plan to follow for 8 weeks, as well as recipe booklets and instructions to minimize difference between diets in sources of fat, fruit and vegetable consumption and meal frequency.

Fatty-acid analysis

The proximate chemical composition of the fish used in the study was carried out according to AOAC methods[20] and fatty acid determination followed the conditions described by Bandarra *et al*.[21] Absolute values were calculated using the corrective factors proposed by Weihrauch *et al*.[22] The culinary treatments were carried out following usual household methods. True retention factors were calculated for total n-3 fatty acids.[23] The n-3 content of the cod (in diet 2) was 0.3 g/day, the salmon (in diet 3) 3.0 g/day and the fish oil capsules (in diet 4) 1.5 g/day. Other sources of n-3 fatty acids were congruent between diet groups. Table 1 shows results from analysis of n-3 and n-6 analysis of erythrocyte phospholipids. The n-6 fatty acids in red blood cells of individuals on the four different diets showed similar values. The percentage of n-3 fatty acids in erythrocyte phospholipids was similar in diets (3) and (4) despite higher intake of group (3) according to the fatty-acid analysis of the salmon and fish oil capsules.

Anthropometric measurements

Anthropometric measurements were performed on three occasions during the 8-week period: at baseline, midpoint and endpoint. Weight was measured in light underwear on a calibrated scale (SECA 708, Germany) in all centers. Height was measured with a calibrated stadiometer, and waist and hip circumferences were measured following standardized methods. Fat mass and fat-free mass were assessed by bioelectrical impedance analysis (Bodystat 1500, Bodystat Ltd, UK). All measurements were carried out using standard measurement procedures as outlined in the research protocol used by each of the three countries participating in the study.

Statistical analysis

Mean and s.d. were used to describe the data. The unadjusted difference in weight loss and changes of other anthropometric measurements between the control group and treatments groups was assessed by independent sample *t*-test. The individuals included in the study were young, healthy adults, except for their overweight or obesity, and hence a normal model was the natural candidate. A linear model for the response in a repeated measures analysis for the difference was set up. The important main effects were assumed to be diet, gender, initial body weight and country. Owing to cultural differences, it was conceivable that there might be interactions between country and diet, as seafood may play a different role in the national diets. Genders may differ in compliance as well as in the response. Therefore, to evaluate the impact of the dietary experiment, the use of linear models for repeated measures with two-way interactions was the approach of choice. The most important interactions were found to be (gender $\times$ initial-weight), (gender $\times$ diet) and (diet $\times$ calories), giving an *F*-value of 89, which is highly significant. The *F*-value of other two-way interactions was 0.79, which is not significant.

The quality of the model was evaluated by residual diagnosis. If the response to treatment is normally distributed then skewness of residuals should be around zero and kurtosis around 3. The skewness for weight reduction was 0.05 for males and –0.13 for females, and the kurtosis was 2.5 and 3.2 for men and women, respectively, showing that the normal approximation is reasonable. If modelling is successful residuals should also show irregular patterns when plotted against various variables and transformations of them. Traditional residual diagnostics were performed. The dropout process was modelled with a binary response model. The impact of the covariates on the probability of dropout was evaluated by a logistic regression. Correlations between weight loss and decrease in other anthropometric variables were illustrated by Spearman's rank correlation analysis.

Results

Baseline characteristics and dropouts

Of a total of 324 randomized individuals to enter into the trial, 278 (86%) completed the randomized intervention. Figure 1 shows the flow of participants. The two most common reasons for dropout were that the subject was unable to follow the restricted diet and/or lack of time to maintain the schedule of clinical visits. The results of the logistic regression suggest no systematic explanation of dropout. Dropouts were equally distributed between intervention diet groups and variables predicting weight loss in the present study were not different between dropouts and those who completed the trial. The baseline characteristics of the subjects are shown in Table 2.

Dietary intake

Habitual dietary intake was assessed by a 2-day food record at baseline before randomization and compliance with the

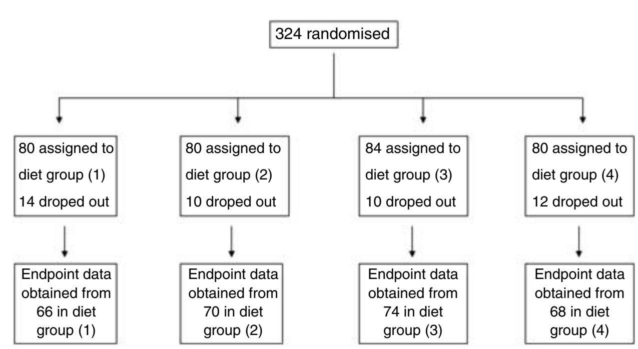

Figure 1 Flow of participants in the study.

Table 2 Characteristics of subjects at baseline ($n = 324$)

	Group 1 (n = *80) Control*		*Group 2* (n = *80) Lean fish*		*Group 3* (n = *84) Fatty fish*		*Group 4* (n = *80) Fish oil*	
	Women (n = *48)*	*Men* (n = *32)*	*Women* (n = *45)*	*Men* (n = *35)*	*Women* (n = *42)*	*Men* (n = *42)*	*Women* (n = *51)*	*Men* (n = *29)*
Weight (kg)	81.7 (6.7)	96.7 (7.2)	84.2 (7.3)	96.3 (7.4)	83.3 (6.9)	97.5 (10.6)	80.5 (7.0)	93.0 (7.8)
Height (m)	1.65 (0.06)	1.79 (0.06)	1.67 (0.06)	1.79 (0.06)	1.66 (0.06)	1.79 (0.08)	1.63 (0.05)	1.77 (0.07)
Body mass index (kg/m^2)	29.9 (1.5)	30.1 (1.5)	30.2 (1.6)	30.2 (1.2)	30.3 (1.5)	30.5 (1.3)	30.1 (1.7)	29.5 (1.2)
Waist circumference (cm)	91.7 (6.1)	100.4 (6.0)	93.2 (6.1)	101.3 (4.4)	92.5 (7.6)	101.4 (5.3)	91.4 (6.4)	98.5 (4.4)
Hip circumference (cm)	111.5 (5.5)	108.9 (3.9)	113.5 (5.7)	107.8 (3.9)	111.9 (4.2)	109.5 (5.4)	110.5 (6.0)	107.2 (4.2)
Fat-free mass (kg)	49.3 (3.9)	72.0 (5.2)	51.3 (5.8)	71.0 (5.5)	51.3 (5.1)	71.9 (7.4)	49.9 (6.9)	69.5 (6.3)
Fat mass (kg)	32.8 (4.7)	24.6 (3.8)	32.9 (4.9)	25.3 (3.3)	32.5 (4.1)	25.5 (4.5)	31.8 (4.4)	23.5 (3.6)

Values are means (s.d.). No differences in anthropometric measurements were observed between diet groups at baseline.

dietary intervention was assessed between weeks 6 and 8. Energy intake was not different between the four intervention diet groups, neither at baseline nor during the intervention. The mean habitual energy intake at baseline was 2022 kcal/day (522 s.d.) among women and 2694 kcal/day (837 s.d.) among men. The intervention diets gave on average 1350 kcal/day (318 s.d.) and 1579 kcal/day (363 s.d.) for women and men, respectively. The contribution of energy-giving nutrients (E%) was not different between the intervention diets except for dietary proteins where a small but significant difference was observed (20E% in diet groups (2) and (3) compared with 18E% and 19E% in diet groups (1) and (4), respectively).

Weight loss

The average reduction in weight was 6.5 kg (s.d. = 3.3 kg) for men and 4.2 kg (s.d. = 2.4 kg) for women. Table 3 shows the mean unadjusted decrease in weight, body mass index (BMI) and other anthropometric measurements in the four groups. Weight loss and decrease in waist circumference was significantly greater in the groups receiving fish or fish oil (cod, salmon or fish oil) when compared with the control group ($P < 0.05$), but in male subjects only.

We chose to use a modelling approach to assess any true difference in weight loss across the intervention diets. This was done to adjust for baseline values and the fact that men and women were not equally distributed in the groups. The base model was taken as:

$$\text{Weight loss} = \text{main effects} + [\text{gender} \times \text{initial weight}] + [\text{gender} \times \text{diet}] + [\text{diet} \times \text{energy intake}]$$

The expected weight loss for an average man (96 kg, assigned to a diet giving1600 kcal/day) after the first 4 weeks of the study, calculated from the model output in Table 4, was 3.55 kg (95% CI, 3·14–3·97) on diet (1), 4.35 kg (95% CI, 3.94–4.75) on diet (2), 4.50 kg (95% CI, 4.13–4.87) on diet (3), and 4.96 kg (95% CI, 4.53–5.40) on diet (4). The expected weight loss for a male weighing 10 kg above the average is 1.60 kg more. Calculating average weight loss from the Icelandic model, a Spanish subject would be expected to lose 0.47 kg more and an Irish subject 0.80 kg less in every diet group. No significant (country × diet) interaction was seen indicating that diet effects were similar across countries. Diets did not differ in their effect on weight loss for females. The expected weight loss for an average woman (82 kg) assigned to a diet giving 1300 kcal/day did not differ between the diet groups being 2.82 kg (95% CI, 2.48–3.16) on diet (1), 2.99 kg (95% CI, 2.64–3.34) on diet (2), 2.83 kg (95% CI, 2.47–3.18) on diet (3), and 3.03 kg (95% CI, 2.69–3.37) on diet (4) in weeks 1–4. The most significant interaction was the (gender × initial weight) interaction. For men, the

Table 3 Decrease in body weight and other anthropometric measurements from baseline after 8-weeks intervention, unadjusted

	Group 1 control (42 women, 24 men)	*Group 2 lean fish (39 women, 31 men)*	*Group 3 fatty fish (37 women, 37 men)*	*Group 4 fish oil (40 women, 28 men)*
Body weight (kg)				
Women	3.9 (2.6)	4.4 (2.3)	3.9 (2.3)	4.4 (2.6)
Men	5.3 (3.0)	6.5 (2.8)	7.0 (3.5)	6.7 (3.6)
All	4.4 (2.8)	5.4 (2.7)	5.5 (3.3)	5.4 (3.2)
Body mass index (kg/m2)				
Women	1.4 (1.0)	1.6 (0.8)	1.4 (0.9)	1.7 (1.0)
Men	1.6 (0.9)	2.1 (0.9)	2.2 (1.1)	2.1 (1.1)
All	1.5 (0.9)	1.8 (0.9)	1.8 (1.0)	1.9 (1.0)
Waist circumference (cm)				
Women	3.8 (2.4)	4.0 (2.6)	4.1 (2.9)	3.8 (2.8)
Men	4.5 (2.3)	6.2 (2.8)	6.6 (3.2)	6.9 (2.7)
All	4.0 (2.4)	5.0 (2.9)	5.4 (3.3)	5.1 (3.1)
Hip circumference (cm)				
Women	3.9 (2.7)	4.4 (2.4)	4.0 (2.1)	4.0 (2.5)
Men	2.7 (1.6)	3.8 (1.8)	4.6 (3.1)	4.0 (2.4)
All	3.5 (2.4)	4.2 (2.1)	4.3 (2.6)	4.0 (2.5)
Fat-free mass (kg)				
Women	1.3 (2.4)	1.6 (2.7)	1.2 (1.2)	0.9 (1.2)
Men	2.1 (1.6)	2.5 (1.8)	2.8 (1.7)	2.6 (1.7)
All	1.6 (2.2)	2.0 (2.4)	2.0 (1.7)	1.6 (1.7)
Fat mass (kg)				
Women	2.8 (2.2)	2.9 (2.9)	2.9 (1.8)	3.4 (2.1)
Men	3.2 (2.6)	4.0 (2.1)	4.2 (2.5)	4.2 (2.5)
All	3.0 (2.3)	3.4 (2.6)	3.5 (2.3)	3.7 (2.3)

Values are means (s.d.).

Table 4 Parameter estimates of base model

	E	*s.e*	t	P
Intercept	11.71	4.77	2.46	0.01
Weight at baseline (kg)	−0.16	0.03	−5.54	0.00
Gender (women)	−13.60	2.69	−5.05	0.00
Energy intake, 1000 kcal	0.22	3.05	0.07	0.94
Country (Spain)	−0.47	0.23	−2.03	0.04
Country (Ireland)	0.80	0.27	2.96	0.00
Diet group (2), lean fish	−14.75	5.89	−2.50	0.01
Diet group (3), fatty fish	−11.07	5.48	−2.02	0.04
Diet group (4), fish oil	−7.05	5.76	−1.22	0.22
Baseline weight * gender (women)	0.15	0.03	5.20	0.00
Energy intake * diet group (2)	8.45	3.56	2.37	0.02
Energy intake * diet group (3)	6.13	3.31	1.85	0.07
Energy intake * diet group (4)	3.41	3.50	0.98	0.33
Gender (women) * diet group (2)	3.44	1.30	2.65	0.01
Gender (women) * diet group (3)	2.98	1.22	2.44	0.02
Gender (women) * diet group (4)	2.33	1.30	1.80	0.07

Example of how expected weight loss in diet group 4 (fish oil) the first 4 weeks of the 8-week intervention is calculated for a female weighing 81.9 kg at baseline (average weight at baseline), receiving 1320 kcal/day (average energy intake of females in the trial): Expected weight loss = 11.71–0.162*81.9–13.60+0.216*1.320–7.049+0.147*81.9+3.41*1.320+2.334.

expected weight loss was a function of initial weight, but this was not the case for women. This means that weight at baseline was positively related to actual weight loss (in kg) in men, but not in women.

A significant (energy intake × diet) interaction (see Table 4) suggested that the impact of calorie reduction was not the same across diets, where the impact seems to be larger in diet group (2) than in the other diet groups.

For the period from weeks 5 to 8, a similar model was estimated. Two variables were added to the model, observed and predicted weight loss in the first period (weeks 1–4). The results show that weight loss during weeks 1–4 was the only significant factor that explained weight loss during weeks 5–8. The estimated weight loss in weeks 5–8 was 0.45 (0.41–0.49) times the observed weight loss in weeks 1–4.

Other anthropometric measurements

Strong correlations were seen between weight reduction and reductions in other anthropometric measurements, both for the first 4 and the last 4 weeks as seen in Table 5. The BMI of males in diet groups (2), (3), (4) decreased more during the 8-week intervention than the control, diet group (1). The reduction of waist circumference was larger in diet group (4) than in diet group (1), but the impact was small. It was observed that reduction in all anthropometric measurements in weeks 5–8 was predominantly predicted by weight reduction in weeks 1–4.

Discussion

The important finding of the current study is that following an energy-restricted diet for 8 weeks containing lean or fatty fish or fish oil resulted in more weight loss than did an isocaloric energy-restricted diet without marine food.

Table 5 Correlation (Spearman *r*) between the decrease in body weight and decrease in other anthropometric measurements from baseline to midpoint (week 4), and from midpoint to end (week 8)

	Weeks 1–4	*Weeks 5–8*
Decrease in body weight	1.0	1.0
Decrease in BMI	0.95	0.98
Decrease in waist circumference	0.60	0.60
Decrease in hip circumference	0.54	0.51
Decrease in fat free mass	0.60	0.60
Decrease in fat mass	0.76	0.71
Decrease in body fat percentile	0.55	0.46

The effect of lean fish as part of an energy-restricted diet is of specific interest as up to now the main hypothesis on the health benefits of fish consumption has been contingent on the effect of n-3 fatty acids. The current study indicates that consuming a diet relatively low in n-3 fatty acids, but high in quality fish proteins gives similar results as a diet rich in n-3 dietary fatty acids and quality fish proteins. If confirmed this might be an important public health information, especially since some populations consume more lean fish than fish rich in n-3 fatty acids.[14,23]

Seafood-derived n-3 fatty acids, as those found in higher amounts in diets (3) and (4) in the present study including fatty fish or fish oil capsules, have been shown to decrease the growth of the adipose cell, probably through stimulated β-oxidation.[12,13,24] Recently, this finding was supported by a study on mice showing that the anti-adipogenic effect of eicosapentaenoic and docoxahexaenoic acids may involve a switch in adipocytes that includes increase in β-oxidation and upregulation of mitochondrial biogenesis.[25] Furthermore, a recent review by Madsen *et al.*[26] also describes that n-3 fatty acids may affect adipocyte differentiation. N-3 polyunsaturated fatty acids decrease adipose tissue mass and the development of obesity in rodents by targeting a set of key regulatory transcription factors involved in adipogenesis and lipid homeostasis.[26] The same set of factors are targeted by n-6 polyunsaturated fatty acids, but their effect seem to be dependent on feeding status and hormonal background and n-6 therefore react either as anti- or proadipogenic agents.[26] However, Garaulet *et al.*[27] have recently showed, for the first time in humans, that n-3 and n-6 fatty acids are related to a reduced adipocyte size according to the tissue localization. A specific effect of seafood n-3 fatty acids may be responsible for more reduction in body weight and associated anthropometric variables of diets (3) and (4), including fatty fish or fish oil capsules, compared to the diet without seafood or marine oil, that is, control diet (1).

However, this cannot solely explain the similarly effective lean fish diet, which had only slightly higher amounts of total n-3 than the control diet (1) without seafood. Fish proteins may be the candidate for this effect.[5–7] Inclusion of fish in a weight loss diet has been shown to have positive effects on several health-related variables in a study of 63 subjects,[3,4,28] but to our knowledge, the effects of seafood to increase weight loss in humans has not been seen before. Inclusion of fish oil in a diet of six adults was found to reduce body fat mass in a study by Couet *et al*.[24] The results of the present study may indicate that there are components of fish, for example, particular combinations of amino acids that may be beneficial to human health beyond the n-3 fatty acids or other bioactive constituents. A plausible candidate is taurine, an amino acid abundant in fish protein. An early study showed that taurine decreases body weight in hyperglycemic obese mice,[14] and ingestion of 3 g taurine per day for 7 weeks has been found to be associated with weight loss in humans.[29] Additionally, a recent study found dietary taurine supplementation to prevent obesity in mice by increased resting energy expenditure.[30] Further studies are needed to examine possible mechanisms. In addition, closer investigation of the effects of portion size and more frequent fish consumption as part of a varied and balanced diet is required. For example, eating fish three times a week would not have been regarded as exceptional in countries like Iceland until relatively recently.

A gender difference was observed in the present study, where the different effects of the intervention diets on weight loss was specifically observed in men but not in women. Furthermore, men lost weight in proportion to their weight at baseline, but this effect was not observed in women. This may partly be explained by the fact that women naturally lost less, during the period of the study, as 30% energy restriction for women was less than for men in absolute values, as their estimated energy requirement is lower. Another potential explanation could be gender difference in response to marine food, as the endogenous conversion of α-linolenic acid into docosahexaenoic acid seems to be greater in women than in men.[31–32]

An important finding in the present study was that reduction in all anthropometric measurements in weeks 5–8 was predominantly predicted by weight reduction in weeks 1–4. This could be interpreted that the weight loss in the first 4 weeks is a proxy variable for compliance to treatment, and that those who comply in the first 4 weeks are more likely to continue the treatment in weeks 5–8, resulting in additive effects on anthropometric variables and potentially diminished risk for chronic diseases. Other studies have shown that weight loss in the beginning of a weight reduction program is an important determinant of further weight loss.[33–35] This could also be due to metabolic determinants.[36] The results indicate that the loss of fat-free mass was greater in the diet groups receiving fish. However, the distribution was large and no significant difference observed in loss of fat-free mass between the four diet groups.

The strength of the study is the design and it is the first human randomized controlled trial to test effects on weight loss from similarly energy-restricted diets containing lean fish, fish rich in n-3 fatty acids and supplemental fish oil vs diet containing no seafood. The statistical model designed to describe effects on weight including important determinants for weight loss is also of high value as it reflects the high impact of gender and initial weight on the outcome, which has to be taken into consideration. There is no reason to believe that the results could not apply in similar populations other than in Iceland, Spain and Ireland as no diet $\times$ country interaction was found, showing that the difference in weight loss between diet groups was seen to a similar degree in all countries. Physical activity was assessed during the intervention period and was found to be similar between diet groups.

A limitation of the current study could be the inclusion of both men and women, as they were found to react differently. However, this could also be interpreted as an additional benefit of the study, as the pronounced gender difference was unexpected. Another limitation could have been uncertainty in dietary intakes of subjects during the study period, but as compliance was tested during the intervention trial and overall results, red blood cell fatty acid composition as well as total weight loss were as expected, this risk was minimized. Probably the main limitation of the study is the relatively short time period, that is, 8 weeks, for observation. The reason for the short time period lies in the main aim of the study, which was to investigate if there was a possible difference between weight-loss-diets that included and excluded food components of marine origin. The study was not designed to assess long-term effects, which we suggest should be considered in future studies.

It remains a significant priority to help overweight individuals to lose weight. How the weight loss is brought about can make a difference to both total kg lost and to reduction of cardiovascular risk factors. The subjects in the present study were 20- to 40-year-old, which means that some are parents or are likely to have children in the near future. Parental influence on children's dietary habits is well known,[37,38] and if fish consumption proves to be effective in assisting weight reduction and improving weight maintenance in humans, the 20–40 year age group is a key target group for dietary interventions aimed to increase fish intake.

In conclusion, in young, overweight men, the inclusion of either lean or fatty fish, or fish oil as part of a hypoenergetic diet resulted in $\sim$1 kg more weight-loss after 4 weeks than did a similar diet without seafood or supplement of marine origin. The addition of seafood to a nutritionally balanced energy-deficient diet may boost weight loss.

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References

1 World Health Organization (WHO). Consultation on obesity. Global prevalence and secular trends in obesity. In: *Obesity: preventing and managing the global epidemic*. WHO: Geneva, Switzerland, 1998, pp 17–40.
2 British Medical Association (BMA) Board of Science. *Preventing childhood obesity. A report from the BMA Board of Science*. BMA publications unit, June 2005.
3 Bao DQ, Mori TA, Burke V, Puddey IB, Beilin LJ. Effects of dietary fish and weight reduction on ambulatory blood pressure in overweight hypertensives. *Hypertension* 1998; **32**: 710–717.
4 Mori TA, Bao DQ, Burke V, Puddey IB, Watts GF, Beilin LJ. Dietary fish as a major component of a weight-loss diet: effect on serum lipids, glucose, and insulin metabolism in overweight hypertensive subjects. *Am J Clin Nut* 1999; **70**: 817–825.
5 Yahia DA, Madani S, Prost E, Prost J, Bouchenak M, Belleville J. Tissue antioxidant status differs in spontaneously hypertensive rats fed fish protein or casein. *J Nut* 2003; **133**: 479–482.
6 Lavigne C, Tremblay F, Asselin G, Jacques H, Marette A. Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats. *Am J Physiol Endocrinol Metab* 2001; **281**: E62–E71.
7 Tremblay F, Lavigne C, Jacques H, Marette A. Dietary cod protein restores insulin-induced activation of phosphatidylinositol 3-kinase/Akt and GLUT4 translocation to the T-tubules in skeletal muscle of high-fat-fed obese rats. *Diabetes* 2003; **52**: 29–37.
8 Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality form coronary heart disease. *N Engl J Med* 1985; **312**: 1205–1209.
9 Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P *et al.* Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med* 1997; **336**: 1046–1053.
10 Bang HO, Dyerberg J. Lipid metabolism in Greenland Eskimos. *Adv Nutr Res* 1980; **31**: 1–32.
11 Dolocek TA, Grandits G. Dietary polyunsaturated fatty acids and mortality in multiple risk factor intervention trial (MRFIT). *World Rev Nutr Diet* 1991; **66**: 205–216.
12 Nakatani T, Kim HJ, Kaburagi Y, Yasuda K, Ezaki O. A low fish oil inhibits SREBP-1 proteolytic cascade, while a high-fish-oil feeding decreases SREBP-1 mRNA in mice liver: relationship to anti-obesity. *J Lipid Res* 2003; **44**: 369–379.
13 Ukropec J, Reseland JE, Gasperikova D, Demcakova E, Madsen L, Berge RK *et al.* The hypotriglyceridemic effect of n-3 FA is associated with increased beta-oxidation and reduced leptin expression. *Lipids* 2003; **38**: 1023–1029.
14 Fujihira E, Takahashi H, Nakazawa M. Effect of long-term feeding of taurine in hereditary hyperglycemic obese mice. *Chem Pharm Bull (Tokyo)* 1970; **18**: 1636–1642.
15 Thorsdottir I, Birgisdottir BE, Halldorsdottir S, Geirsson RT. Association of fish and fish liver oil intake in pregnancy with infant size at birth among women of normal weight before pregnancy in a fishing community. *Am J Epidemiol* 2004; **160**: 460–465.
16 Martinez-Gonzalez MA, Varo JJ, Santos JL, De Irala J, Gibney M, Kearney J *et al*. Prevalence of physical activity during leisure time in the European Union. *Med Sci Sports Exerc* 2001; **33**: 1142–1146.
17 Cankayali I, Demirag K, Kocabas S, Resat Moral A. The effects of standard and branched chain amino acid enriched solutions on thermogenesis and energy expenditure in unconscious intensive care patients. *Clin Nutr* 2004; **23**: 257–263.
18 Salvino RM, Dechicco RS, Seidner DL. Perioperative nutrition support: who and how. *Cleve Clin J Med* 2004; **71**: 345–351.
19 Nordic Nutrition Recommendations 2004. *Integrating Nutrition and Physical Activity* 4th edn. Nordic Council of Ministers: Copenhagen, 2004.
20 AOAC. *Official Methods of Analysis* 16th edn, 4th Revision, Vol I e II. Association of Official Analytical Chemistry, AOAC International, Washington, DC, 1998.
21 Bandarra NM, Batista I, Nunes ML, Empis JM, Christie WW. Seasonal changes in lipid composition of sardine *Sardina pilchardus*. *J Food Sci* 1997; **62**: 40–43.
22 Weihrauch JL, Posati LP, Anderson BA, Exler J. Lipid conversion factors for calculating fatty acids contents in foods. *J Am Oil Chem Soc* 1977; **54**: 36–40.
23 Dahl L, Bjorkkjaer T, Graff IE, Malde MK, Klementsen B. Fish–more than just omega 3. *Tidsskr Nor Laegeforen* 2006; **126**: 309–311.
24 Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int J Obes Relat Metab Disord* 1997; **21**: 637–643.
25 Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal N *et al.* Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. *Diabetologia* 2005; **48**: 2365–2375.
26 Madsen L, Petersen RK, Kristiansen K. Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. *Biochim Biophys Acta* 2005; **1740**: 266–286.
27 Garaulet M, Hernandez-Morante JJ, Lujan J, Tebar FJ, Zamora S. Relationship between fat cell size and number and fatty acids composition in adipose tissue from different fat depots in overweight/obese humans. *Int J Obes* 2006; **30**: 899–905.
28 Mori TA, Burke V, Puddey IB, Shaw JE, Beilin LJ. Effect of fish diets and weight loss on serum leptin concentration in overweight, treated-hypertensive subjects. *J Hypertens* 2004; **22**: 1983–1990.
29 Zhang M, Bi LF, Fang JH, Su XL, Da GL, Kuwamori T, *et al.* Beneficial effects of taurine on serum lipids in overweight or obese non-diabetic subjects. *Amino Acids* 2004; **26**: 267–271.
30 Tsuboyama-Kasaoka N, Shozawa C, Sano K, Kamei Y, Kasaoka S, Hosokawa Y *et al*. Taurine deficiency creates a vicious circle promoting obesity. *Endocrinology* 2006; **147**: 3276–3284.
31 Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* 2002; **88**: 411–420.
32 Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr* 2002; **88**: 355–363.
33 Packianathan I, Sheikh M, Boniface D, Finer N. Predictors of programme adherence and weight loss in women in an obesity programme using meal replacements. *Diabetes Obes Metab* 2005; **7**: 439–447.
34 Elfhag K, Rössner S. Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain. *Obes Rev* 2005; **6**: 67–85.
35 Astrup A, Ryan L, Grunwald GK, Storgaard M, Saris W, Melanson E *et al.* The role of dietary fat in body fatness: evidence from a preliminary meta-analysis of ad libitum low-fat dietary intervention studies. *Br J Nutr* 2000; **83**: S25–S32.
36 Labayen I, Diez N, Parra D, Gonzalez A, Martinez JA. Basal and postprandial substrate oxidation rates in obese women receiving two test meals with different protein content. *Clin Nutr* 2004; **23**: 571–578.
37 Feunekes GI, de Graaf C, Meyboom S, van Staveren WA. Food choice and fat intake of adolescents and adults: associations of intakes within social networks. *Prev Med* 1998; **27**: 645–656.
38 Cook S, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988–1994. *Arch Pediatr Adolesc Med* 2003; **157**: 821–827.