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The effect of farmed trout on cardiovascular risk markers in healthy men

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Increased intake of marine long-chain *n*-3 PUFA (*n*-3 LCPUFA) may decrease the risk of CVD and reduce mortality by lowering serum TAG and blood pressure (BP). Furthermore, *n*-3 LCPUFA may affect novel CVD risk markers related to inflammation and vascular function. The objective of the present study was to examine the effect of farmed trout on novel and traditional CVD risk markers in healthy men, and to evaluate whether this was affected by the aquacultural feed regime. We performed a parallel, 8-week intervention study in which sixty-eight healthy male volunteers were randomised to consume either a daily meal with 150 g farmed trout raised on either marine or vegetable-based feed, or a reference meal containing 150 g chicken. Twenty-four hour BP, pulse wave velocity, augmentation index, fatty acid composition of erythrocyte (RBC), and concentrations of TAG, HDL-cholesterol, LDL-cholesterol, glucose, insulin, C-reactive protein (CRP) and other markers of inflammation were measured at weeks 0 and 8. RBC content of total *n*-3 LCPUFA, both EPA and DHA, was significantly higher among men consuming trout raised on marine feed compared with men consuming the vegetable-fed trout or chicken. The three intervention groups did not differ significantly with respect to any of the other outcome variables, although there were trends towards associations between the changes in RBC *n*-3 LCPUFA and those in BP and CRP. In the present study, we conclude that we could not confirm the fish oil-induced reduction in CVD risk markers after daily consumption of trout with high or low *n*-3 LCPUFA content. However, trout raised on vegetable-based feed had less pronounced impact on RBC *n*-3 LCPUFA status.

***n*-3 PUFA: Vascular function: Blood pressure: Plasma lipid profile**

A large number of prospective studies have shown that regular fish consumption is related to a lower risk of CVD, such as stroke[1] and CHD[2]. The health-promoting effect of fish has been ascribed to the long-chain *n*-3 PUFA (*n*-3 LCPUFA), EPA and DHA. A meta-analysis of the randomised intervention trials performed showed that *n*-3 LCPUFA may reduce mortality in patients with CHD[3]. *n*-3 LCPUFA may protect against CVD by lowering serum TAG[4], blood pressure (BP)[5] and heart rate[6], as has been shown in randomised trials with fish oil supplementation.

In addition, *n*-3 LCPUFA may affect novel CVD risk markers related to inflammation and vascular function. Chronic low-grade inflammation and increased adhesiveness for circulating leucocytes to vascular endothelial cells is believed to play a key role in the process leading to atherosclerosis and CVD[7]. Increased plasma concentrations of the acute-phase reactant C-reactive protein (CRP) have been shown to be associated with CVD[8], and circulating concentrations of adhesion molecules are considered to indicate a pro-inflammatory state in the vasculature and to be predictive of CVD risk[9]. A number of studies have examined the effect of *n*-3 LCPUFA supplementation on inflammatory markers in healthy subjects[10–15]. The majority of these studies have shown that *n*-3 LCPUFA supplementation has little or no effect on CRP, whereas the effect on cell adhesion molecules is more complex and uncertain. Vascular function may also play a central role in the development, progression and clinical manifestations of atherosclerosis, and a number of studies indicate that *n*-3 LCPUFA may positively improve vascular function[16–22], but there are some studies that do not find such an effect[23].

Only few well-designed randomised intervention trails have examined the effect of increased fish intake on CVD risk markers in healthy and high-risk subjects[24–31], but the results are inconclusive. Fish rich in *n*-3 LCPUFA have been shown to reduce serum TAG in some studies[24,25,27,28],

Abbreviations: AIx, augmentation index; BP, blood pressure; CRP, C-reactive protein; HDL-C, HDL-cholesterol; *n*-3 LCPUFA, long-chain *n*-3 PUFA; PWV, pulse wave velocity; RBC, erythrocyte; sVCAM, soluble vascular cell adhesion molecule.

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but not in all studies[26,30], and the effect on inflammation is inconclusive[27–29,31]. Additional studies have focused on the effect of other fish components, such as fish protein and vitamin D, on CVD risk markers in animals and human subjects. These studies suggest that fish protein may improve BP[32,33] and insulin sensitivity[34], decrease plasma CRP[35] and modulate lipoprotein metabolism[36,37]. Vitamin D has been shown to improve CVD risk markers such as endothelial function[38].

In Denmark, farmed fish contribute increasingly to the overall fish consumption, due to the reduced availability of wild fish and an increasing demand. The most important fish farmed in Denmark is the rainbow trout (*Oncorhynchus mykiss*). However, the aquaculture sector is facing major challenges due to limited access to marine raw material resources. Therefore, vegetable oil and protein are introduced as alternative ingredients in feed for fish farming. Such change in feed composition may influence the human health effect of the trout. Presumably, the content of *n*-3 LCPUFA will decrease whereas that of *n*-6 PUFA will rise, as has been shown in other fish species depending on the vegetable oil used[28]. However, only a single study has examined clinical effects of fish raised on different types of feeds in CVD patients[28]. The overall objective of the present study is to examine the effect of intake of farmed trout on novel and traditional CVD risk markers in healthy men, and to evaluate whether the fish feed composition (100 % marine or vegetable-based feed) had any effect on the measured parameters.

Subjects and methods

Subjects

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Local Research Ethical Committee of Copenhagen and Frederiksberg (H-KF 325441). Written informed consent was obtained from all the subjects after they had received oral and written information about the study. We recruited healthy adult men from Copenhagen and the surrounding areas by advertising in the local media. Men, who were interested in the study, contacted the principal investigator, who interviewed them by telephone using a detailed screening questionnaire. Those who met the inclusion criteria were invited to take part in a physical examination. Eligible participants were apparenty healthy males aged 40–70 years with no history of CVD, inflammatory disease, diabetes, or other significant medical history, no regular use of medication known to affect the outcome measures, e.g. anti-inflammatories, hypertension medication or statins. In addition, participants had to be non-smokers or smokers of <7 cigarettes/week, had to have a BP $<160/100$ mmHg and a BMI between 20 and 30 kg/m^2, were not allowed to take fish oil supplements, had to follow a weight-reducing diet, had to be regular blood donors, or had to be trained athletes or heavy exercisers. Those who used vitamin and/or mineral supplements before the study were instructed to continue during the study period. Six participants used multivitamin supplements throughout the study (one in the marine trout group, two in the vegetable trout group and three in the chicken group). One participant from the marine trout group smoked an average of three cigarettes per week, and one participant in the chicken group smoked eight cigarettes per week.

Study design

We performed a randomised, parallel, 8-week intervention study in adult males. The volunteers were randomised to consume either a daily meal with 150 g farmed trout fillet either raised on a pure marine diet (marine trout) or on a pure vegetable diet (vegetable trout), or a reference meal containing 150 g chicken.

A weekly meal plan was provided, which included seven different meals, i.e. fresh salad, lasagne, steamed fillet with carry sauce, steamed fillet with spicy potatoes, thai meat balls, steamed fillet with tomato sauce and meat balls with bacon. The trout and chicken meals were identical in all respects other than the main ingredient being trout or chicken, and for the chicken meals, the addition of 3·5 g rapeseed oil to provide the same amount of fat per meal. The fatty acid composition of the fish and chicken fillets is shown in Table 1. The daily intake of 150 g marine trout, vegetable trout or chicken provided an amount of 3·4 g/d (2·0 and 0·9 g/d of DHA and EPA, respectively), 0·8 and 0·2 g/d

Table 1. Fatty acid composition of the fish and chicken fillets

(Mean values and standard deviations, weight percentages from three separate determinations)

	Marine trout		Vegetable trout		Chicken*	
Variables	Mean	SD	Mean	SD	Mean	SD
Total SFA (%)	25·0	0·2	15·2	0·3	20·8	1·4
Total MUFA (%)	32·0	1·0	51·8	1·0	47·2	1·8
Total PUFA (%)	35·7	1·0	30·3	0·8	32·1	2·9
Linoleic acid (C18 : 2*n*-6) (%)	3·7	0·1	15·1	0·2	22·1	1·7
Arachidonic acid (C20 : 4*n*-6) (%)	0·7	0·04	0·8	0·04	1·8	0·3
α-Linolenic acid (C18 : 3*n*-3) (%)	1·2	0·02	3·5	0·1	3·2	0·4
C18 : 4*n*-3	1·5	0·01	0·9	0·03	–	–
C20 : 4*n*-3	1·1	0·02	0·3	0·01	–	–
EPA (C20 : 5*n*-3) (%)	6·9	0·2	1·3	0·1	0·3	0·04
Docosapentaenoic acid (C22 : 5*n*-3) (%)	2·6	0·04	0·5	0·04	0·8	0·1
DHA (C22 : 6*n*-3) (%)	16·3	0·7	5·1	0·8	0·5	0·1
n-6/*n*-3 PUFA	0·16	0·01	1·48	0·12	3·33	0·25

* Values for chicken are given with the inclusion of 3·5 ml rapeseed oil per 150 g fillet.

n-3 LCPUFA, respectively. The vitamin D_3 and 25-hydroxy vitamin D_3 contents of the marine trout were 0·62 and $<0{\cdot}1$ μg/100 g, respectively, and the content of both vitamin D_3 and 25-hydroxy vitamin D_3 of the vegetable trout was $<0{\cdot}1$ μg/100 g, and that of the chicken was $<0{\cdot}1$ and 0·1 μg/100 g, respectively. The average energy and macronutrient content of trout and chicken meals were similar. Mean meal energy was 1856 kJ, and the contribution of macronutrients was protein 37 g, carbohydrate 26 g and fat 22 g. The volunteers visited the department twice a week during lunch or dinner throughout the study period (at the end of the intervention period, this was reduced to once a week for some of the subjects). At these visits, they consumed the freshly prepared daily meal (the salad meal and the steamed fillet with spicy potatoes), and received frozen meals until their next visit. The five handout meals were produced in a single batch and were frozen at −30°C until use. The volunteers were instructed to consume their habitual diets throughout the study period, but were instructed to avoid consumption of any seafood. Furthermore, the volunteers were instructed to consume one study meal per day as lunch or dinner, and to keep daily records of meal consumption and well-being in a study diary. Compliance was assessed using the study diaries, as well as using an analysis of erythrocyte (RBC) fatty acid composition.

The volunteers visited the department for two examinations during the study: at the beginning of the study (week 0) and after the intervention period (week 8). During the visits, we measured height (only at week 0), body weight, waist and hip circumference, 24 h BP, pulse wave velocity (PWV) and augmentation index (AIx). Furthermore, we collected a 12 h fasting blood sample for the assessment of fatty acid composition of RBC, plasma concentrations of TAG, HDL-cholesterol (HDL-C), LDL-cholesterol, and serum concentration of CRP, IL-6, soluble vascular cell adhesion molecule (sVCAM)-1, insulin and glucose. Blood samples were collected during the morning after 15 min supine rest (a total of 90 ml blood during the entire study). The volunteers consumed a standardised low-fat meal providing a maximum of 15 g fat the evening before the collection of blood. The volunteers kept a 3 d weighed food record for 1 week before the study and during the last week of the intervention period to estimate habitual dietary intake. Energy and macronutrient intake were calculated using the Dankost 2000 dietary assessment software (National Food Agency, Herlev, Denmark).

Laboratory measurements

The fatty acid composition and vitamin D content of the trout and chicken were determined. The meat (50 g) was minced, and 10 g (in duplicate) were used for the determination of lipid content. Muscle lipids were extracted using a chloroform–methanol mixture (1:1, v/v) according to the protocol of Bligh & Dyer[39]. The lipids were transmethylated with B trifluoride in methanolic NaOH[40]. The resulting fatty acid methyl esters were dissolved in *n*-heptane, and separated on a HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with a flame ionisation detector and an Omegawax 320 fused silica capillary column (30 m × 0·32 mm internal diameter × 0·25 μm) (Supelco, Belafonte, PA, USA). The initial oven temperature was 160°C, immediately raised by 3°C/min to 200°C, held for 1 min, further raised by 3°C/min to 220°C and held for 12 min. Peaks from 14 : 0 to 22 : 6 (*n*-3) were identified from retention times of commercial standards (Nu-Chek-Prep, Elysian, MN, USA). Each fatty acid was quantified by calculating its peak area relative to the total peak area. Vitamin D_3 and 25-hydroxy vitamin D_3 in the fish and the chicken were determined as described previously[41,42]. Briefly, the internal standards of vitamin D_2 and 25-hydroxy vitamin D_2 were added to the samples, and all vitamers were saponified with ethanolic KOH. After extraction of the unsaponifiable matter with diethyl ether–petroleum ether (1:1, v/v), the purification of the extract included a solid-phase and a preparative HPLC step with silica and amino columns. The final separation, detection and quantification of the vitamers were performed by a HPLC system (Waters, Milford, MA, USA) equipped with reversed-phase columns, diode array detector and UV detectors. The detection and quantification levels were 0·02 and 0·1 μg/100 g, respectively, for both of the vitamin D metabolites.

Fatty acid composition of RBC was determined on lipid from thawed RBC from heparinised blood samples, which were haemolysed in redistilled water and extracted by the Folch procedure[43], transmethylated with B trifluoride in methanolic NaOH, and extracted by heptane. Fatty acid methyl esters from 12 : 0 to 22 : 6 (*n*-3) were separated by GLC, as described previously[44]. The fatty acid compositions of all RBC samples were determined in duplicate, and the results were expressed as the percentage area of each fatty acid relative to fatty acid peaks together. Plasma vitamin D was measured with DiaSorin LIAISON® 25-hydroxy vitamin D TOTAL (DiaSori, Inc., Stillwater, OK, USA). The LIAISON® 25-hydroxy vitamin D TOTAL assay is a direct competitive chemiluminescence immunoassay for quantitative measurement of total 25-hydroxy vitamin D in serum or plasma[45]. We found that precision ranges (CV, CV%) were as stated by the manufacturer, within-run 3·2–8·1 % and total precision 6·9–12·7 %.

The analyses of plasma CVD risk markers were performed by standard methods. The plasma lipid profile was determined in blood drawn into a 7 ml EDTA tube (no. 367655; Becton Dickinson, Meylan Cedex, France), which was centrifuged at 2000 ***g*** for 10 min at 20°C and stored at −80°C until analysis. total cholesterol, HDL-C and TAG were measured on a Cobas Mira + analyser (Roche Diagnostic, Basel, Switzerland) with enzymatic kits from Roche (Roche Diagnostic cholesterol oxidase–*p*-aminophenazone, HDL-C-plus 2nd generation, and glycerol phosphate oxidase–*p*-aminophenazone, respectively). The LDL-cholesterol concentrations were calculated using the Friedewald formula[46]. The intra- and inter-assay CV% were 0·9 and 1·6 %, 2·6 and 4·0 %, and 0·9 and 3·2 % for total cholesterol, HDL-C and TAG, respectively. Glucose was measured in blood drawn into a 2 ml NaFI tube (Becton Dickinson no. 368520), centrifuged at 2200 ***g*** for 15 min at 4°C and stored at −80°C until analysis. Glucose was measured with the use of an enzymatic endpoint method (Hexokinase) (Gluco-quant Glucose/HK; Roche Diagnostics) using an ABX Pentra 400 chemistry analyser (ABX Pentra, Horiba ABX, Montpellier, France); intra-assay CV was 1·9 %. Blood drawn into a 10 ml tube with no additives (Becton Dickinson no. 368430) was used for the analysis of high-sensitive CRP, IL-6, sVCAM-1 and insulin. It was

centrifuged at 2200 *g* for 15 min at 4°C and stored at −80°C. CRP was measured with a chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, CA, USA) using a Pentra 400 analyser (Horiba Diagnostics). Intra- and inter-assay CV for CRP were 3·6 and 2·3 %, respectively. IL-6 and sVCAM-1 concentrations were determined using human IL-6 (Quantikine HS600; R&D Systems Europe Limited, Abingdon, UK) and sVCAM-1 (Parameter BBE3; R&D Systems Europe Limited) immunoassay kits. Intra- and inter-assay CV were 4·2 and 3·1 %, and 5·7 and 6·0 % for IL-6 and sVCAM-1, respectively. Insulin was measured on an Immulite 1000 analyser by solid-phase, two-site chemiluminescent immunometric assay (Immulite/immuliter 1000 insulin; Diagnostic Products Corporation). Intra- and inter-assay CV for insulin were 2·8 and 4·9 %, respectively. Insulin resistance was estimated using the homeostatic assessment model and calculated according to the formula[47]:

Homeostatic assessment model
= fasting plasma insulin (μU/ml)
× fasting plasma glucose (mmol/l)/22·5.

Pulse wave analysis and pulse wave velocity measurements

All the pulse wave measurements (AIx and PWV) were performed using an applanation tonometer (SPT-301B; Millar, Houston, TX, USA) and the SphygmoCor® hardware and software (version 7.1; Atcor Medical, Sydney, Australia). The brachial BP was assessed by oscillometry (UA-787 Digital BP monitor; A&D Medical, Tokyo, Japan) using the average of the last two out of three BP recordings. The method of AIx and PWV measurements has been described in detail elsewhere[48]. AIx is a surrogate measure of arterial stiffness, defined as the proportion of the central pulse pressure that is due to the late systolic peak and the reflected pulse wave. Briefly, AIx was estimated using an algorithm, the 'generalised transfer function' determined by a pulse wave analysis based on a mean of ten radial pulse wave forms and a brachial BP. Aortic PWV was determined as the difference in travel time of the pulse wave between the femoral and carotid artery. The time difference was determined by relating both signals to the start of the pulse wave determined from an electrocardiogram. The surface distance between the two recording sites was then measured, thus allowing PWV to be determined (velocity = distance/time).

Ambulatory blood pressure monitoring

All volunteers underwent 24 h ambulatory BP monitoring using the Spacelabs 90 217-15Q ambulatory BP monitor (Spacelab, Inc., Washington, DC, USA). The appropriate sized cuff was placed on the non-dominant arm, and measurements were made every 15 min between 07.00 and 23.00 hours, and every 30 min during the night. Mean BP was calculated from the readings during the entire 24 h period.

Power calculations

We used the method of least standardised difference to calculate the number of participants needed in the present study. The study was designed to demonstrate a difference in plasma TAG between the marine trout and the control (chicken) groups. The inclusion of twenty men/group gave the study enough power (90 %) to detect a significant difference ($P<0·01$) of 0·28 mmol/l (20 %) plasma TAG. This is the mean difference observed after the consumption of 0·4–4 g *n*-3 LCPUFA/d in patients with CVD according to the recent Cochrane meta-analysis[49]. The standard deviation of mean change in plasma TAG was set to 0·22 as earlier reported[50].

Statistical analysis

Data describing the characteristics of the volunteers are summarised as the means and standard deviations. Data on the outcome of the study are expressed either as means with their standard errors or as the median and 25th, 75th percentiles. Data were analysed in Statistical Analysis System 9.1 (SAS Institute©, Inc., Cary, NC, USA) using a mixed model ANOVA with treatment as fixed factors, subjects as random factor and the baseline measurements as a covariate. The covariate × treatment interaction was included to test for varying slopes/interactions. Data on total RBC *n*-3 LCPUFA, plasma TAG and serum CRP were log transformed to obtain normally distributed residuals. Differences were considered significant when $P<0·05$. We performed the bivariate associations between the changes in RBC *n*-3 LCPUFA and in outcome variables by Pearson's correlation. The following mixed model analysis initially included the baseline measurement and changes in dietary variables (total energy intake, macronutrient composition (energy from protein and fat/carbohydrate), intake of fibres and fat quality (ratio of PUFA to SFA and relative content of MUFA)). The dietary variables were excluded one by one based on their *P*-value until a final model was found that included only those dietary parameters that had a *P*-value$<0·1$ at baseline and the RBC *n*-3 LCPUFA variable.

Results

Seventy-five male volunteers were recruited for the study, and sixty-eight of these volunteers completed the study according to the protocol. One participant dropped out for personal reasons, two were dropped out due to illness during the study period, and four were dropped out because they did not like the study meals. The three groups were comparable with respect to baseline characteristics of the volunteers with no significant differences in age, anthropometric measurements, estimated energy and macronutrient intake (Table 2). In addition, there were no significant differences between the three groups in any of the baseline measurements of the outcome variables assessed in the study (data not shown). During the intervention period, body weight ($P=0·314$) or waist to hip ratio ($P=0·093$) did not differ between the three intervention groups. Furthermore, there were no significant differences between the groups in the estimated intake of energy ($P=0·151$), fat ($P=0·244$), protein ($P=0·757$), carbohydrate ($P=0·220$) and total fibre ($P=0·471$).

Compliance assessed using study diaries showed that the consumption of study meals during the intervention period was 99 %. Furthermore, changes in the fatty acid composition

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Table 2. Baseline characteristics of the men participating in each of the three diet groups
(Mean values and standard deviations)

	Marine trout (*n* 23)		Vegetable trout (*n* 23)		Chicken (*n* 22)		
Variables	Mean	SD	Mean	SD	Mean	SD	*P**
Age (years)	52	9	54	7	53	9	0·643
Height (cm)	178·5	5·6	179·0	4·3	181·9	5·7	0·076
Weight (kg)	77·4	9·1	80·1	8·0	82·6	8·3	0·130
BMI (kg/m^2)	24·2	2·3	25·0	2·4	25·0	2·1	0·455
Waist (cm)	91·5	8·0	94·0	8·6	93·0	8·0	0·593
Waist:hip ratio	0·93	0·05	0·94	0·06	0·93	0·07	0·826
Energy (MJ/d)	10·1	0·5	10·2	0·6	10·4	0·5	0·934
Fat (% of energy)	30	1	31	1	33	1	0·155
Protein (% of energy)	15	1	16	1	16	1	0·607
Carbohydrate (% of energy)	49	1	48	1	47	1	0·517
Fibre (g/d)	30	3	28	3	28	2	0·745

**P*-values for the difference between the groups (ANOVA).

of RBC from the beginning to the end of the intervention period show good agreement between the intake of *n*-3 LCPUFA and the incorporation of these fatty acids, thus indicating good compliance to the study protocol (Table 3). The men consuming the marine trout and vegetable trout had a significant higher RBC concentration of total *n*-3 LCPUFA, EPA and DHA at the end of the intervention compared with the men consuming chicken. Furthermore, the men in the marine trout intervention group had a significant higher RBC concentration of total *n*-3 LCPUFA, EPA and DHA compared with the men in the vegetable trout intervention group. There was a similar, but reciprocal, change in total *n*-6 PUFA and linoleic acid. The mean 25-hydroxy vitamin D status of the subjects at the beginning of the intervention period was 47 (SD 23) mmol/l, and the status decreased with 33 (20–44) % for the subjects, who participated in the intervention during the fall, and increased with 31 (−5–64) % for the subjects, who participated during spring. However, there were no significant differences between the intervention groups ($P=0·442$).

There were no significant differences in plasma total cholesterol, TAG, LDL-cholesterol and HDL-C concentrations between the three groups at the end of the intervention period (Table 4). The mean changes in TAG from baseline to week 8 was −0·14 (SD 0·35), −0·08 (SD 0·51) and −0·13 (SD 0·42) mmol/l for the marine trout, vegetable trout and chicken intervention groups, respectively. In order to control for the effect of changes in the carbohydrate intake during the intervention periods, changes in carbohydrate were included in the statistical model as a controlling factor, but this did not reveal any significant difference between the changes in plasma TAG concentration in the three groups ($P=0·935$). In addition, there was no significant difference in the changes in serum concentrations of CRP, IL-6, sVCAM-1, insulin and glucose or in homeostatic assessment model between the three groups during the intervention period. Furthermore, there were no significant differences in the change in PWV, AIx, heart rate, or in the systolic or diastolic BP between the three groups during the intervention period (Table 5).

The incorporation of *n*-3 LCPUFA in RBC tended to be associated with a reduction in diastolic blood pressure and an increase in CRP, most pronounced when expressed as the *n*-3 to *n*-6 PUFA ratio (Pearson *r* 0·225, $P=0·074$, *n* 64) and EPA (*r* 0·289, $P=0·019$, *n* 64), respectively. The association for BP was improved, when the model was adjusted for changes in diet. The overall model r^2 for the change in diastolic blood pressure after adjustment for change in relative intake of MUFA was 0·697 ($P=0·018$ for RBC *n*-6/*n*-3 PUFA), and

Table 3. Fatty acid composition of erythrocytes in the trout or chicken group at baseline and 8-week intervention period
(Mean values with their standard errors)

	Marine trout				Vegetable trout				Chicken				
	Week 0		Week 8		Week 0		Week 8		Week 0		Week 8		
Variables	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P**
Total SFA (%)	40·5	0·2	39·8	0·3	40·8	0·4	40·0	0·4	40·7	0·4	40·8	0·4	0·0682
Total MUFA (%)	19·9	0·3	18·9	0·3	19·3	0·3	19·0	0·3	19·8	0·2	19·8	0·4	0·0696
Total PUFA (*n*-6) (%)	28·1	0·5	25·7^c	0·4	27·5	0·3	27·6^b	0·4	28·1	0·6	29·1^a	0·4	<0·0001
Linoleic acid (C18 : 2*n*-6) (%)	12·5	0·3	10·9^c	0·3	12·2	0·4	11·5^b	0·3	12·0	0·4	12·5^a	0·3	<0·0001
AA (C20 : 4*n*-6) (%)	10·8	0·3	10·8	0·3	10·8	0·3	11·4	0·4	11·5	0·4	11·7	0·4	0·2716
Total PUFA (*n*-3) (%)	9·3	0·6	13·7^a	0·6	9·2	0·3	10·2^b	0·3	9·5	0·5	8·3^c	0·4	<0·0001
EPA (C20 : 5*n*-3) (%)	0·9	0·1	2·5^a	0·2	1·0	0·1	1·1^b	0·1	1·1	0·2	0·7^c	0·1	<0·0001
DHA (C22 : 6*n*-3) (%)	4·2	0·2	7·0^a	0·2	4·6	0·2	5·8^b	0·2	5·0	0·3	4·2^c	0·3	<0·0001

AA, arachidonic acid.
^{a,b,c} Mean values with unlike superscript letters differ for *n*-3 PUFA with $P<0·0001$ and for *n*-6 PUFA with $P<0·01$.
**P*-values for the treatment effect analysed using a mixed model ANOVA.

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Table 4. Plasma lipid concentrations and serum concentrations of circulation markers of inflammation of the trout or chicken group at the beginning and end of the intervention period

(Mean values with their standard errors)

	Marine trout				Vegetable trout				Chicken				
	Week 0		Week 8		Week 0		Week 8		Week 0		Week 8		
Variables	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P**
TAG (mmol/l)	1·02	0·08	0·88	0·09	1·28	0·13	1·20	0·18	1·11	0·11	0·99	0·10	0·702
TC (mmol/l)	4·74	0·17	5·06	0·23	5·05	0·19	5·18	0·25	4·67	0·13	4·74	0·13	0·475
LDL-C (mmol/l)	2·89	0·15	3·05	0·19	3·17	0·17	3·17	0·20	2·83	0·09	2·93	0·13	0·565
HDL-C (mmol/l)	1·20	0·05	1·23	0·04	1·15	0·04	1·19	0·05	1·18	0·05	1·15	0·22	0·296
Glucose (mmol/l)	5·62	0·35	5·66	0·43	5·65	0·47	5·68	0·47	5·64	0·60	5·61	0·53	0·826
Insulin (pmol/l)	32·9	18·0	39·7	19·9	35·6	20·9	36·4	16·8	34·4	16·3	37·7	19·1	0·261
HOMA-IR	1·39	0·80	1·68	0·88	1·54	1·01	1·56	0·78	1·47	0·80	1·60	0·92	0·312
CRP (mg/l)	0·68	0·22	1·23	0·41	0·87	0·29	0·95	0·22	0·53	0·11	0·68	0·14	0·482
IL-6 (ng/l)	1·10	0·12	1·17	0·17	1·14	0·14	1·28	0·25	1·24	0·16	1·33	0·20	0·430
sVCAM-1 (ng/ml)	644	38	637	37	707	41	707	40	647	28	630	28	0·437

TC, total cholesterol; LDL-C, LDL cholesterol: HDL-C, HDL cholesterol; HOMA-IR, homeostatic model assessment-insulin resistance; CRP, C-reactive protein, sVCAM-1, soluble vascular cell adhesion molecule-1.

**P*-values for the treatment effect analysed using a mixed model ANOVA.

the r^2 for systolic blood pressure after adjustment for change in relative intake of dietary fibre and protein was 0·595 ($P=0·037$ for RBC *n*-6/*n*-3 PUFA, both *n* 64).

Discussion

The results from the present study showed a significantly higher increase in RBC *n*-3 LCPUFA status following the intake of traditionally marine-fed trout compared with a daily intake of trout raised on vegetable-based feed after 2 months. These differences reflected differences in the *n*-3 LCPUFA content of the trout meat. However, the intake of both types of trout resulted in a significant increase in the RBC *n*-3 LCPUFA concentrations compared with the intake of chicken or baseline values. In contrast to what we expected, the intake of marine trout was not associated with any reduction in plasma TAG or clear beneficial effects in any of the other CVD risk markers that we assessed – although some trends were observed for CRP and BP. It is therefore not possible to make any firm conclusion with respect to the effect of the aquacultural feed regime on the health-promoting effect of the fish from the results of the present study.

Some previous studies have reported beneficial effects of fish interventions on classical CVD risk markers such as plasma TAG, HDL-C and BP, in healthy lean and overweight subjects[24–27,51,52]. Some of these studies compared the effect of fish with no intervention or the effect of vegetable oil capsules[25,51,52]. Some made comparisons between fatty and lean fish[27,52], and only two of the studies made a comparison with a meat diet[24,26]. The results from the trials are difficult to compare as the effects will depend on health status of the included subjects, dose, duration and *n*-3 LCPUFA composition of the fish. The subjects in the Moore study varied in weight loss across groups, which made the evaluation of the results difficult. The most well-controlled studies appear to be the ones by Lindqvist *et al.*[26] and Gunnarsdottir *et al.*[52], who tried to keep a constant diet and macronutrient composition. The length of their studies were comparable to that of the present study, but they both had more subjects per group and supplied only about half the dose of *n*-3 LCPUFA (as herring or salmon, respectively) as we did in our marine trout group, and their subjects were somewhat younger but overweight. None of these two studies found any marked differences in plasma lipid profile or BP between the fatty fish diets and their pork/chicken or cod diet.

Table 5. Pulse wave velocity (PWV), pulse wave analysis, heart rate (HR) and blood pressure of the trout or chicken group at the beginning and end of the intervention period

(Mean values with their standard errors)

	Marine trout				Vegetable trout				Chicken				
	Week 0		Week 8		Week 0		Week 8		Week 0		Week 8		
Variables	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P**
PWV (m/s)	7·52	0·19	7·57	0·19	7·62	0·26	7·51	0·18	7·37	0·26	7·50	0·24	0·682
AIx (%)	19·52	2·04	19·00	2·22	19·48	2·24	19·46	2·28	19·00	2·52	17·23	2·45	0·575
AIx75 (%)	9·74	2·06	9·76	2·20	10·54	2·43	10·04	2·72	8·73	2·64	7·95	2·42	0·872
HR (beats per minute)	55	1	56	2	54	2	55	2	54	1	56	1	0·576
BPsys (mmHg)	121	2	119	2	123	2	121	2	123	2	123	2	0·197
BPdia (mmHg)	77	1	75	1	77	2	76	1	77	2	78	2	0·155

AIx, augmentation index; BPsys, systolic blood pressure; BPdia, diastolic blood pressure.

**P*-values for the treatment effect analysed using a mixed model ANOVA.

Randomised interventions with *n*-3 LCPUFA from fish oil show a consistent reduction in plasma TAG[4]. A first choice of possible explanatory factors for the missing effect of *n*-3 LCPUFA from marine trout on plasma TAG in the present study would be the dose and specific type of *n*-3 LCPUFA, the healthiness of the subjects and the power. However, we have previously reported a significant reduction in plasma TAG after a similar *n*-3 LCPUFA dose in a study with sixty-six healthy young men[10]. Furthermore, a number of studies have shown that *n*-3 LCPUFA are incorporated just as effective from fish as from fish oil provided that intake is similar both in dose of *n*-3 LCPUFA and in frequency[53,54]. The power would in theory have been greater in a crossover design like the studies by Lindquist *et al.*[26] and Lara *et al.*[51], but the risk of carryover effects in studies with *n*-3 LCPUFA would be a problem in such a design. Our study was based on a power calculation, but the recruited subjects were more heterogeneous than expected. The observed SD on the mean change in TAG during the intervention was 0·43 mmol/l compared with 0·22 mmol/l, which was what our power calculation was based on. Another plausible explanation could be the choice of chicken as control, since chicken had a relatively high content of PUFA. The present results were also in agreement with the not so marked effects in the Lindqvist *et al.*[26] study that used chicken/pork as control. Furthermore, the Danish chicken appears to have a high content of *n*-3 PUFA, which resulted in an intake of 0·2 g/d of *n*-3 LCPUFA. With respect to the clinical effect of *n*-3 LCPUFA on CVD mortality, this appears to reach a maximum at an intake of about 0·3 g/d[55]. Furthermore, there may have been differences in the diet of the groups that we did not pick up with our dietary registrations, possibly a difference in the intake of carbohydrate that could potentially counterbalance the effect of *n*-3 LCPUFA on plasma TAG, as a high carbohydrate intake has been shown to increase plasma TAG[56]. Our statistical analysis did not indicate that this would be the case, and the dietary data did not indicate any such differences between the groups. However, such dietary data may not be accurate enough, and a full dietary intervention is needed in order to avoid unintended dietary changes that could confound the effects. Many previous studies have shown a beneficial effect on BP, which we also could not confirm. However, the BP-lowering effect is most pronounced in elderly hypertensive subjects[5]. We excluded the subjects who had a treatment demanding hypertension, therefore our subjects were all more or less normotensive, but all were elderly, and their CVD risk profile reflects their age (BMI was a bit on the overweight side, total cholesterol was about 5 mmol/l, glucose was also about 5 mmol/l and homeostatic model assessment was well above 1). Furthermore, we used 24 h ambulatory BP determinations, which provide a more sensitive and accurate measurement compared with the oscillometrically determined BP. The randomisation process did not result in any significant differences between the three groups at baseline, but there was an unfortunate tendency towards a more healthy CVD risk profile in the marine trout group that to some extent could have modified the effect of the *n*-3 LCPUFA intake.

Since no effect of marine-fed trout was detected, it is not possible to draw any clear conclusions as to the effect of the aquacultural feed. We did not expect in our power calculation to see a significant difference between the two trout groups, but rather expected a dose–response in the reduction in plasma TAG, chicken, vegetable-fed trout, marine-fed trout groups. In accordance with our hypothesis, there was a significant difference in the RBC *n*-3 LCPUFA content between the two trout groups. A number of controlled studies have shown that increased consumption of fish with a high *n*-3 LCPUFA content reduces serum TAG[24,28,30,57], whereas other studies that used fish species with a low content of *n*-3 LCPUFA have reported no changes in TAG[30,58]. Such results support the hypothesis that the *n*-3 LCPUFA content of fish is a major determinant of the health effects, which was also indicated in the present study by significant or near significant associations between the changes in RBC *n*-3 LCPUFA and BP plus CRP in the present study. Like the present study, a recent study showed that changing the feed from fish oil to rapeseed oil in salmon fish farming produced fillets with a lower content of *n*-3 LCPUFA[28]. In that study, CVD patients consuming the tailor-made salmon fillets with the high content of *n*-3 LCPUFA of marine origin had lower concentration of CVD risk markers such as serum TAG, sVCAM-1 and IL-6 when compared with the patients eating the salmon fillets with the low content of *n*-3 LCPUFA raised on vegetable feed[28]. The present study and other studies[59] indicate that fish and *n*-3 LCPUFA affect also novel inflammation-related parameters. Again, since no effect of the marine-fed trout was observed on the classical CVD risk markers in the present study, we cannot draw any conclusion from the present results with respect to the lack of effect on the novel CVD risk markers. Some studies have observed beneficial effects of *n*-3 LCPUFA on inflammation-related clinical diseases in the absence of significant effects on immune markers of inflammation[59].

To our surprise, the changes in vitamin D status in the volunteers did not differ significantly between the three dietary groups. However, the marine-fed trout in our study had < 1 μg vitamin D/100 g, which was only slightly higher than in trouts raised on vegetable feed and the chicken. This could be due to low vitamin D content in the feed, as observed previously in salmons fed a low vitamin D feed as first feeding, but not so if the vitamin D-deficient feed was fed for the last 4 months of breeding[60,61]. The vitamin D content of the feeds was not measured in the present study, but similar BioMAR feeds have previously been shown to contain the recommended amounts of vitamin D for trouts (J Holm, unpublished results). However, other studies have previously found that farmed salmon only contained 25 % of vitamin D compared with wild salmon[62]. The reason for the difference in vitamin D in farmed and wild fish is unknown. Trout is regarded as a fatty fish and as such should contain 7–8 μg vitamin D/100 g[63]. Intake of trout with this level of vitamin D would give an intake of approximately 12 μg vitamin D, which would be expected to result in an increase in vitamin D status of 24 nmol/l[64]. This again should have prevented the decrease in vitamin D status during the fall and should have given rise to a more pronounced increase during spring. With the increasing amounts of farmed fish on the market, it is essential to investigate in more detail why farmed fish have lower contents of vitamin D and how this may be increased, as it might otherwise have effects on human health.

Conclusion

In this fish intervention study, we could not confirm the previous results, mainly from fish oil intervention trials, which have shown a beneficial effect of an increased *n*-3 LCPUFA intake on CVD risk markers. This may be due to a more heterogeneous response in diet interventions compared with pharmacological interventions with oil capsules, lack of power or the health profile of the subjects. Since no significant differences were found on CVD risk markers between the diet groups, we could not distinguish between the health effects of a traditionally grown trout and that of a trout raised on vegetable feed. However, the vegetable feed resulted in a decrease in the *n*-3 LCPUFA content of both the trout itself and the subjects who consumed the trout, which would indicate potentially less health effect. Thus, in this respect, the trout raised on vegetable feed may be equivalent to a lean type of fish.

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