# No effect of salmon fish protein on 2-h glucose in adults with increased risk of type 2 diabetes: a randomised controlled trial

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## Abstract

The association between fish consumption and decreased risk of CVD is well documented. However, studies on health effects of fish consumption suggest that other components than *n*-3 PUFA have beneficial cardiometabolic effects, including effects on glucose metabolism. The aim of the present study was to investigate effects of salmon fish protein on cardiometabolic risk markers in a double-blind, randomised controlled parallel trial. We hypothesised that daily intake of a salmon fish protein supplement for 8 weeks would improve glucose tolerance in persons with increased risk of type 2 diabetes mellitus (T2DM). Our primary outcome measure was serum glucose (s-glucose) 2 h after a standardised oral glucose tolerance test. In total, eighty-eight adults with elevated s-glucose levels were randomised to 7·5 g of salmon fish protein/d or placebo, and seventy-four participants were included in the analysis. We found no significant effect of salmon fish protein supplementation on our primary outcome or other markers related to glucose tolerance, serum lipids, weight or blood pressure compared with placebo. The present study does not support the hypothesis that daily intake of a salmon fish protein supplement for 8 weeks improves glucose tolerance in persons with increased risk of T2DM.

**Key words: Glucose metabolism: Salmon protein: Prediabetes: Randomised controlled trials**

Type 2 diabetes mellitus (T2DM) is a considerable contributor to the global burden of disease[1]. In 2019, worldwide prevalence of T2DM among adults was estimated to 8·4 %, where only half being diagnosed. In addition, 7·5 % of the adult population were estimated to have impaired glucose tolerance[2].

There is a strong correlation between diabetes and CVD[3], which is the number one cause of death globally[4] and a major cause of mortality in people with diabetes[5]. Adults with diabetes has a two-three times increased risk of CVD, and CVD events generally occur at an earlier age in people with diabetes than people without diabetes[5]. A healthy diet is important to prevent CVD and T2DM,[6] and the association between fish consumption and decreased risk of CVD is well documented[7–9]. The beneficial effects of fish consumption have largely been attributed to marine *n*-3 PUFA present in fatty fish[10]. However, studies on health effects of lean fish consumption suggest that other components than *n*-3 PUFA have beneficial cardiometabolic effects[11–14].

Both lean and fatty fish contain other potential health-promoting components such as taurine, vitamin D, vitamin B$_{12}$, iodine, Se[15] and more unspecified components such as bioactive peptides[16].

Peptides with a specific amino acid sequence, and with known bioactivity, have been isolated from by-products from lean and fatty fish[17,18]. *In vitro* and animal studies have suggested that fish protein has beneficial effects on, for example, cardiometabolic markers, including markers related to blood glucose metabolism[16,19–21]. Fish protein peptides are formed during digestion or from enzymatically treatment, and it has been hypothesised that peptides may act locally in the gut or peripherally[16,21]. Animal studies have shown improved post-prandial glucose regulation, albeit higher weight gain[21], resistance to high-fat-diet-induced obesity[22,23], and reduced plasma lipids such as TAG and total cholesterol[22,24] when fed a diet rich in hydrolysed salmon protein. Clinical trials with fish protein given

as supplements, mainly from lean fish, have suggested beneficial cardiometabolic effects[25–30], including improved glucose metabolism[29]. However, clinical trials in humans with protein supplements derived from fatty fish are sparse, and the studies are small and with inconsistent results on cardiometabolic risk markers[26,27,31–33].

The overall aim of the FishMeal human intervention study was to investigate effects of salmon fish protein on cardiometabolic risk markers. We hypothesised that daily intake of a salmon fish protein supplement for 8 weeks would improve glucose tolerance in persons with increased risk of T2DM. Our primary outcome was changes from baseline in serum glucose (s-glucose) measured after a 2-h oral glucose tolerance test (2-h OGTT). Secondary outcomes were changes from baseline in other markers related to glucose tolerance: fasting s-glucose, fasting serum insulin (s-insulin), 2-h OGTT-s-insulin, homeostatic model assessment of insulin resistance (HOMA-IR) and HbA1c. Other pre-specified outcomes were changes from baseline in body weight and markers related to lipid metabolism: TAG and total, LDL- and HDL-cholesterol.

## Methods

### Participants

The study was conducted at the University of Oslo, Norway, from August 2018 to September 2019. We recruited participants through advertisements in social media and medical practices at the University of Oslo. The text in the advertisement was directed at people at risk of T2DM. After a telephone interview, we invited eligible participants to a screening visit to further check eligibility criteria. Inclusion criteria were ≥20 years of age and elevated blood glucose defined as either fasting s-glucose ≥ 5·6 mmol/l, 2-h OGTT-s-glucose ≥ 6·5 mmol/l or HbA1c ≥40 mmol/mol (≥5·8 %). Exclusion criteria were diabetes (defined as fasting s-glucose ≥ 7·0 mmol/l, 2-h OGTT-s-glucose ≥ 11·1 mmol/l or HbA1c ≥40 mmol/mol (≥5·8 %)), high fish/seafood intake (>450 g/week), fish or shellfish allergy and age-related elevated blood pressure (≥70 years: ≥180/110 mmHg, >40–70< years: ≥170/100 mmHg and ≤40 years: ≥160/100 mmHg). Further exclusion criteria were use of prescription drugs related to diabetes, inflammation or systemic use of corticosteroids, or unstable use (defined as change of dose during the last 3 months) of lipid-lowering drugs, thyroxine, blood pressure-lowering drugs and drugs affecting appetite. In addition, we excluded participants with unstable use (defined as change of dose during the last month) of dietary supplements including $n$-3 PUFA, daily use of protein supplement powder and participants who were pregnant, breast-feeding or planning pregnancy. Furthermore, all participants had to have a stable body weight (defined as ±5 %) during the last 3 months and not be planning changes in body weight during the intervention period.

### Ethics

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. All participants gave their written informed consent, and the Regional Ethics Committee for Medical Research in South-East Norway approved the study. The

study was registered at ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT03764423). The post-prandial 'Fish protein Ex Vivo' study, assessing uptake of the study product, was registered as a separate study (ClinicalTrials.gov Identifier: NCT04078958).

### Study design

We conducted an 8-week double-blind, randomised controlled parallel study. Before the baseline visit, all participants performed a 2–4-week washout period where intake of fish and seafood were reduced to a maximum of one serving (150 g) per week. We instructed participants in both groups to consume ten capsules together with a meal three times per d for 8 weeks (in total thirty capsules per d). All participants were advised to maintain their usual lifestyle habits throughout the study, without changing their physical activity and dietary habits including supplement use, except for a reduction in fish and seafood intake to a maximum of one serving (150 g) per week. Clinical and blood laboratory assessments were performed at baseline and after 8 weeks of follow-up. In addition, the participants came to the study centre after 4 weeks of follow-up to receive more of the study product. We sent a text message 2–3 d before all visits as a reminder of how to prepare for the upcoming visit.

### Blinding and randomisation

Participants were stratified by sex (male and female) and age (<50 years, ≥50 years) prior to a block randomisation with use of an external statistician (Health Services Research Unit (HØKH), Akershus University Hospital, Lørenskog, Norway and Faculty Division Akershus University Hospital, University of Oslo, Blindern, Oslo, Norway). The randomisation allocations, selected consecutively, were sent to the product packaging personnel on demand, according to strata information of newly recruited participants.

To ease the management for the participants, capsules were packed in blister sheets (thirty capsules per sheet) and delivered in boxes (seven sheets per box). Boxes (fish protein and placebo) were identical in appearance and were only identifiable by numbers on the containers. The study was double-blinded, as neither the participants, the study investigator collecting data nor the outcome adjudicators knew which group the participants were assigned to. The randomisation code was concealed from the study investigators until the statistical analyses were completed.

### Study product

The experimental group received capsules containing salmon fish protein (250 mg/capsule), microcrystalline cellulose (240 mg/capsule), antioxidants (tocopherols and rosemary extract) and excipients (magnesium stearate: 5 mg/capsule, tricalsiumphosphate: 5 mg/capsule and silisiumdioxide: 2·5 mg/capsule). The placebo group received capsules containing microcrystalline cellulose (250 mg/capsule) and antioxidants and excipients similar to the fish protein capsules, but without amino acids. The salmon fish protein contained 69·7 g of protein and 13·2 g of fat/100 g. Table 1 shows the amino acid composition and main groups of fatty acids of the salmon fish protein used in the present study. In the fish protein group, the daily

**Table 1.** Characterisation of the encapsulated salmon fish protein

|  | g/100 g | mg/daily dose |
|---|---|---|
| Crude fat | 13·2 | 990 |
| Fatty acids |  |  |
| SFA | 2·3 | 173 |
| MUFA | 5·4 | 405 |
| PUFA | 5·1 | 383 |
| *n*-3 Fatty acids | 3·2 | 240 |
| EPA (20 : 5*n*-3) | 0·6 | 45 |
| DHA (22 : 5*n*-3) | 1·4 | 105 |
| Crude protein | 69·7 | 5228 |
| Amino acid profile |  |  |
| Alanine | 3·98 | 299 |
| Arginine | 4·09 | 307 |
| Aspartic acid | 6·04 | 453 |
| Cysteine + cystine | 0·78 | 59 |
| Glutamic acid | 7·86 | 590 |
| Glycine | 5·18 | 389 |
| Hydroxyproline | 0·89 | 67 |
| Ornithine | <0·05 | 0 |
| Proline | 3·36 | 252 |
| Serine | 2·97 | 223 |
| Taurine | 0·72 | 54 |
| Tyrosine | 2·19 | 164 |
| Essential amino acids |  |  |
| Histidine | 1·57 | 118 |
| Isoleucine | 2·59 | 194 |
| Leucine | 4·65 | 349 |
| Lysine | 4·73 | 355 |
| Methionine | 1·84 | 138 |
| Phenylalanine | 2·69 | 202 |
| Threonine | 2·91 | 218 |
| Tryptophan | 0·82 | 61 |
| Valine | 3·25 | 244 |

dosage of capsules provided 7·5 g of salmon fish protein, corresponding to a total of 5·2 g of salmon protein. Mowi ASA supplied the salmon fish protein and Optipharma AS produced the capsules in transparent bovine gelatine capsule shells (96 mg of gelatine/capsule) (ACG Europe Ltd). Before and after encapsulation, the fish protein and capsules were stored at 5·5°C, and participants were instructed to store the capsule containers at 4°C during the intervention period. Before encapsulation, and regularly during the intervention period, the content of unwanted micro-organisms (histamine, aerobe micro-organisms, *Escherichia coli*, and *Salmonella*) were analysed in the fish protein. Before encapsulation, we also analysed the content of contaminants (Eurofins Food & Feed Testing Norway AS). We did not detect any increase in unwanted micro-organisms in the fish protein during the intervention.

## Uptake of study product

To investigate whether fish protein was taken up into the circulation, we performed a post-prandial analysis of serum amino acids 1 h after intake in five healthy participants. In short, five healthy, male participants were recruited from the University of Oslo from October to November 2019. Inclusion criteria were >20 years of age and BMI between 18·5 and 24·9 kg/m². Exclusion criteria were known diabetes, elevated blood pressure, pregnancy, breast-feeding or allergy/intolerance to fish. Participants arrived fasting on the morning of the post-prandial test and consumed thirty capsules containing a total of 5·2 g of salmon protein (the same amount as the daily dose in the present study) with 0·5 litres of water. Blood samples were taken at fasting and 60 min after capsule intake. Participants were not allowed to consume dietary supplements or fish the day before the test.

Serum amino acid concentrations were measured by HPLC–tandem MS (HPLC-MS/MS), as previously described[34]. Chromatographic separation was performed on a Phenomenex Kinetex Core Shell C18 system (100 × 4·6 mm, 2·6 μm), with an aqueous solution of formic acid (0·5 %) and heptafluorobutyric acid (0·3 %), and acetonitrile. Linear calibration curves of the peak area ratios of analytes and internal standards were used for quantification.

## Compliance

The participants received boxes with capsules at baseline and after 4 weeks of follow-up and were instructed to deliver all blister sheets, both empty and full, at the end-of-study visit. Compliance was assessed by capsule count. The number of capsules consumed during the intervention period were counted and divided by the number of capsules scheduled for the intervention period[35]. Participants with compliance less than 70 % would be excluded from the analysis.

## Blood sampling and standard laboratory analysis

Participants were instructed to avoid consumption of alcohol and doing vigorous physical activity the day before blood sampling. Venous blood samples were drawn after an overnight fast (≥10 h). Serum was obtained from silica gel tubes (Becton, Dickinson and Company) and kept at room temperature for >30–60< min, until centrifugation (1500 ***g***, 15 min). Plasma was obtained from $K_2$EDTA tubes (Becton, Dickinson and Company), immediately placed on ice, and centrifuged within 10 min (2000 ***g***, 4°C, 15 min). Lithium-heparin tubes (Becton, Dickinson and Company) and $K_2$EDTA tubes with whole blood were kept at room temperature. Serum and plasma concentrations of fasting glucose, insulin, HbA1c, TAG, total cholesterol, LDL-cholesterol, HDL-cholesterol, high-sensitive C-reactive protein, creatinine, estimated glomerular filtration rate, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase and Hg, and glucose and insulin after a 2-h OGTT were measured by standard methods at an accredited routine laboratory (Fürst Medical Laboratory).

## Oral glucose tolerance test

An OGTT was conducted at baseline and at the end-of-study visit. Venous blood samples were drawn, and within 10 min, the participants were instructed to drink a 75-g anhydrated glucose drink (Esteriplas) in less than 5 min. Participants were instructed to remain fasting, remain in the waiting room and refrain from any activity until the post-prandial blood samples were drawn 120 min after finishing the glucose drink.

## Clinical assessment

We measured body weight on a digital scale (Seca GmbH) in light clothing without shoes and height with a stadiometer (Seca GmbH). Blood pressure was measured by a Carecape

V100 monitor (GE Healthcare) in a sitting position, on the non-dominant arm after a 10 min rest. We obtained three measurements with a 1-min interval, and calculated the average value of the last two measurements.

## Dietary assessment

Habitual dietary intake was assessed prior to the intervention through a semi-quantitative FFQ designed to capture dietary habits during the last year[36]. The FFQ included questions about intake of 270 food items, including six questions about cold cuts and spreads made of fish and twelve questions about fish eaten for dinner. The options for frequency of consumption ranged from several times per d to once a month, with options for portion sizes based on household units such as slices, pieces and spoons. The same FFQ was used to assess the participants' diets during the 8-week intervention.

## Statistical analysis

Power calculations estimated that 120 participants (including a 20 % dropout rate) were required to obtain 80 % of power with a type I error of 5 % to detect a clinically relevant difference between the two groups of 0·4 (sd 0·7) mmol/l in changes from baseline in 2-h OGTT-s-glucose. Descriptive data are presented as means and standard deviations or medians and quartiles (Q1–Q3) for continuous variables or as frequencies and percentages for categorical variables. We used paired $t$ tests to evaluate differences in energy and nutrient intake between the groups. Differences between the groups in primary, secondary and other pre-specified outcomes were tested with a linear regression model (outcome variable ~ intervention group + outcome variable at baseline), hereafter called crude model. We performed the same analysis adjusting for strata (age and sex) and weight change in addition to the outcome variable at baseline (outcome variable ~ intervention group + outcome variable at baseline + age + sex + weight change), hereafter called the adjusted model. Skewed variables (fasting s-insulin, 2-h OGTT-s-insulin, HOMA-IR, TAG and weight) were log-transformed before analysis. Results from the regression analysis are presented as B-coefficients with 95 % CI or logB-coefficients with 95 % CI for skewed variables. $P < 0·05$ was considered significant. The models were checked for independence and normality of the residuals. Statistical analyses were performed in Stata/MP 16.1 (StataCorp LLC)[37].

## Results

In total, 717 participants were assessed for eligibility, eighty-eight were randomly assigned, eighty-three received allocated interventions and seven were lost to follow-up. Thus, seventy-six participants completed the study. All participants had a capsule compliance >70 %. Two participants were non-compliant with the approved study protocol and were not included in the statistical analysis (Fig. 1). Baseline characteristics of the
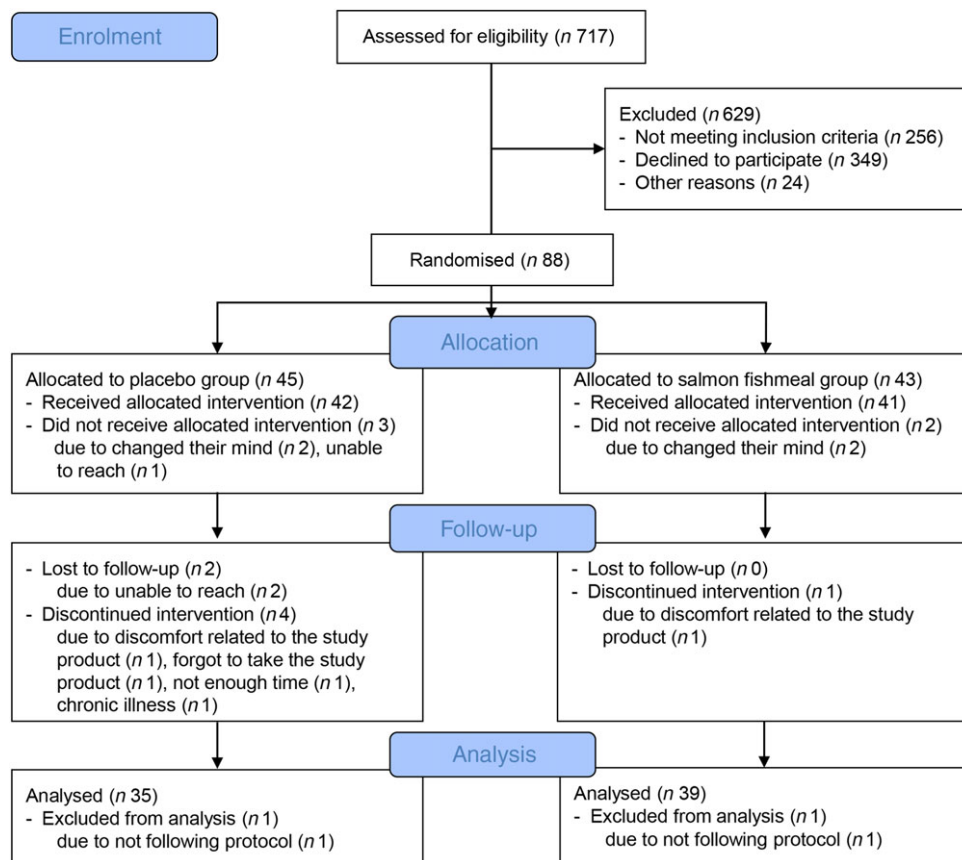


**Fig. 1.** Flow diagram of the study participants.

**Table 2.** Subject characteristics at baseline
(Mean values and standard deviations; median values and quartiles (Q1–Q3); frequencies and percentages)

| | Fish protein (*n* 39) | | Placebo (*n* 35) | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| Descriptives | | | | |
| Age (years) | 54·5 | 10·2 | 56·7 | 11·0 |
| Sex, female | | | | |
| *n* | 24 | | 23 | |
| % | 61·5 | | 65·7 | |
| BMI (kg/m$^2$) | 34·0 | 5·2 | 32·9 | 3·9 |
| Daily tobacco use | | | | |
| *n* | 3 | | 8 | |
| % | 7·7 | | 22·9 | |
| CVD history* | | | | |
| *n* | 0 | | 1 | |
| % | 0 | | 2·9 | |
| Lipid-lowering drug use | | | | |
| *n* | 9 | | 7 | |
| % | 23·1 | | 20·0 | |
| Blood pressure-lowering drug use | | | | |
| *n* | 12 | | 11 | |
| % | 30·8 | | 31·4 | |
| Blood biochemistry | | | | |
| hsCRP (mg/l) | | | | |
| Median | 3·4 | | 3·3 | |
| Q1–Q3 | 1·8–6·0 | | 2·2–6·0 | |
| Creatinine (μmol/l) | 65 | 12 | 64 | 11 |
| eGFR (ml/min per 1·73 m$^2$) | 97 | 13 | 97 | 13 |
| ASAT (U/l) | 25 | 7 | 24 | 6 |
| ALAT (U/l) | | | | |
| Median | 28 | | 29 | |
| Q1–Q3 | 21–44 | | 23–38 | |
| γ-GT (U/l) | | | | |
| Median | 29 | | 31 | |
| Q1–Q3 | 20–55 | | 20–41 | |
| Hg (nmol/l) | | | | |
| Median | 6 | | 7 | |
| Q1–Q3 | 5–8 | | 5–10 | |

hsCRP, high-sensitive C-reactive protein; eGFR, estimated glomerular filtration rate; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; γ-GT, γ-glutamyl transferase.
* CVD history includes heart attack and angina.

seventy-four participants included in the present study are shown in Table 2. The participants were 56 (Q1–Q3 48–64) years of age, with a mean BMI of 33·5 (SD 4·7) kg/m$^2$, and 64 % were female.

### Primary and secondary outcomes

At baseline, fasting s-glucose was 5·4 (SD 0·5) mmol/l in the fish protein group and 5·7 (SD 0·6) mmol/l in the placebo group, and 2-h OGTT-s-glucose was 5·8 (SD 1·4) mmol/l in the fish protein group and 6·3 (SD 1·5) mmol/l in the placebo group. During the intervention period, we found no statistically significant differences on fasting s-glucose, 2-h OGTT-s-glucose, fasting s-insulin, HOMA-IR or HbA1c, whereas 2-h OGTT-s-insulin was significantly increased in the crude model (logB 0·23 (95 % CI 0·01, 0·45), $P < 0.05$). Results on primary and secondary outcomes using both the crude and the adjusted model are shown in Table 3.

### Other pre-specified outcomes

During the intervention, no significant difference were found for total cholesterol, LDL-cholesterol, HDL-cholesterol and TAG between the groups (Table 4). Median weight increase was 1·0 (Q1–Q3 −0·2 to 2·0) kg in the fish protein group and 0·4 (Q1–Q3 −0·8 to 1·3) kg in the placebo group ($P = 0.08$).

### Energy and macronutrient intake

At baseline, median daily energy intake was 9295 (Q1–Q3 7931–11 760) kJ/d in the fish protein group and 9257 (Q1–Q3 7931–10 618) kJ/d in the placebo group. There were no significant changes in the macronutrient, sugar, fibre and energy intake between the groups during the intervention period (Table 5). The fish protein group reported a reduction in energy intake of 559 (Q1–Q3 −1278 to 462) kJ/d and the placebo group reported a reduction of 971 (Q1–Q3 −2828 to 417) kJ/d ($P = 0.24$). Contribution of energy and macronutrients from the study products are not included in the analysis of dietary data.

### Systolic and diastolic blood pressure

We also measured systolic blood pressure and diastolic blood pressure. In both groups, 31 % of the participants

**Table 3.** Primary and secondary outcomes*
(Mean values and standard deviations; median values and quartiles (Q1–Q3); B-coefficients and 95 % confidence intervals)

| | | Fish protein (n 39) | | | | Placebo (n 35) | | | | Linear regression change in the fish protein group relative to the placebo group | | | | | |
| | | Baseline | | Change | | Baseline | | Change | | Crude values | | Adjusted values | | | |
| | n | Mean | SD | Mean | SD | Mean | SD | Mean | SD | B | 95 % CI | B | 95 % CI | P† | P‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fasting s-glucose (mmol/l) | 73 | 5·4 | 0·5 | 0·2 | 0·4 | 5·7 | 0·6 | 0·0 | 0·4 | 0·08 | −0·10, 0·25 | 0·02 | −0·16, 0·20 | 0·37 | 0·80 |
| 2-h OGTT-s-glucose (mmol/l) | 72 | 5·8 | 1·4 | 0·5 | 1·6 | 6·3 | 1·5 | −0·4 | 1·5 | 0·61 | −0·06, 1·27 | 0·48 | −0·21, 1·16 | 0·07 | 0·17 |
| Fasting s-insulin (pmol/l) | 73 | | | | | | | | | 0·06 | −0·09, 0·21 | 0·05 | −0·10, 0·21 | 0·42 | 0·52 |
| Median | | 94 | | −1 | | 110 | | −7 | | | | | | | |
| Q1–Q3 | | 64–140 | | −18 to 19 | | 63–153 | | −15 to 11 | | | | | | | |
| 2-h OGTT-s-insulin (pmol/l) | 73 | | | | | | | | | 0·23 | 0·01, 0·45 | 0·21 | −0·02, 0·44 | 0·04 | 0·07 |
| Median | | 512 | | 33 | | 566 | | −29 | | | | | | | |
| Q1–Q3 | | 196–726 | | −58 to 250 | | 306–710 | | −218 to 87 | | | | | | | |
| HOMA-IR | 73 | | | | | | | | | 0·09 | −0·08, 0·26 | 0·07 | −0·11, 0·24 | 0·31 | 0·43 |
| Median | | 3·86 | | 0·02 | | 4·46 | | −0·3 | | | | | | | |
| Q1–Q3 | | 2·4–5·6 | | −0·5 to 1·0 | | 3·0–5·9 | | −0·7 to 0·6 | | | | | | | |
| HbA1c (mmol/mol) | 73 | 41 | 3·4 | 0 | −1 to 2 | 40 | 3·3 | 0 | −1 to 1 | 0·01 | −0·84, 0·87 | −0·22 | −1·09, 0·64 | 0·98 | 0·61 |

s-glucose, Serum glucose; OGTT, oral glucose tolerance test; s-insulin, serum insulin; HOMA-IR, homeostatic model assessment of insulin resistance.
* Differences between the groups in primary and secondary outcomes were tested with a linear regression model. Skewed variables (fasting s-insulin, 2-h OGTT-s-insulin and HOMA-IR) were log-transformed before analysis. The regression coefficient expresses the mean difference between the groups. A negative regression coefficient in this table represents a reduction in the fish protein group compared with the placebo group, and a positive regression coefficient represents an increase.
† P for difference between the fish protein group and placebo group using crude values: end-of-study values adjusted for group and baseline values.
‡ P for difference between the fish protein group and placebo group using adjusted values: end-of-study values adjusted for group, baseline values, age, sex and weight change.

**Table 4.** Other pre-specified outcomes*
(Mean values and standard deviations; median values and quartiles (Q1–Q3); B-coefficients and 95 % confidence intervals)

| | | Fish protein (n 39) | | | | Placebo (n 35) | | | | Linear regression change in the fish protein group relative to the placebo group | | | | | |
| | | Baseline | | Change | | Baseline | | Change | | Crude values | | Adjusted values | | | |
| | n | Mean | SD | Mean | SD | Mean | SD | Mean | SD | B | 95 % CI | B | 95 % CI | P† | P‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total cholesterol (mmol/l) | 74 | 5·4 | 1·2 | −0·3 | 0·6 | 5·2 | 0·9 | −0·1 | 0·4 | −0·15 | −0·36, 0·06 | −0·15 | −0·37, 0·07 | 0·16 | 0·17 |
| LDL-cholesterol (mmol/l) | 74 | 3·8 | 1·1 | −0·3 | 0·4 | 3·5 | 0·9 | −0·1 | 0·4 | −0·15 | −0·33, 0·03 | −0·13 | −0·30, 0·04 | 0·10 | 0·14 |
| HDL-cholesterol (mmol/l) | 74 | 1·3 | 0·3 | 0 | 0·2 | 1·4 | 0·4 | 0·0 | 0·2 | −0·04 | −0·12, 0·04 | −0·03 | −0·10, 0·05 | 0·33 | 0·49 |
| TAG (mmol/l) | 73 | | | | | | | | | 0·07 | −0·06, 0·20 | 0·06 | −0·07, 0·19 | 0·26 | 0·39 |
| Median | | 1·65 | | 0·00 | | 1·45 | | −0·04 | | | | | | | |
| Q1–Q3 | | 1·21–2·13 | | −0·26 to 0·26 | | 1·08–2·05 | | −0·27 to 0·11 | | | | | | | |
| Weight (kg) | 74 | | | | | | | | | 0·01 | −0·00, 0·02 | 0·01 | −0·00, 0·02 | 0·07 | 0·08§ |
| Median | | 99·2 | | 1·0 | | 96·0 | | 0·4 | | | | | | | |
| Q1–Q3 | | 80·7–114·9 | | −0·2 to 2·0 | | 86·7–106·7 | | −0·8 to 1·3 | | | | | | | |
| SBP (mmHg)‖ | 71 | 119 | 13 | 1·6 | 8·2 | 122 | 16 | 2·5 | 11·3 | −1·7 | −6·1, 2·8 | −1·8 | −6·4, 2·9 | 0·45 | 0·45 |
| DBP (mmHg)‖ | 71 | 71 | 10 | −0·5 | 4·7 | 71 | 10 | −0·9 | 5·7 | 0·3 | −2·2, 2·7 | −0·3 | −2·7, 2·1 | 0·82 | 0·79 |

SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Differences between the groups in other pre-specified outcomes were tested with a linear regression model. Skewed variables (TAG and weight) were log-transformed before analysis. The regression coefficient expresses the mean difference between the groups. A negative regression coefficient in this table represents a reduction in the fish protein group compared with the placebo group, and a positive regression coefficient represents an increase.
† P for difference between the fish protein group and placebo group using crude values: end-of-study values adjusted for group and baseline values.
‡ P for difference between the fish protein group and placebo group using adjusted values: end-of-study values adjusted for group, baseline values, age, sex and weight change.
§ P for difference between the fish protein group and placebo group using adjusted values: end-of-study values adjusted for group, baseline values, age and sex.
‖ Three participants had not taken their antihypertensive medication before one of the study visits and were excluded from the statistical analysis of SBP and DBP.

**Table 5.** Energy and nutrient intake*
(Median values and quartiles (Q1–Q3))

| | | Fish protein (*n* 39) | | | | Placebo (*n* 35) | | | | |
| | | Baseline | | Change | | Baseline | | Change | | |
| | *n* | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | Median | Q1–Q3 | *P* |
|---|---|---|---|---|---|---|---|---|---|---|
| Energy (kJ/d) | 74 | 9295 | 7940–11 760 | −559 | −1278 to 462 | 9257 | 7931–10 618 | −971 | −2828 to 417 | 0·24 |
| Protein (E%) | 74 | 16·6 | 15·9–18·7 | −0·4 | −2·5 to 1·3 | 16·9 | 15·3–18·6 | 0·2 | −1·1 to 1·4 | 0·22 |
| Fat (E%) | 74 | 36·2 | 31·6–39·9 | −0·5 | −4·3 to 1·9 | 35·2 | 33·5–38·6 | 0·2 | −3·9 to 2·8 | 0·40 |
| Saturated (E%) | 74 | 13·5 | 11·8–15·5 | 0·3 | −0·7 to 1·4 | 11·9 | 11·1–15·0 | 0·2 | −0·9 to 1·3 | 0·95 |
| Monounsaturated (E%) | 74 | 13·0 | 11·7–15·2 | −0·4 | −2·3 to 0·7 | 13·5 | 11·9–14·6 | 0 | −2·0 to 1·5 | 0·32 |
| Polyunsaturated (E%) | 74 | 5·9 | 4·8–6·9 | −0·6 | −1·4 to 0·3 | 5·8 | 4·6–7·2 | 0 | −1·0 to 0·9 | 0·18 |
| Carbohydrates (E%) | 74 | 40·2 | 36·7–47·8 | 1·0 | −0·8 to 5·2 | 41·6 | 35·9–45·4 | 0·2 | −2·9 to 2·4 | 0·11 |
| Fibre (E%) | 74 | 2·4 | 1·8–2·7 | 0·1 | −0·4 to 0·2 | 2·5 | 2·0–3·0 | 0 | −0·3 to 0·2 | 0·56 |
| Sugar (E%) | 74 | 5·0 | 3·3–8·3 | −0·4 | −1·8 to 1·7 | 4·4 | 2·9–7·0 | 0·2 | −1·2 to 1·7 | 0·64 |
| Alcohol (E%) | 74 | 1·7 | 0·5–3·9 | 0 | −0·7 to 0·2 | 2·1 | 0·5–7·5 | 0 | −0·7 to 0·7 | 0·60 |

E%, percentage of total energy intake.
* Differences in energy and nutrient intake between the groups were tested with the Mann–Whitney test.

used blood pressure-lowering drugs (Table 2). At baseline, systolic blood pressure was 119 (sd 13) mmHg and diastolic blood pressure was 71 (sd 10) mmHg in the fish protein group and 122 (sd 16) mmHg and 71 (sd 10) mmHg in the placebo group. During the intervention period, there were no significant changes in systolic blood pressure ($P = 0.45$) or diastolic blood pressure ($P = 0.79$) between the groups (Table 4).

### Uptake of study product

Post-prandial analysis of serum amino acids was performed 1 h after intake in five healthy participants. A non-significant increase in plasma levels of most amino acids were seen (Supplementary Table S1 and Supplementary Fig. S1).

### Discussion

In the present study, we investigated the effects of a daily intake of salmon fish protein on several cardiometabolic risk markers among adults with increased risk of T2DM. We found no beneficial effect of salmon fish protein supplementation on markers related to glucose tolerance, serum lipids, weight or blood pressure compared with the placebo group. The present study does not support the hypothesis that daily intake of a salmon fish protein supplement (7·5 g/d) for 8 weeks improves glucose tolerance in persons with increased risk of T2DM.

To the best of our knowledge, this is the first clinical trial exploring the health effect of a fatty fish protein supplement in adults with elevated blood glucose levels.

Few clinical trials, of which three were randomised controlled trials (RCT) ongoing for 6–12 weeks, have investigated health effects of protein supplements from fatty fish[26,31,32]. In line with the results in the present study, no between-group differences in markers related to glucose regulation or lipid metabolism were observed in overweight adults (*n* 77) assigned to 2·5 g of protein/d (8 weeks) from either herring, salmon, cod or casein/whey, except from lower glucose AUC in the casein/whey group than the salmon group[31] nor did Nenseter *et al.* observe improvement in risk factors for CHD in adults with hypercholesterolemia (*n* 70) from 10 g of fish powder/d (12 weeks) from

herring in patients following the National Cholesterol Education Program Step I Diet[32]. In contrast, an RCT on lean fish protein in overweight adults (*n* 34) assigned to a cod protein supplement (3 g/d for 4 weeks and 6 g/d for 4 weeks) demonstrated lower fasting glucose and glucose, insulin and C-peptide after a 2-h OGTT in the cod supplement group than the placebo group[29]. However, these findings were not supported in a later RCT conducted by the same research group in overweight or obese adults (*n* 42) consuming 6 g of protein/d (8 weeks) from cod residuals[38]. An RCT in overweight adults (*n* 110) on 1·4 or 2·8 g/d (90 d) of blue whiting protein hydrolysate given as part of a food supplement significantly improved body composition, decreased body weight, and increased cholecystokinin and glucagon-like peptide-1 compared with whey protein[28]. Both doses provided equal results. None of the RCT using fish protein supplements has found between-group effects on markers related to glucose tolerance. However, investigating the acute effect of fish protein supplementation, a double-blind crossover post-prandial trial in healthy participants (*n* 41) found that 20 mg of cod protein hydrolysate/kg body weight consumed before a standardised breakfast meal reduced post-prandial insulin concentrations, without affecting blood glucose, compared with casein[25]. In addition to the use of different protein doses between the studies, different fish species have different amino acid composition that may explain the inconsistent results. Including large amounts of fish in the diet, between-group effects are reported on insulin sensitivity and post-prandial C-peptide in studies with lean fish[11,12]. Salmon consumed as whole fillets (750 g/week) have shown improved post-prandial glucose response and less increase in C-peptide response in an RCT in overweight or obese adults (*n* 65) compared with cod fillet (8 weeks)[39]. In the present study, participants reported a protein intake of about 93 g/d (results not shown). In addition, the fish protein capsules provided 5·2 g of protein/d for participants in the fish protein group, the same protein amount as in approximately 25 g of salmon fillet (175 g/week). These results suggest that the daily dose of fish protein provided in the present study may be too low to detect an effect and indicate that fish protein may have to be consumed in larger amounts than what a supplement can provide.

In the present study, the participants in the fish protein group had a non-significant weight gain and less reduction in reported energy intake during the intervention period compared with the placebo group. In contrast, Framroze *et al.* found that 16 g of salmon protein hydrolysate/d consumed together with breakfast, in a 6-week RCT in overweight participants (*n* 48), reduced BMI by 5·6 % compared with whey protein isolate, which did not affect weight[26]. In addition, similar to the use of different fish species and protein doses, different protein sources used as control diets make it difficult to compare results. Most intervention studies have compared lean fish with fatty fish or a non-seafood diet containing equal amounts of protein from lean meat, poultry, eggs and dairy products.

Although daily intake of a salmon fish protein supplement did not improve the cardiometabolic risk markers we investigated in the present study, we did not detect any harmful effects of the supplement. The High Level Panel of Experts on Food Security and Nutrition has presented utilisation of by-products as one of the solutions to reduce food losses and waste[40]. Thus, the potential for fish by-products utilised for human consumption should be further investigated, for example, adding fish protein to food products.

Norway is one of the world's largest aquaculture and fishing nations. In 2018, 27 % of all catch from the fishery and aquaculture industry ended as by-products, mainly utilised for animal feed production[41]. Only 13 % of the by-products are used for human consumption[41]. In a sustainability perspective, it is important to explore available food resources at our disposal. With the expected growth in the aquaculture industry, protein-rich by-products will become even more available. Such by-products should ideally be utilised for human consumption[42].

Bastos *et al.* found that adding fish residue flour to wheat bread resulted in products with higher content of protein, essential fatty acids and minerals, and lower contents of carbohydrates[43]. The sensory acceptancy for bread with fish residue flour was better than or as good as bread without fish flour. Groups that could benefit from enriched products are those with increased protein needs, if ensuring high-quality protein in the final product.

The strengths of the FishMeal human intervention study is the randomised controlled double-blind design and the frequent follow-up of the participants. The inclusion of participants with increased risk of T2DM, and thus potentially high benefit of a supplement influencing glucose tolerance, is a strength compared with other studies on fish protein[25,29,31,38]. A strength of the study is also that we performed a post-prandial uptake study to investigate that the fish protein capsules were taken up by the body. The main limitation of the present study is that we did not fulfil our power calculations indicating that 100 participants needed to complete the study to detect a clinically relevant difference between the two groups of 0·4 mmol/l in 2-h OGTT-s-glucose at the end-of-study visit. However, an increase in 2-h OGTT-s-glucose of 0·48 mmol/l (not significant) from intake of fish protein among seventy-four participants with increased levels of either fasting s-glucose, 2-h OGTT-s-glucose or HbA1c decreases the risk of a type II error. In addition, the use of FFQ as a dietary registration method to register changes in the diet during the intervention must be pointed out as a limitation of the study. However, as the intervention consisted of taking a supplement and the participants were instructed not to change their dietary habits, we did not expect any dietary changes.

In conclusion, in the present study, a daily intake of 7·5 g of salmon fish protein did not affect glucose tolerance markers among participants with increased risk of diabetes. However, in a sustainability perspective, salmon fish protein utilised for human consumption could be a valuable protein supplement or ingredient.

## Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0007114521000040

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