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Randomized Control Trials

Type 2 diabetes preventive effects with a 12-months sardine-enriched diet in elderly population with prediabetes: An interventional, randomized and controlled trial

D.A. Díaz-Rizzolo [a, b, c, *], A. Serra [a], C. Colungo [c, d], A. Sala-Vila [e, f], A. Sisó-Almirall [c, d, g], R. Gomis [a, b, g, h, i]

[a] Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) – Hospital Clinic of Barcelona, Barcelona, Spain
[b] Universitat Oberta de Catalunya, Barcelona, Spain
[c] Primary Healthcare Transversal Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Spain
[d] Primary Care Centre, CAPSBE, Barcelona, Spain
[e] IMIM – Hospital del Mar Medical Research Institute, Barcelona, Spain
[f] Fatty Acid Research Institute, Sioux Falls, SD, USA
[g] University of Barcelona, Barcelona, Spain
[h] Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Spain
[i] Department of Endocrinology and Nutrition, Hospital Clinic of Barcelona, Barcelona, Spain

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SUMMARY

Background: Fish could play a role in preventing type 2 diabetes (T2D) but there has been little specification about the type of fish and the preventive mechanism involved in its health claim. The sardine is a source of omega-3 and taurine that, in isolation or in synergy, would produce T2D-delaying through different molecular mechanism.
Hypothesis: The consumption of twice a week of sardine, during one year would reduce T2D-developing risk in a population with prediabetes (preDM) and old age.
Design: 152 subjects with fasting glucose between 100-124 mg/dL aged ≥65 yo were recruited from three primary care centers in Barcelona and were randomly distributed among two interventional groups: control group (CG) and sardine group (SG). Both groups received same T2D-prevention nutritional during a year but only SG had to add 200 g of sardine per week. All variables were collected before to start and at the end of the diet. (ClinicalTrials.gov: NCT03557541).
Results: 152 people were randomized into CG (n=77) and SG (n=75) with 18 and 12 drop outs respectively. Subjects in SG, significantly compared to CG, decreased percentage classified-individuals in a very high risk group to develop T2D according to FINDRISC (p=0.035). In addition to increasing HDL-cholesterol and adiponectin and decreasing triglycerides (p<0.05) and blood pressure (<0.05), SG showed a lower HOMA-IR (p=0.032). The consumption of sardine characteristics nutrients as omega-3, EPA and DHA, vitamin D, fluorine and taurine were higher for SG (p<0.05). These results agreed with the increased of taurine, fatty acid (FA) omega-3 and bile acids circulating metabolites (p<0.05). Changes erythrocyte membrane FA were detected only in SG with a decrease of 5 omega-6 FA (p<0.001) and an increase of 3 omega-3 FA types (p<0.001).
Conclusion: We conclude that a year T2D-prevention diet with sardine supplementation has a greater protective effect against developing T2D and CV events.

* Corresponding author. Diabetes and Obesity Research Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) – Hospital Clinic of Barcelona, Barcelona, Spain.
E-mail addresses: dadiaz@clinic.cat, didi.rizzolo@gmail.com (D.A. Díaz-Rizzolo).

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1. Introduction

Age is considered one of the most important risk factors for developing type 2 diabetes (T2D) [1] due to β-cell defects in insulin secretion. Furthermore, even without diagnosed obesity, insulin resistance (IR) can also appear in this age group due to regional adipose tissue distribution [2]. The prevalence of both T2D and prediabetes (preDM) increases significantly with age [3], with new-onset T2D in the ≥65 years old population increasing 1.5-fold as compared to the total population [4]. In the majority of European countries, the prevalence of T2D is 10–20% in people aged 60–80 years, while the prevalence of preDM is 15–20% for people over 60 years of age [5].

As preDM is a reversible condition, many therapies have been developed to reduce the conversion ratio to T2D. Most of them aim to reduce body weight through nutrition interventions, as weight loss is associated with a lower T2D incidence [6]. This is an especially sensitive issue for the elderly, the group with the highest prevalence of T2D, as weight loss in ≥65 year old individuals has been reported to cause loss of lean body mass, bone mineral density and fat mass, leading to different health problems [7]. Some epidemiological studies have shown that weight loss in the elderly leads to increased mortality [8,9]. For this reason, perhaps a more suitable diet for this age group would be one based on changing dietary patterns instead of focusing on caloric restrictions [10], in order to avoid malnutrition [11].

Fish consumption has demonstrated beneficial effects on cardiovascular risk factors and against cardiovascular diseases (CVD) [12], but it has shown contradictory effects in relation to the incidence of T2D [13]. It is thought that the positive association between fish-rich diets and T2D could be due to environmental contaminants in fish such as persistent organic pollutants, which may induce abdominal obesity, impair insulin sensitivity and reduce glucose intake [14,15]. On the other hand, potential benefits of fish were attributed to the presence of omega-3 fatty acids (FA) EPA and DHA, both with an anti-inflammatory role [16], capable of increasing membrane fluidity, the amount of insulin receptors and the action of insulin [17].

Moreover, the beneficial action of fish oil could also be due to its enormous content of proteins and amino acids, thanks to their role in increasing satiety, which facilitates weight loss [18], and also thanks to their ability to boost insulin secretion in response to ingestion [19].

Sardines are one of the oily fishes richest in omega-3 FA, moreover, they are loaded in proteins and amino acids. Among these is taurine, found in sardines in concentrations of 147 mg/100 g per serving [20], depending on the species, which is thought to have hypoglycemic, antioxidant and anti-inflammatory actions [21], all of which may prove relevant in the prevention of CVD and T2D. Aside from their favorable nutritional composition, sardines are also one of the fish with the lowest content of contaminants, and, since they are available during the whole year, they are affordable for most consumers, regardless of socioeconomic status. Thus, they are proposed as a good candidate for nutrition interventions aimed at preventing T2D progression and diminishing CVD risk.

Many dietary factors, nutrients and/or bioactive compounds mediate the relationship between food intake and health. For this reason, it is also important to elucidate the mechanisms involve in the preventive effects of certain foods. The response to a specific diet is highly dependent on many different processes in the body which are influenced by the diet itself, including metabolomic profile and membrane erythrocyte fatty acid (MEFA) composition.

The purpose of this study was to investigate the preventive effects of a diet rich in sardines against T2D in older persons (≥65 years old) with preDM. We hypothesize that the consumption of sardines twice a week for one year reduces the risk of T2D in an elderly high-risk group.

2. Methods

2.1. Study design

This interventional, randomized and controlled trial was conducted at three primary care centers of the Consorci d'Atenció Primaria de Salut de la Barcelona Esquerra (CAPSBE) in Barcelona, Spain. Informed consent was obtained for research from the Ethics Committee of Hospital Clinic de Barcelona, and the study was performed in accordance with the Code of Ethics of the World Medical Association and the 1975 Helsinki Declaration, as revised in 1983. The clinical phase was performed from May 2014 to June 2016, and biochemical parameters were extracted, stabilized and preserved during the clinical phase and then later analyzed from July 2018 to January 2019.

2.2. Subjects

Individuals, male or female, aged at least 65 years old, with an impaired fasting glucose of 100–125 mg/dL, were invited to join the nutrition intervention as long as informed consent was obtained prior to the start of any activity related to the study. Exclusion criteria were the following: suspected or confirmed hypersensibility/allergy to sardines, chronic treatment with oral steroids and/or NSAIDS, treatment with oral antidiabetic drugs or insulin, treatment with immunosuppressive drugs, diagnosis of an active neoplasia, diagnosis of HIV/AIDS, abnormal hepatic profile (>6-fold normal values), diagnosis of acute psychiatric syndrome, presence of acute concomitant disease requiring more than 7 days for recovery, major cardiovascular event (ictus or myocardial infarction) during the month prior to randomization and any other condition considered as inopportune by researchers.

A database of 929 possible participants from CAPSBE was created, who were classified according to age and glycemia criteria between 100 and 125 mg/dL. The calculation of the sample size for the study of new-onset T2D, with a power of 90% and a level of significance of 0.05, resulted in a required total of 164 study participants. 583 possible participants were called randomly until the first 202 volunteers meeting study criteria and interested in participating were recruited. After the initial explanatory visit, 182 subjects signed informed consent, and the first clinical visit was conducted, during which baseline blood samples were obtained. After this first non-interventional visit, 15 people declined to continue. Of the 167 remaining participants, new-onset T2D was observed in 15 individuals, who were then eliminated following exclusion criteria. A total of 152 participants were then randomized and proceeded to begin the nutrition intervention, however, 30 subjects later abandoned the study due to various reasons: 36.7% reported being tired of the intervention, 33.3% for health reasons and 30% for other reasons, including family issues, transportation problems, etc. Therefore, in the end, a final total of 122 participants started and finished the nutrition intervention of the study (Fig. 1).

2.3. Randomisation and masking

Using random-number tables, participants were assigned at a ratio of 1:1 to either a control group (CG) or sardine group (SG) by the researcher. All study personnel performing assessments were masked to group assignment.

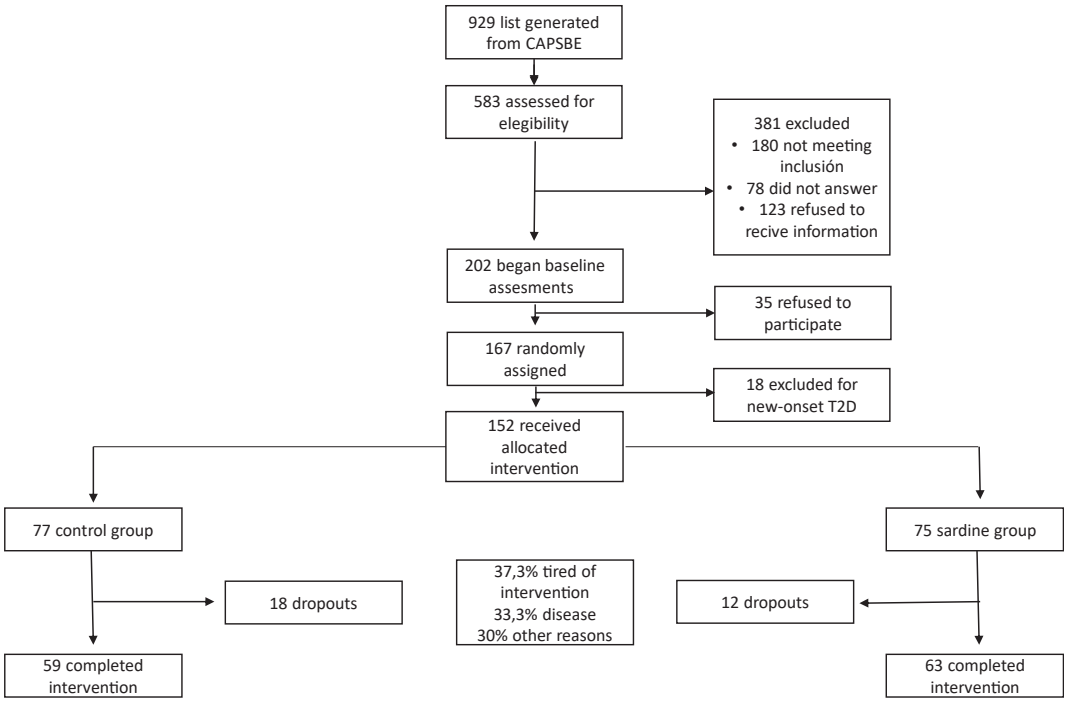

Fig. 1. Flowchart of study participation.

2.4. Procedures

Participants were randomized into two groups: CG and SG. Both groups received the same nutrition education based on clinical guidelines issued by the American Diabetes Association, which promotes the consumption of vegetables, legumes and whole grains, favors increased seafood and lean meats as substitutes for red and processed meat, and recommends reducing added sugar and avoiding ultra-processed foods [22]. Nutrition education in group was provided prior to the start of the study and then every four months during its execution, in order to reinforce nutritional knowledge among participants. The SG received 200 g of canned sardines in olive oil per week, which were provided to them by researchers every 4 months. The SG consumed a serving of sardines twice a week, each serving consisting of 100 g, which fulfills the health and safety recommendations issued by Spanish Agency for Food Safety and Nutrition and nearly doubles the average consumption of taurine and omega-3 fatty acids EPA + DHA, as previously observed by our group in (from 56 mg/day to 98 mg/day, and from 0.30 g/day to 0.58 g/day, respectively) [20].

Six visits were conducted during the year of the clinical study. Values were obtained during the baseline visit (V0) before providing nutrition education and then during the last visit held after one year following either the CG or SG diet (V12).

2.5. Outcomes

Information obtained during V0 and V12 included anthropometric measurements and body composition by Lunar iDXA (GE Healthcare). In addition, blood pressure was recorded and blood samples taken, which were sent for biochemical analysis by the Biomedical Diagnostic Centre (CDB) at Hospital Clinic i Provincial de Barcelona. During both visits, different questionnaires were used: a 3-day dietary record analyzed by the DIAL program, created by the Complutense University of Madrid, in order to obtain information regarding nutritional patterns; and VREM, a short questionnaire adapted from the Minnesota Leisure Time Physical Activity Questionnaire specialized in aged population, in order to obtain information regarding physical activity. T2D risk was calculated using the Finnish Diabetes Risk Score (FINDRISC) questionnaire as either “low”, “medium”, “high” or “very high”. In order to assess the progression of blood glucose levels, we consider high blood glucose “yes” or “not”, according to the levels presented in each moment, at V0 and V12, by each participant.

Fasting blood samples were collected in order to measure different parameters. Serum was separated to determine metabolomic profile and mass spectrometry, coupled with ultra-high performance liquid chromatography (UHPLC-MS), was carried out to profile fatty acids (FA), bile acids (BA), steroids and lysoglycerophospholipids and amino acids. Red blood cells were obtained to check MEFA composition, and the amount of each FA was expressed as a percentage of the total identified fatty acids in the sample. The omega-3 index was calculated as the sum of the percentages of EPA and DHA in relation to all FA detected.

2.6. Statistical analysis

Descriptive data are presented as the mean with standard deviation (SD) or standard error of the mean (SEM), and number and percentage (%) for categorical outcomes. Groups were compared with themselves by paired test at the beginning (V0) and at the end (V12) of the study. After that, unpaired tests were made to compare differences between CG and SG. After checking for normality by Kolmogorov–Smirnoff test, non-parametric Mann–Whitney U test was used for the comparison of continuous outcomes when normality and equality of variance could not be assumed. Student's t test was used for the rest of continuous outcomes and Chi-square test for categorical outcomes. To investigate potential metabolic differences between patients taking the sardine supplement and patients who did not take the sardine supplement during the nutritional intervention, data were first normalized by dividing the data of each metabolite obtained for each patient at timepoint V12

per the data of each metabolite obtained for each patient at time-point V0. This was performed in order to avoid the differences per patient. Then, univariate statistical analyses were also performed calculating group percentage changes and unpaired Student's t-test p-value (or Welch's t test where unequal variances were found) for the comparison.

The number by which the p-value was understood as significant was ≤0.05. All analyses were conducted using the IBM SPSS Statistics 23 software package.

2.7. Role of the funding source

The research presented in the present paper received a Recercaixa 2013 grant from "La Caixa" Banking Foundation, which was not involved with the study design, data collection, analysis or interpretation of Results. The corresponding author had full access to all data from the study and holds final responsibility for their publication.

3. Results

3.1. Baseline and randomization

Our recruited subjects were 44.74% female and 55.26% male. Their mean age was 71.20 ± 5.15 years, while the mean duration of preDM at the beginning of the study was 4.84 ± 3.38 years. No differences between SG and CG were observed in terms of age, sex or duration of preDM. Clinical characteristics of both groups were similar, as well as anthropometric measurements and body composition (Table 1).

3.2. Blood pressure, blood chemistry, anthropometrics and body composition

Results obtained during the last visit (V12) were compared with all data collected before the start of the study, at V0 (Table 2). The two groups decreased anthropometric measurements equally in weight, body mass index (BMI), waist and hip circumference and index and percentage of approximation to ideal weight. In addition, certain body composition parameters improved in both groups. In particular, CG and SG decreased equally in centile fat mass, total percentage of fat mass, fat mass percentage in the torso and especially in the abdomen, and quantity of visceral fat. Only the decrease in fat mass percentage in the hips was stronger in CG than in SG (−2.65 ± 1.85 and −1.27 ± 2.85, respectively, with $p = 0.025$ between groups). On other hand, systolic blood pressure (SBP) and diastolic blood pressure (DBP) decreased −4.34 ± 13.32 and −2.24 ± 69.86, respectively, but only in SG ($p = 0.014$ and 0.020, respectively).

3.3. Physical activity and balance of energy expenditure/intake

Energy expenditure was compared with energy intake for each participant pre- and post-intervention, to monitor any changes (Fig. 2). Harris-Bennedict formula was used to evaluate daily energy expenditure. Both CG and SG showed similar energy expenditure pre- and post-intervention (1306.418 ± 301.11 and 1364.679 ± 258.10 kcal/day, respectively, at V0, and 1333.795 ± 193.69 and 1372.120 ± 181.30 kcal/day, respectively, at V12, with $p > 0.05$), and no changed were observed in either of the two groups (27.378 ± 289.35 and 7.521 ± 249.41 kcal/day, respectively, with $p = 0.704$ and with $p > 0.05$).

Analyses of 3-day dietary records revealed that both CG and SG significantly decreased energy intake post-intervention (2027.26 ± 606 and 1952.26 ± 508.44 kcal/day, respectively, at V0, and 1686.12 ± 333.88 and 1858.71 ± 1081.98 kcal/day, respectively, at V12, with $p < 0.001$ for CG and $p = 0.004$ for SG), without any difference between groups (−383.18 ± 620.70 and -102.50 ± 1193.06, with $p = 0.350$).

3.4. Nutritional pattern

Changes in nutrient intake were observed after the intervention (Supplementary Table 1). SG significantly increased the proportion of kcal derived from proteins (1.73 ± 3.90, with $p < 0.001$) while CG decreased total g of proteins (−13.77 ± 37.73, with $p = 0.025$). Among amino acids, the only notable change was observed in the consumption of taurine, which increased significantly only in SG (43.22 g ± 95.28, with $p < 0.001$, as compared to CG, with $p < 0.001$).

The consumption of total lipids decreased in CG (−19.63 g ± 37.98, with $p < 0.001$) and increased in SG (0.97 g ± 87.62, with $p = 0.027$). Both CG and SG showed a decrease in saturated fatty acid (SFA) intake (- 7.63 g ± 9.93 and −4.07 g ± 9.28, respectively, with $p < 0.05$), but the decrease was greater in CG ($p = 0.05$). Also, in both CG and SG, an equally lower consumption of monounsaturated fatty acid (MUFA) was observed (- 8.36 g ± 24.98 and −5.35 g ± 12.92, with $p < 0.05$). CG also demonstrated a decreased intake in polyunsaturated fatty acid (PUFA) (−1.66 g ± 5.01 with $p = 0.012$). Moreover, omega-3 FA consumption was decreased in CG and increased in SG (−0.63 g ± 1.64 and 1.07 g ± 2.16, with $p < 0.05$ and comparing groups $p < 0.001$). In particular, a higher consumption of EPA and DHA were seen in SG (0.50 g ± 0.63 and 0.55 g ± 0.88 with $p < 0.001$, as compared to CG with $p < 0.001$).

CG and SG equally decreased consumption of carbohydrates (−24.42 g ± 54.85 and −21.40 g ± 55.50, respectively, with p-value<0.05), especially simple carbohydrates (−15.38 g ± 28.70 and −8.50 g ± 26.04, respectively, with $p < 0.05$). Of note, differences pre- and post-intervention in both CG and SG were due to intake of glucose (−1.95 g ± 5.70 and −1.44 g ± 4.99, respectively, with $p < 0.05$). CG also showed a lower consumption of fructose, sucrose and maltose (−2.62 g ± 7.37, −5.14 g ± 12.78 and −0.17 g ± 0.58, respectively, with $p < 0.05$), and SG showed a higher consumption of lactose (2.11 g ± 6.77, with $p = 0.032$).

At the end of the study, both CG and SG decreased consumption of iron (−8.60 mg ± 17.49 and −4.15 mg ± 14.41, respectively, with $p < 0.05$ and, when comparing groups, $p = 0.026$) and of aluminum (−117.77 μg ± 400.73 and −81.57 μg ± 304.95, respectively, with $p < 0.05$). CG showed a decreased intake of calcium, iron, zinc, potassium copper, chromium, phosphorus and fluorine (−189.26 mg ± 476.87, −8.60 mg ± 17.49, −17.21 μg ± 75.21, −888.71 mg ± 1842.88, −1.07 mg ± 2.58, −67.76 μg ± 153.18, −241.29 mg ± 584.89 and −143.85 μg ± 363.88, respectively, with $p < 0.05$). SG showed a greater intake of fluorine (33.58 μg ± 396.99 with $p = 0.035$, and when comparing groups, $p < 0.001$) and a reduced intake of iron (−4.15 mg ± 14.41, with $p = 0.049$, but this decrease was lower when compared to CG, $p = 0.026$).

Changes in vitamin consumption also were observed. CG showed a lower intake of vitamins A, B1, B2, B3, B5, B6, B9 and K (−467.20 μg ± 1654.65, −0.21 mg ± 0.41, −0.69 mg ± 1.05, −2.53 mg ± 13.26, −1.00 mg ± 2.09, −0.25 mg ± 0.78, −52.19 μg ± 127.28 and −158.82 μg ± 287.39, respectively, with $p < 0.05$). Also, SG showed a higher intake of vitamins B12 and D (7.89 g ± 10.99 and 1.87 μg ± 4.39, respectively, with $p < 0.05$ and, when compared to CG, $p < 0.05$).

Ultimately, micronutrients were also affected by both nutrition interventions. For example, CG demonstrated a decreased consumption of retinol and phytosterols (−331.79 μg ± 1638.91 and −3.27 μg ± 64.16 respectively with $p < 0.05$), while SG demonstrated an increased consumption of phytic acid (0.09 g ± 0.27 with $p = 0.022$).

Table 1
Baseline characteristics from both control group (CG) and sardine group (SG).

	CG	SG	p
Sex, % female	48.1	41.3	0.405
Age, years	71.44 (5.17)	70.96 (5.16)	0.566
Personal history			
Evolution IFG, years	5.13 (3.55)	4.55 (3.19)	0.288
Family history DM, % cases	44.2	34.7	0.231
Drugs			
Antihypertension, % consumers	64.9	61.3	0.645
Antithrombotic, % consumers	19.5	32	0.077
Cholesterol-lowering drugs, % consumers	37.7	45.3	0.377
Gastric protector, % consumers	23.4	22.7	0.917
Thyroid hormone therapy, % consumers	9.1	12	0.559
Antidepressant, % consumers	18.2	22.7	0.493
Bronchodilators, % consumers	5.2	5.3	0.969
Opioid analgesics, % consumers	11.7	6.7	0.284
Other, % consumers	70.1	61.3	0.253
Toxic habits			
Smoking, % cases	6.5	9.3	0.244
Previous smoking, % cases	44.2	54.7	
Occasional drinking, % cases	55.8	20.8	0.376
Daily drinking, % cases	52.1	30.1	
Blood pressure			
Systolic blood pressure, mmHg	134.387 (17.81)	135.687 (18.69)	0.672
Dyastolic blood pressure, mmHg	78.776 (9.89)	80.243 (9.34)	0.362
Blood chemistry			
HbA1c, %	6.026 (0.43)	6.041 (0.37)	0.813
Glucose, mg/dL	104.026 (10.43)	104.907 (10.89)	0.611
Insulin, mU/L	14.652 (5.79)	16.780 (8.07)	0.069
Total cholesterol, mg/dL	204.377 (30.32)	194.773 (31.73)	0.056
Cholesterol- HDL, mg/dL	51.571 (12.43)	49.120 (11.58)	0.211
Cholesterol-LDL, mg/dL	131.437 (25.67)	123.124 (25.26)	0.051
Tryglicerides, mg/dL	110.948 (48.67)	115.453 (54.27)	0.591
AdipoQ, μg/mL	11.043 (5.65)	10.276 (4.33)	0.352
Anthropometrics			
Weight, kg	77.432 (11.70)	79.340 (12.07)	0.324
BMI, kg/m^2	29.289 (3.86)	29.131 (4.13)	0.809
Waist, cm	99.371 (11.22)	99.992 (11.06)	0.744
Hips, cm	10.699 (8.81)	106.176 (7.72)	0.564
Percentage approximation ideal weight, %	132.517 (17.49)	131.683 (18.32)	0.774
Body composition			
Centile fat mass, index	88.382 (14.81)	89.438 (16.52)	0.767
Fat mass total, %	41.568 (6.05)	39.706 (6.51)	0.191
Fat mass, trunk %	46.438 (5.72)	44.514 (6.83)	0.182
Fat mass in abdomen, %	52.126 (5.90)	49.429 (7.09)	0.072
Fat mass in hips, %	44.418 (9.43)	42.386 (8.83)	0.319
Visceral fat, g	2104.97 (889.23)	2229.63 (826.38)	0.504

Values are expressed as mean (SD) for quantitative data, and mean % of subjects for frequency variables. In continuous outcomes, for non-parametric values Mann–Whitney U test was used and for parametric Student's t test was used. In categorical values, Chi-squared was used.
p in bold are <0.05.
BMI: body mass index, CG: control group, DBP: diastolic blood pressure, HbA1c: glycated hemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, preDM: prediabetes, SBP: systolic blood pressure, SG: sardine group, TAG: triacylglycerides.

3.5. Membrane erythrocytes fatty acids

Among the 23 FA studied in the erythrocyte membrane, only PUFAs showed significant differences at the end of the study (Supplementary Table 2). SG showed a decrease in the composition of omega-6 FA: eicosadienoic acid, dihomo-y-linolenic, arachidonic acid, adrenic acid and osbond acid (-0.02 ± 0.04, -0.11 ± 0.19, -1.00 ± 1.27, -0.44 ± 0.39 and -0.10 ± 0.09, respectively, with $p < 0.001$). On the other hand, SG also demonstrated an increase in omega-3 FA: EPA, Clupanodonic acid and DHA (0.79 ± 0.68, 0.59 ± 0.46 and 0.45 ± 0.82, respectively, with $p < 0.001$).

Omega-3 index was calculated for each participant (Fig. 3), and in CG and SG a similar baseline index was observed (6.29 ± 1.45 and 6.64 ± 1.22, with $p = 0.161$). However, after one year of nutrition intervention, only SG showed an increase in the index (final index number of 7.90 ± 1.33, with $p < 0.001$ when comparing pre- and post-, and an increase of 1.25 ± 1.25, with $p < 0.001$ between groups).

3.6. Metabolomics

A similar metabolic profile was obtained when comparing samples taken at baseline (V0) from SG and CG, suggesting that there were no significant basal differences between groups before starting the nutrition intervention (data not shown).

A greater number of significantly altered metabolites were found in the serum samples from SG as compared to number of altered metabolites in CG. In fact, 50 versus 27 metabolites out of 407 were found to be significantly altered in samples from SG and CG at V12 as compared to V0, respectively. In particular, taurine (0.28 fold change with $p = 0.029$) and EPA (1.10 fold change with 0.011) only changed in SG (data not shown).

Data were normalized for metabolite and for group at V0 and V12 time points, in order to compare Resultsbetween groups. 33 out of 407 metabolites were found to be significantly altered in the serum samples from SG as compared to CG. Metabolite classes and ratios were also calculated for the purpose of comparing groups.

Table 2
Comparison pre and 12-months post intervention for anthropometrics, blood pressure and body composition.

	CG			SG			p[a]	p[b]	p[c]
	V0	V12	Dif.	V0	V12	Dif.			
Blood pressure									
Systolic blood pressure, mmHg	134.39 (17.81)	133.28 (12.73)	−1.30 (18.13)	135.68 (18.69)	129.72 (13.28)	−4.34 (13.32)	0.340	**0.014**	0.565
Diastolic blood pressure, mmHg	79.13 (9.03)	79.13 (8.18)	−0.49 (7.71)	80.08 (9.02)	77.85 (7.83)	−2.24 (6.86)	0.660	**0.020**	0.222
Anthropometrics									
Weight, kg	77.41 (12.48)	74.72 (12.18)	−2.69 (3.30)	79.44 (11.81)	77.67 (12.02)	−1.77 (3.23)	**<0.001**	**<0.001**	0.128
BMI, kg/m^2	29.29 (3.86)	28.13 (3.73)	−1.01 (1.18)	29.13 (4.13)	28.25 (3.98)	−0.65 (1.21)	**<0.001**	**<0.001**	0.185
Waist, cm	98.49 (11.77)	94.95 (10.98)	−3.54 (4.93)	99.95 (10.61)	97.13 (11.10)	−2.82 (4.75)	**<0.001**	**<0.001**	0.454
Hips, cm	106.99 (8.81)	103.81 (7.43)	−1.88 (4.39)	106.18 (7.72)	104.29 (6.93)	−1.46 (4.31)	**0.005**	**0.010**	0.662
Waist/Hip, index	0.93 (0.08)	0.91 (0.07)	−0.02 (0.05)	0.95 (0.07)	0.93 (0.08)	−0.02 (0.05)	**<0.001**	**0.020**	0.817
Percentage approximation ideal weight, %	132.51 (17.49)	127.12 (16.67)	−4.57 (5.41)	131.68 (18.32)	127.63 (17.97)	−2.90 (5.52)	**<0.001**	**<0.001**	0.341
Body composition									
Centile fat mass, index	88.38 (14.82)	82.13 (18.63)	−6.86 (7.07)	89.44 (16.52)	85.24 (17.51)	−5.34 (9.39)	**<0.001**	**0.001**	0.064
Fat mass total, %	41.51 (6.23)	39.11 (6.61)	−0.94 (8.64)	39.65 (6.17)	38.20 (6.89)	−1.45 (2.57)	**<0.001**	**<0.001**	0.720
Fat mass, trunk %	46.58 (5.78)	43.68 (6.30)	−1.30 (9.05)	44.50 (6.36)	42.80 (7.64)	−1.70 (3.62)	**<0.001**	**<0.001**	0.797
Fat mass in abdomen, %	52.24 (5.90)	48.82 (7.02)	−1.63 (10.21)	49.65 (6.76)	47.28 (8.18)	−2.36 (3.85)	**<0.001**	**<0.001**	0.672
Fat mass in hips, %	44.17 (9.70)	41.52 (9.60)	−2.65 (1.85)	42.20 (8.52)	40.93 (8.91)	−1.27 (2.85)	**<0.001**	**0.010**	**0.025**
Lean mass total, g	41203.62 (8812.54)	42050.14 (7820.59)	1981.17 (7632.85)	45707.10 (7331.35)	45958.24 (7284.54)	1598 (8827.35)	0.320	0.230	0.848
Visceral fat, g	2104.97 (889.23)	1895.83 (922.36)	−209.14 (341.58)	2229.63 (826.38)	2078.12 (852.47)	−151.51 (317.56)	**<0.001**	**<0.001**	0.471

Values are expressed as mean (SD) for quantitative data. In continuous outcomes, for non-parametric values Mann–Whitney U test was used and for parametric Student's t test was used.
p in bold are <0.05.
BMI: body mass index, CG: control group, DBP: diastolic blood pressure, Dif: Differences V0 vs V12, SBP: systolic blood pressure, SG: sardine group, V0: visit pre-intervention, V12: visit post-intervention.
[a] Comparison among V0 vs V12 for CG.
[b] Comparison among V0 vs V12 for SG.
[c] Comparison among differences between CG and SG.

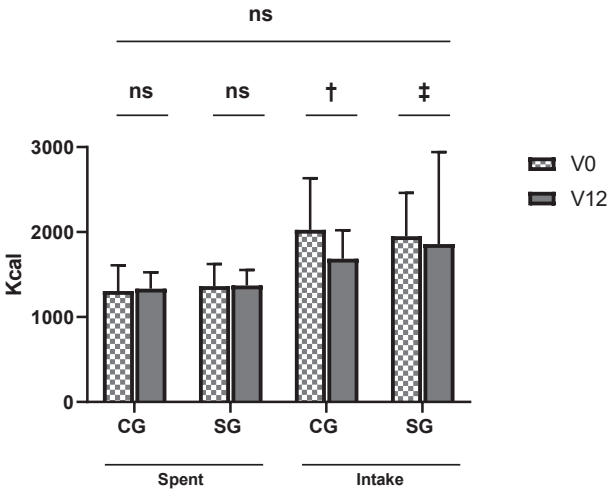

Fig. 2. Energy balance from each group pre and 12-months post intervention comparing energy intake recorded from 3-days dietary record and energy expended, calculated taking into consideration basal energy expenditure and physical activity. Values are expressed as mean ± SD. CG: control group, SG: sardine group, V0: visit pre-intervention, V12: visit post-intervention, ns: non significant. † Significant difference of energy intake (V0 vs V12) for CG. ‡ Significant difference of energy intake (V0 vs V12) for SG.

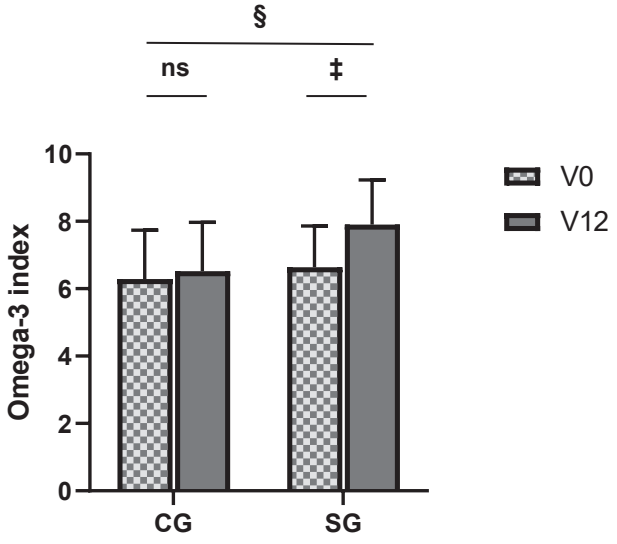

Fig. 3. Comparison omega-3 index pre and 12-months post intervention for both intervention groups. Values are expressed as mean ± SD. CG: control group, SG: sardine group, V0: visit pre-intervention, V12: visit post-intervention, ns: non significant. ‡ Significant difference of energy intake (V0 vs V12) for SG. § Significantly differences between CG and SG for V12 risk groups.

Interestingly, glycine conjugated bile acids (GCBA), taurine conjugated bile acids (TCBA), non-esterified FA omega-3 (NEFA_omega_3) and 1-monoetherglycerophosphoethanolamine O plasmanyles (MEPE.O_plasmanyles) were significantly increased in SG as compared to CG at V12 (Table 3).

Total amino acids (AAs_total) increased in both CG and SG but aromatic amino acids (ArAAs) and branched chain amino acids (BCAAs) only increased in CG. On the other hand, total bile acids (Bas_total) and free bile acids (FBA), cholesteryl esters (ChoE) and diacylglycerophosphocholine (DAPC) increased following intervention with a sardine-rich diet (Table 3).

3.7. Percentage of T2D onset, preDM and normoglycemic values

At the start of the study, all participants, in both CG and SG, presented preDM, but after a year of nutrition intervention, the

Table 3
Significantly altered metabolic classes and ratios found for the different comparisons performed.

	V0: SG vs CG		CG: V12 vs V0		SG: V12 vs V0		V12: SG vs CG	
Metabolic class	log_2 (fold change)	p	log_2 (fold change)	p	log_2 (fold change)	p	log_2 (fold change)	p
Amino acids								
AAs_total	-0.01	0.811	0.10	**<0.001**	0.11	**0.008**	0.02	0.715
ArAAs	0.09	0.099	0.12	**0.036**	0.10	0.139	-0.01	0.846
BCAAs	0.06	0.347	0.13	**0.034**	0.09	0.170	-0.03	0.734
Bile acids								
BAs_total	-0.51	0.250	0.09	0.226	1.33	**0.038**	0.94	0.114
FBA	-0.74	0.246	0.92	0.319	1.89	**0.047**	0.60	0.467
GCBA	-0.40	0.419	-0.3	0.087	0.98	0.924	1.33	**0.023**
TCBA	0.12	0.869	-0.17	0.559	1.40	0.957	1.74	**0.015**
Cholesteryl esters								
ChoE	-0.20	0.218	0.04	0.545	0.41	**<0.001**	0.19	0.119
Fatty acids								
NEFA_omega_3	-0.29	0.189	0.10	0.734	0.45	**0.024**	0.41	**0.033**
Glycerophospholipids								
DAPC	-0.15	0.292	-0.05	0.141	0.24	**0.047**	0.17	0.142
MEPE.O_plasmanyles	-0.32	0.200	-0.19	**0.007**	0.33	0.524	0.52	**0.029**

Metabolites were first normalized. Univariate statistical analyses were performed calculating group percentage changes and unpaired Student's t-test p-value or Welch's test for non-parametric values.
AAs_total: total amino acids, ArAAs: aromatic amino acids, BAs_total: total bile acids.
BCAAs: branched-chain amino acid, CG: control group, ChoE: cholesteryl esters, DAPC: diacylglycerophosphocholine. FBA: free bile acids, GCBA: glycine conjugated bile acids, MEPE.O_plasmanyles:1-monoetherglycerophosphoethanolamine O plasmanyles, NEFA_omega_3: non esterified FA omega-3, SG: sardine group, TCBA: taurine conjugated bile acids, V0: visit pre-intervention, V12: visit post-intervention.
Colors mean that red decreased and green increased.
p in bold are <0.05.

percentage decreased equally (p = 0.769) to 63.6% and 61.3%, respectively. Around a third of the subjects from each group (p = 0.528) reverted to normoglycemic values and, although 5.2% of CG subjects showed new T2D onset as compared to only 2.7% of SG, this difference was not significant, corresponding to 4 and 2 subjects, respectively (p = 0.424).

3.8. T2D risk

Before the start of the nutrition intervention (V0), the FINDRISC questionnaire was used to evaluate T2D risk, assigning subjects to one of 5 groups: low, slightly elevated, moderate, high and very high. The evaluation was repeated after one year, taking into consideration the newly obtained data in V12 (Table 4). When subjects were distributed according to T2D risk groups, there were no differences between SG and CG prior to the intervention (0.088), but significant differences became evident afterwards (0.035). Most noteworthy is the fact that, following the nutrition intervention, 29.6% of SG subjects had left the "very high" risk group (from 37.3% to 7.7%), while only 4.9% of CG subjects had managed to do so (from 27.3% to 22.4%), a finding which is significantly different (p = 0.021) (Fig. 4).

Table 4
Distribution and comparison pre and 12-months post intervention type 2 diabetes risk groups by FINDRISC questionnaire.

V0	Low	Slightly elevated	Moderate	High	Very high	p
CG	0.0%	2.6%	6.5%	63.6%	27.3%	0.088
SG	0.0%	4.0%	14.7%	44.0%	37.3%	
V12	Low	Slightly elevated	Moderate	High	Very high	p
CG	0.0%	27.6%	19.0%	31.0%	22.4%	**0.035**
SG	6.2%	21.5%	18.5%	46.2%	7.7%	

Values are expressed as mean percentage of distribution of each risk group; both CG and SG and Chi-squared analyses was used.
Bold signifies statistically significant differences in the risk distribution after 12 months of intervention between both groups.
CG: control group, SG: sardine group, V0: visit pre-intervention, V12: visit post-intervention, FINDRISC: The Finnish Diabetes Risc Score.

3.9. T2D risk factor markers

A biochemical comparison showed that (Table 5) both CG and SG equally decreased glycated hemoglobin (HbA1c) (−0.10% ± 0.25

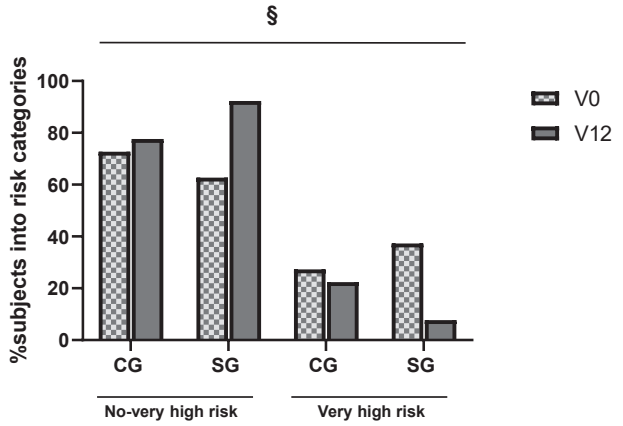

Fig. 4. Distribution and comparison pre and 12-months post intervention type 2 diabetes risk groups by FINDRISC as "very high" or not. Values are expressed mean % of subjects into risk categories. FINDRISC: The Finnish Diabetes Risc Score, CG: control group, SG: sardine group, V0: visit pre-intervention, V12: visit post-intervention. § Significantly differences between CG and SG for V12 risk groups.

and −0.07% ± 0.21, with p = 0.010 and 0.011, respectively), glucose fasting concentrations (−3.56 mg/dL ±12.79 and −3.10 mg/dL ±10.26, with p = 0.040 and 0.020, respectively). Only CG significantly decreased total cholesterol (−6.46 mg/dL ±24.58, with p = 0.032), while SG significantly increased cholesterol-HDL (3.43 mg/dL ±7.37, with p = 0.003 and between groups p = 0.045). Triacylglyceride (TAG) concentrations decreased (−11.13 mg/dL ±35.33, with p = 0.006) while adiponectin increased (1.27 μg/mL ±3.16 with, p < 0.001), but only in SG.

β-cell function was calculated by HOMA-β formula, with no significant difference observed. However, when measuring insulin resistance by HOMA-IR, data obtained from both CG and SG showed a significant decrease only in subjects who had followed a sardine-enriched diet during the nutrition intervention (−0.47 ± 1.68, with p = 0.032) (Fig. 5).

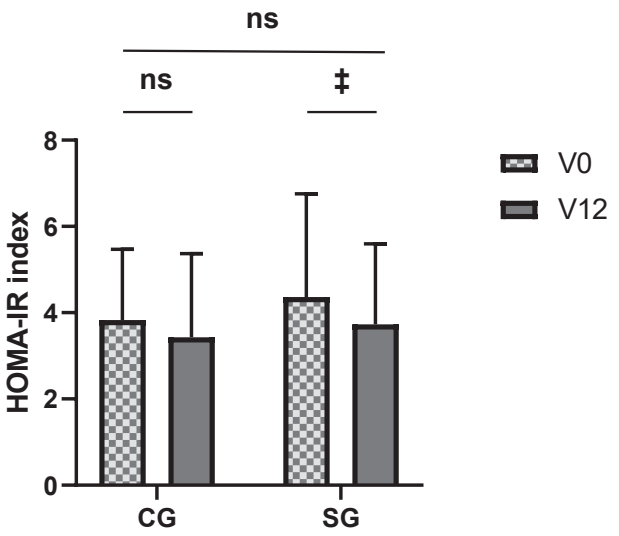

Fig. 5. Comparison HOMA-IR for both groups between pre and 12-months post intervention. Values are expressed as mean ± SD. CG: control group, SG: sardine group, V0: visit pre-intervention, V12: visit post-intervention, ns: non significant. ‡ Significant difference of energy intake (V0 vs V12) for SG.

4. Discussion

Diet has demonstrated an important role in preventing several chronic diseases through multiple associated mechanisms. In particular, the prevention of T2D through changes in dietary patterns and, more specifically, by supplementing with a specific food or nutrient has been widely investigated.

Despite this, a review of the evidence obtained in models using certain nutrients and food extracts reveals that the consumption of sardines for the prevention of T2D has never been studied before [23].

Our Resultsshowed that, in both the sardine and control groups, a personalized control of dietary patterns following specific recommendations to prevent T2D, showed an improvement in the management of body weight, BMI and waist and hip circumference, as well as an improvement in body composition. This is probably because both groups followed the same base T2D-preventive diet, with the one exception of sardine supplementation, and, although they did not modify their physical activity, both groups reduced their daily caloric intake through food. It should be noted that, although both of them reduced their body weight, only the SG group decreased both SBP and DBP. Previously, only lean fish consumption had demonstrated an improvement in BP, not fatty fish consumption [24], perhaps because the species studied excluded those with a higher taurine content such as sardines. Taurine supplementation has been demonstrated to reduce BP [25], and EPA and DHA supplementation has also shown similar improvements [26]; thus, it would be logical to deduce that the individuals of the group following a diet that stressed the consumption of a food rich in nutrients, taurine, EPA and DHA, could also enjoy beneficial effects on BP.

As for the consumption of specific nutrients, both dietary interventions resulted in a decreased consumption of SFA, MUFA, carbohydrates and simple sugars, which could be associated with a decrease in T2D risk [27]. In addition to restricted calories, there were nutrients that decreased only in the group undergoing a standard T2D-preventive intervention (CG), including protein, lipids, PUFA, omega-3 FA, minerals (such as calcium, zinc, chromium, fluoride and phosphorous), vitamins (such as A, K, B1, B2, B, B6 and B9) and antioxidants (such as phytosterols and retinol). However, despite the same caloric restrictions, SG showed an increase in nutrients such as taurine, EPA, DHA, total omega-3 FA, minerals (such as calcium, iodine, zinc, phosphorous and fluoride) and vitamins (such as B12 and D), in addition to other antioxidants including lycopene, and total tocopherols. This may be due to the fact that sardines are very rich in many of these nutrients, while the olive oil present in the sardine can may contain others [28].

Table 5
Comparison pre and 12-months post intervention for blood chemistry markers.

	CG			SG			p[a]	p[b]	p[c]
	V0	V12	Dif.	V0	V12	Dif.			
HbA1c, %	6.03 (0.43)	5.95 (0.38)	−0.10 (0.25)	6.04 (0.37)	5.97 (0.38)	−0.07 (0.21)	**0.010**	**0.011**	0.736
Glucose, mg/dL	104.51 (10.48)	100.95 (11.26)	−3.56 (12.79)	104.94 (10.62)	101.89 (10.18)	−3.10 (10.26)	**0.040**	**0.020**	0.828
Insulin, mU/L	14.65 (5.80)	13.45 (6.74)	−0.82 (5.57)	16.75 (8.07)	15.23 (7.93)	−0.93 (5.98)	0.171	0.167	0.897
Total cholesterol, mg/dL	204.38 (30.33)	198.81 (32.59)	−6.46 (24.58)	194.77 (31.73)	191.44 (35.40)	−0.10 (26.36)	**0.032**	0.828	0.090
Cholesterol- HDL, mg/dL	51.57 (12.43)	53.20 (13.69)	0.23 (9.75)	28.64 (11.24)	51.77 (12.85)	3.43 (7.37)	0.915	**0.003**	**0.045**
Cholesterol-LDL, mg/dL	130.88 (25.93)	126.14 (26.72)	−4.74 (23.43)	121.82 (25.25)	121.49 (30.04)	−0.33 (24.65)	0.140	0.920	0.331
Triglycerides, mg/dL	110.95 (48.68)	101.37 (50.05)	−8.53 (30.70)	115.45 (54.28)	97.73 (36.97)	−11.13 (35.33)	0.077	**0.006**	0.501
AdipoQ, μg/mL	11.04 (5.65)	11.39 (4.54)	0.52 (3.09)	10.132 (4.174)	11.23 (4.22)	1.27 (3.16)	0.083	**<0.001**	0.403

Values are expressed as mean (SD) for quantitative data. In continuous outcomes, for non-parametric values Mann–Whitney U test was used and for parametric Student's t test was used.
CG: control group, Dif: Differences V0 vs V12, HbA1c: glycated hemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, SG: sardine group, TAG: triacylglycerides, V0: visit pre-intervention, V12: visit post-intervention.
p in bold are <0.05.
[a] Comparison among V0 vs V12 for CG.
[b] Comparison among V0 vs V12 for SG.
[c] Comparison among differences between CG and SG.

Moreover, the increased intake of vitamin D, calcium and zinc has been linked to protective effects against T2D [29,30].

MEFA composition reflects diet profile over the course of months [31], thus, the increase in EPA and DHA in SG could be considered as a dietary biomarker of good adherence to the nutrition intervention carried out. On the other hand, the increase in these omega-3 FA in SG could indicate a T2D preventive effect through the increase in the fluidity of cell membranes which is an important mediator that links intake and metabolism of FAs to T2D risk [32].

Moreover, we observed a decrease in five different omega-6 FA subtypes only in SG, which could also indicate certain protective effects against T2D, as an increase in these circulating FA has been associated with an increased risk of T2D [33–35]. Specifically, in the scientific literature, the omega-6 FA subtypes have been observed increases in the population with DM [36], they have been described as pro-inflammatory [36,37], predictors of worsening of hyperglycemia [38], have been seen in a higher proportion in relation to diabetological patients with severe complications [39] and they have been associated with a higher risk of incidence of T2D [40]. These subtypes have also been increased in obese population [41] and were associated with a decrease in insulin sensitivity [42]. Furthermore, there was a decrease in the proportions of some of theme in MEFA related to higher dietary EPA and DHA intakes [43].

The increased of membrane erythrocytes omega-3 FA and decreased omega-6 FA in the erythrocytes membrane result in a decrease in the omega-6/omega-3 FA ratio, which has been associated with a decreased risk of developing T2D by [44]. In other words, considering that the omega-3 index has been proposed as the dietary intake of omega-3 FA [45] from fish consumption [46] and, in particular, from a sardine-enriched diet [20], we can consider it a biomarker of a correct adherence to the nutrition intervention carried out in SG. Also, as demonstrated previously [47], an increase in this index is related with a protective effect against CVD, which would suggest a reduced risk of developing cardiovascular complications in subjects with preDM [48].

Metabolomics has elucidated many aspects of the pathological pathways underlying T2D, which could potentially serve as markers of dysglycemic states and also answer questions regarding the conversion from predDM to T2D.

Based on previous metabolomic studies of nutrition interventions, the metabolites in our study show a very similar circulating pattern among subjects from both groups. Some differences have been observed in CG following a standard T2D-preventive diet. A decrease in circulating TAG, lysophosphatidylethanolamines (LPE), phosphatidylcholines (PC) and lysophosphatidylcholines (LPC) have been observed in CG and can be explained by the weight loss of this group since these metabolites previously have shown positive association with BMI [49]. In particular, the decrease of all these metabolites means a positive change in T2D prevention. TAG and LPE metabolites have been associated with an increased risk of T2D [50,51], Furthermore, a reduction in PC could reduce fasting insulin and improve glucose tolerance [52], as it has been observed that T2D alters PC metabolism [53]. LPC, for its part, induces proinflammatory cytokines [54], which has been related with a higher risk of developing T2D [55].

Previous studies have shown correlations among levels of LPC, PC and also sphingomyelins (SM) in the plasma of obese subjects, supporting the theory of lipotoxicity by which an increased supply through diet leads to an excess of lipid storage in tissues, leading to IR [56]. This may explain why, in our study, SG, following a one-year nutrition intervention implicating a higher daily consumption of lipids, showed elevated levels of certain lipid subtypes. Moreover, our Results are in line with a recent study in a Spanish cohort that concluded that certain LPC, LPE, SM and ChoE were inversely associated with T2D risk [57]. Our results also showed an increase in SG and a decrease in CG of different glycerophospholipids, which were found to be inversely associated with the risk of dysglycemia in the RISC study (Relationship between Insulin Sensitivity and Cardiovascular disease) [58] and were also found to be reduced in subjects with impaired fasting glucose and untreated T2D as compared to healthy controls [59]. Hence, SG showed an increase in DAPC, associated with a higher risk of T2D [60], but this link appears only in certain DAPC subtypes. Specifically, C32:1, C36:1, C38:3, and C40:5 are the T2D risk metabolites subtypes [61] and none of them appear increased in SG.

In addition to an increase in certain circulating essential amino acids, perhaps due to improved protein content in diet, an increase in total BCAA and ArAA has also been studied as a potential predictor of the future development of T2D [62].

High dietary intake of BCAA has been associated with a decrease in T2D risk [63] by increasing the glucose uptake capacity of insulin-sensitive tissues through an improvement in protein anabolism and muscle synthesis [64]. Nonetheless, in obese subjects, an increase in BCAA has been linked with the development of T2D [65] because cause or exacerbate IR through mechanisms involving activation of the molecular target of rapamycin (mTOR) [66]. Moreover, increased levels of BCAA circulating in IR subjects indicate a reduced BCAA catabolism in key tissue [67]. This reduced catabolism is hypothesized to limit tissue concentrations of amino acids derivatives important to normal metabolism [67]. Despite these observed trends, it is important to point out that circulating BCAAs are not only dependent on dietary intake [63].

For their part, ArAAs have be found to be increased in IR obese subjects [68] and in non-T2D individuals who later develop T2D [69], thus they are useful to predict T2D development. In particular, tryptophan and histidine have been linked to obesity and T2D [62] and tyrosine to IR in obesity state [70], while phenylalanine has been proposed as a predictor of both T2D and CVD [71,72]. In our study, we observed an increase in phenylalanine only in CG as well as in total ArAAs and BCAAs, while SG, despite showing an increase in total circulating amino acids, did not show an increase in any of these two metabolic classes. For this reason, we could deduce that a sardine-enriched diet helped create a protective effect against T2D and CVD through circulating amino acids composition. Other amino acids that were only increased only in SG were taurine and glycine. Both have been described as metabolomic markers of a reduced risk of T2D incidence [73] and have shown an effect on the decrease of HbA1c and glucose [74], as well as an inverse relationship with IR [75,76].

We observed an increase in total circulating BA metabolites in SG, which is generally increased in relation to meta-inflammatory diseases, such as obesity and T2D, or in subjects with IR [77]. In spite of this, BA increases in SG were due to increases in GCBA and TCBA (Table 3), which have been associated with fish consumption [78]. BA coupled with either taurine or glycine in the liver is associated with availability of these amino acid substrates, and it is suspected that a taurine-rich diet may increase the concentrations of TCBA [79]. Therefore, the observed levels of augmented taurine in SG could be related to the reported increase in TCBA. On the contrary, despite TCBA having been observed higher in T2D [79], other studies have demonstrated that BA are strongly correlated with adiponectin and inversely with glucose and TAG, as improved insulin sensitivity after weight loss contributes to a relative increase in BA synthesis and absorption [80].

Lifestyle intervention, including diet and physical activity, during 3 years has demonstrated a reversion from preDM to normoglycemia in 23–25% of subjects [81]. In our study, we observed a reversion to normal glucose levels in 31–36% of both groups after only one year of nutrition intervention. Moreover, the

conversion from preDM to T2D in the adult population is 10.6% [82], with an increase observed in population ≥65 years old [4]. At the end of our one-year study, we observed a new-onset T2D of 2.7% and 5.2% in SG and CG, respectively. Both groups decreased HbA1c and glucose levels, as other nutrition intervention studies had previously demonstrated [83]. Moreover, a greater T2D preventive effect was observed in SG following nutrition intervention with a sardine-enriched diet, as determined by FINDRISC scoring. Also, an improvement in lipids profile was observed in SG, in accordance with Results obtained in previous studies with fatty fish in T2D subjects. In particular, as our nutrition intervention with a sardine-enriched diet corroborates, fatty fish has demonstrated a greater effect, compared to lean fish, on decreasing TAG and increasing HDL in T2D subjects [84–86] and overweight/obese subjects with high CVD risk [87–91]. Both increased HDL and decreased TAG have been related with CVD protection [92]. Finally, due to the important role of adiponectin on insulin sensitivity [93], the increase of this hormone observed after nutrition intervention with sardines in SG could translate into a T2D protective effect. Moreover, this can be corroborated by the effects related with the decrease in IR that we observed through improvement in HOMA-IR.

In summary, we conclude that, in comparison with a conventional T2D-prevention diet, a sardine-enriched diet promotes: a decrease in SBP and DBP values; a replacement of the omega-6 FA of the erythrocyte membrane by omega-3 FA; an increase in omega-3 index, which has been proposed as an anti-CV risk marker; changes in circulating metabolites associated with a protective effect and decrease of T2D and CVD risk; an improvement in blood chemistry with a decrease in TAG and an increase in HDL-cholesterol levels, which are associated with low CVD risk; an increase in adiponectin, a hormone whose increase is associated with lower T2D risk and IR; and finally, a decrease in IR as measured by HOMA-IR, along with a decrease in the number of subjects classified as being at a very high risk for developing T2D, according to FINDRISC.

One limitation of the present study is that, despite having included a higher number of participants than the sample calculation indicated, 42 participants did not finish the study. Specifically, 30 participants dropped out before completion. However, the number who abandoned the study was not higher in the sardine group than in the control group, therefore, the prescribed amount of 200gr sardine per week appears to be well-tolerated, even with a long clinical follow-up. Another possible limitation of the study to bear in mind is the use of canned sardines in olive oil, which, despite easing distribution and consumption, present differences in MUFA content as compared to fresh sardines. Although this may have modified the pattern of lipids consumed, the SG group decreased their overall MUFA consumption. This could be due to the fact that the use of olive oil added to meals did not occur when they consumed the canned sardines and, therefore, there are no differences in its consumption with respect to CG. The consumption of canned sardines in olive oil does not interfere with the obtained interventions result, as there were no differences between SG and CG regarding the consumption of MUFA, so all observed study effects must be produced by variations in other nutrients present in sardines.

The evident improvement made by the control group in reducing T2D risk cannot be correlated with the few new cases of T2D appearing in the two groups, as 4 and 2 subjects with new disease occurrence, respectively, are not significantly different data. Follow-up time was long, enabling mechanistic improvements in markers to appear that can indicate improvement in T2D prevent effects is occurring but maybe the follow-up time not enough to indicate normoglycemia reversion classification.

Our data were collected prospectively, eliminating potential recall bias and allowing high quality correlation between clinical symptoms and data recorded.

In conclusion, a one-year, sardine-enriched T2D-preventive diet in an elderly population with preDM exerts a greater protective effect against developing T2D and cardiovascular events, by improving anthropometric parameters, blood chemistry profile, lipid composition in erythrocytes membranes and metabolomics data.

Author statement

DA Díaz-Rizzolo and R Gomis designed the study. DA Díaz Rizzolo and C Colungo performed clinical visits. DA Díaz Rizzolo and A Serra collected samples and codified data. DA Díaz-Rizzolo, A Sisó-Almirall and R Gomis were responsible for interpretation the Results. All of the authors participated in the critical revision of the manuscript. All authors gave final approval of the version to be submitted.

Conflict of interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.03.014.

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