https://doi.org/10.46389/rjd-2023-1195
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2023; volume 30, issue 3, pages 336-344

Original Article

Modulation of hyperglycemia and oxidative stress by purified fish proteins (*Sardina pilchardus*) in type 1 diabetic rats

Nora Benaicheta [1, 2] *, Zoheir Mellouk [1], Malika Bouchenak [1], Dalila Ait Yahia [1], Farida Ouda Boukortt [1]

[1] Department of Biology, Faculty of Natural and Life Sciences, Laboratory of Clinical and Metabolic Nutrition, University Oran 1, Ahmed Ben Bella, Oran, Algeria

[2] Department of Biology, Faculty of Sciences and Technology, University of Relizane, Ahmed Zabana, Relizane, Algeria

* Correspondence to: Benaicheta Nora, Department of Biology, Faculty of Natural and Life Sciences, Laboratory of Clinical and Metabolic Nutrition, University Oran 1, Ahmed Ben Bella, 31100 Oran, Algeria. Phone: 0021341 581944; Fax: 0021341581944; E-mail: nbenaicheta@gmail.com

Received: 16 December 2022 / Accepted: 20 April 2023

Abstract

The aim of this study was to assess the effect of purified fish proteins (*Sardina pilchardus*) on glycemia, serum lipids and redox status in streptozotocin-induced diabetic rats. Diabetes was induced in rats by an intraperitoneal injection of streptozotocin. Diabetic rats were divided into two groups (n=6) and were fed, for 28 days, diets containing 20% casein (CAS) or sardine proteins (SP). The results showed that glycemia lowered since d14 by 22% and remained stable until d28, and HbA1c was reduced by 40% in SP *vs.* CAS. A significant decrease was noted in serum TC and TG concentrations with SP *vs.* CAS. In the SP group, TBARS lowered in serum, liver, kidney, heart and adipose tissue compared to the CAS group. The carbonyl concentration decreased in the liver, kidney and brain, with SP. Nitric oxide concentrations were diminished in the liver, kidney and adipose tissue, whereas the value increased in muscle (+56%). The SOD activity enhanced in the liver, kidney and adipose tissue. The same catalase activity was increased in the liver, kidney and heart in SP *vs.* CAS. SP has a beneficial effect on hyperglycemia. In addition, they have hypocholesterolemia and hypotriglyceridemic effects and attenuate oxidative stress.

Keywords: antioxidant enzymes, carbonyls, nitric oxide, peroxidation, rats, sardine proteins, streptozotocin.

Introduction

Diabetes mellitus (DM) is a severe metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, lipid, and protein metabolism, resulting in defects in the action and secretion of insulin [1].

Type 1 diabetes is a chronic disease that results from an autoimmune destruction of pancreatic -cells. Therefore, insulin deficiency and hyperglycemia are the main outcomes of type 1 diabetes, accounting for 5–10% of all diabetic cases [2].

Dyslipidemia frequently accompanies type 1 diabetes and represents an important component of the disease, imposing cardiovascular risk. The key components of diabetic dyslipidemia are elevated plasma low-density lipoproteins (LDL), triglycerides (TG), and lowered high-density lipoprotein (HDL-C) [3].

In diabetic conditions, hyperglycemia promotes oxidative stress and induces excess generation of reactive oxygen species (ROS) via auto-oxidation of glucose and non-enzymatic protein glycation, which may cause disruption of cellular functions and oxidative damage to membranes [4]. An elevation of ROS can cause an impairment of the antioxidant defense system or an inability to scavenge the oxidative damage [5]. Diet is a key factor in the control of human health. In fact, an important component of a healthy dietary pattern is fish. Fish consumption has been associated with both CVD protective effects [6] and particularly positive health effects of n-3 fatty acids (FA) [7]. Other studies have been focusing on the effects of various fish proteins on body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat-fed rats [8]. Few previous studies concluded the role of fish protein in managing and treating diabetes. It has been demonstrated that

© 2023 The Authors. Romanian Journal of Diabetes, Nutrition and Metabolic Diseases published by Sanatatea Press Group on behalf of the Romanian Society of Diabetes Nutrition and Metabolic Diseases. This is an open access article under the terms of the Creative Commons Attribution License (CC-BY-NC-ND 3.0).

fish proteins and the peptides born of their enzymatic breakdown improved glycemia and insulin sensitivity in rats [9–11] and prevented skeletal muscle insulin resistance [9]. This effect was obtained by improving membrane glucose-transporter in the skeletal muscle of obese rats [12]. In addition, fish proteins have been reported to exert interesting regulatory functions in rats, such as hypoglycemic, hypolipidemic [11, 13], antioxidant [14] and anti-inflammatory effects [10, 15]. Sardine proteins decreased serum glucose and HbA1C in streptozotocin-diabetic rats. In rats with fructose-induced metabolic syndrome, sardine proteins with or without fructose lowered plasma insulin levels and displayed decreased insulin resistance [10]. In type 2 diabetic rats, SP administration reduced glucose concentrations, HOMA-IR and HbA1c and increased glucose tolerance [11].

In oxidative stress, fish proteins played an important role in the antioxidant defense of blood, heart and kidney in spontaneously hypertensive diabetic rats [14]. Some authors have reported the antioxidant activity of fish peptides on human leukocytes and cultured cells [16].

The present study investigated the effects of purified fish protein (*Sardina pilchardus*) on glycemia, serum lipid parameters and tissue redox status in streptozotocin-induced diabetic rats.

Material and methods

Fish proteins preparation

Fresh sardine (*Sardina pilchardus*) from the Mediterranean coast of Oran (fished from October to February) was cleaned, eviscerated, bone removed, filleted, dried and finally pounded. The sardine flour was delipidated (8h, 12 cycles) using hexane: ethyl acetate (1:1, v:v) as a solvent in a Soxhlet-type apparatus, followed by purification of proteins [17]. The purity of proteins was assayed by the Kjeldhal method using a Kjellfossauto analyzer. The composition was 93% of proteins and 0.2% lipids.

Animals and diets

Male Wistar rats (Pasteur Institute, Algeria) 260±10 g were housed in stainless steel cages in a room with controlled lighting (12-h light: dark cycle), constant temperature (24°C) and relative humidity (60%). The rats were fed standard laboratory chow and were allowed free access to food and water for 6 days. After an overnight fast, diabetes was induced in the rats by a single intraperitoneal injection of streptozotocin (STZ)(Sigma, l'Isled'Abeau Chenes, France) (60 mg/kg of body weight) [18] diluted with 0.05 ml/L of citrate buffer (pH 4.5). Fasting glycemia was measured at the beginning of the experiment (d_0) and two days (d_{48h}) after STZ injection from a tail blood sample by using a glucometer and glucostix (Accu-Chek active, Roche Diagnostics, Meylan, Germany). The diabetic animals with blood glucose levels higher than 4.77±0.12mM, 48 hours after streptozotocin injection, were randomly divided into two groups of six rats each and fed, for 4 weeks, a diet containing 20% casein (CAS) or sardine proteins (SP) combined with 5% of mixed oils (3.9% olive, 1% nut, 0.1% sunflower, with n-6/n-3 = 7). The diet composition was previously described by Mellouk *et al.* [13].

Food and water were provided *ad libitum*. Weekly, the animals were weighed, and fasting glycemia was measured.

This study was approved by our Institutional Ethical Committee for Animal Research (agreement number 45/DGLPAG/DVA. SDA.14). The General Guidelines for the Use of Living Animals in Scientific Investigations Council of European Communities were followed [19].

Blood and tissue samples

At four weeks, after overnight food deprivation, the rats were anesthetized with sodium nesdonal (60 mg/kg BW). Blood was withdrawn from the abdominal aorta. The serum was obtained by low-speed centrifugation at 1000 x g for 20 min at 4°C. The liver, heart, kidney, gastrocnemius muscle, brain, and adipose tissue were removed immediately, rinsed with cold NaCl buffer (0.9%), dried and weighed. An aliquot of 100 mg of each tissue was stored at -70°Cuntil analysis. Serum and red blood cell pellets were collected and stored for further biochemical analysis.

Biochemical analysis

Serum glucose, total cholesterol (TC) and triacylglycerols (TG) and glycated haemoglobin (HbA1c) were previously described [11].

Oxidative stress evaluation

Lipid peroxidation

Serum and tissue lipid peroxidation was assayed by estimating the thiobarbituric acid reactive substances (TBARS).

- Serum lipid peroxidation was evaluated according to the method of Quintanilha *et al.* [20] using malondialdehyde (Sigma-Aldrich, l'Isled'Abeau, France) as a standard. One milliliter of diluted serum (protein concentration nearly 2 mg/mL) was added to 2 mL of thiobarbituric acid (TBA) (final concentration 0.017 mM) plus butylated hydroxyl toluene (BHT) (final concentration 3.36 µM) and incubated for 15 min at 100°C. After cooling and centrifugation, the absorbance of the supernatant was measured at 535 nm. Results were expressed as µmol of TBARS/mL of serum;
- Tissue homogenates lipid peroxidation was measured by the method of Salih *et al.* [21]. One hundred mg of tissues (liver, heart, kidney, muscle, brain and adipose tissue) were homogenized in 1.5 ml of trichloroacetic acid (TCA, 10%). Homogenate was incubated with 0.25 mL of TBA (0.02 M) and BHT for 30 min in a boiling water bath, and then the tubes were placed for 45 min in a cold water bath. The absorbance was measured at 532 nm and TBARS concentrations were calculated following the formula:

$$C\ (\mu mol\ MDA/g\ tissue) = ODx10^4 x\ sample\ weight^{-1}\ x\ TCA\ volume\ x\ 0.641\ x\ dilution$$

Protein oxidation in tissue homogenates

Tissues carbonylated proteins were determined according to the method of Levine *et al.* [22] using the 2,4-dinitrophenylhydrazine (DNPH). A sample (1mg protein/ml) was divided into two portions of 1 ml each, a test and a blank. To one portion (test), 0.5 ml of 10 mM DNPH in 2.5 MHCl was added, while to the other portion (blank), 0.5 ml of 2.5 MHCl alone was added. They were then incubated at room temperature for 1h, to the dark, with vortexing every 10 min. The samples were precipitated with 20% trichloroacetic acid (TCA, final concentration), vortexed and centrifuged at 11000 x g, 3 min at 20°C. The supernatant was discarded, and the pellet (with DNPH alone) was washed 3 times with 1 ml of ethanol: ethyl acetate (1:1, v/v). The final pellets were dissolved in 0.6 ml of 6 M guanidine-HCl and left for 15 min at 37°C. The reactive carbonyl content was calculated from its pick absorption around 250–370 nm using a molar absorption coefficient ε=22000.M^{-1}.cm^{-1}.

Nitric oxide (NO) in tissue homogenates

NO determination was performed using the Griess reagent (sulphanilamide and n-naphthyl ethylenediamine) [23]. Homogenate tissues were clarified by zinc sulfate solution. NO_3 was then reduced to NO_2 by cadmium overnight at 20°C under shaking.

Samples were added to the Griess reagent and incubated for 20 min at room temperature. The absorbance was measured at 540 nm. Sodium nitrite was used for the standard curve.

Antioxidant enzymes activity

The superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by kit (Cayman Chemical Company, USA). This activity utilizes a tetrazolium salt to detect superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The absorbance was measured at 450 nm.

The catalase (CAT, EC 1.11.1.6) activity was determined by Aebi. [24] method using the decomposition rate of hydrogen peroxide. The absorbance was measured at 420 nm.

Statistical analysis

Values were expressed as mean±SEM of six rats per group. Statistical analysis was performed using the Student "t" test. Differences were considered significant between the sardine protein group and casein group at $P<0.05$.

Results

Body weight and food intake

The body weight of STZ-induced diabetic rats fed CAS or SP was reduced during the experiment and was similar in the two groups of rats. From d0 until d28, a 15% and 12% reduction was noted in CAS-group and SP-group, respectively. However, there was no change in food intake between both groups (Table 1).

Biochemical analysis

Serum glucose and glycated haemoglobin HbA1C

Serum glucose and HbA1 Clowered significantly by 31% and 40%, respectively, in the diabetic rats who consumed SP compared to CAS (Table 2).

 https://doi.org/10.46389/rjd-2023-1195 © 2023 The Authors

Table 1: Body weight and food intake in diabetic rats at D28 fed casein or sardine proteins.

	CAS	SP
Body weight (g)	202.80±12.00	210.00±10.56
Food intake g.day^{-1}.rat	17.59±4.01	17.53±3.65

Note: Data are mean±SEM of six rats per group. Statistical analysis was performed using the Student t-test. The differences were considered significantly different between sardine proteins (SP)and casein (CAS) at P<0.05.

Serum total cholesterol (TC) and triacylglycerols (TG) concentrations

A significant decrease was noted in serum TC (26%) and TG (12%) concentrations with sardine proteins *vs.* casein (Table 2).

Oxidative stress evaluation

Serum and tissues TBARS contents

Serum TBARS concentrations were decreased by 28% in the SP group *vs.* the CAS group (Table 2). Liver, kidney, heart and adipose tissue TBARS contents were respectively reduced by 31%, 57%, 17% and 24%, with the SP diet compared to the CAS diet. Both groups had no significant brain and muscle differences (Figure 1).

Tissues carbonyls and NO concentrations

Carbonyl concentrations were decreased in the liver (18%), kidney (28%) and brain (15%) of rats consuming sardine proteins compared to those consuming casein, whereas there was no significant difference in muscle and heart (Figure 2).

Tissue NO contents lowered in the liver (51%), kidney (37%) and adipose tissue (37%), while an increase was noted in muscle (56%) in the SP group compared to the CAS group (Figure 3).

Antioxidant enzyme activities

SP diet, compared to the CAS diet, enhanced SOD activity in the liver (61%), kidney (77%) and adipose tissue (66%). No difference was noted in the heart, brain and muscle (Figure 4). Catalase activity was increased by 63%, 14% and 26%, respectively, in the liver, kidney and heart in the sardine proteins group than in the casein group (Figure 5).

Discussion

The effect of purified sardine protein intake was investigated on the modulation of redox status in streptozotocin-induced diabetic rats.

The present study showed a decreased body weight in rats fed casein or sardine proteins from the beginning of the experiment to d28 and remained similar in both groups of rats, whatever the diet consumed.

After 48h of STZ injection, high glycemia was observed in both groups of rats. Nevertheless, from d14 to d28, a significant decrease in glycemia was observed in rats who consumed sardine proteins diet compared to the casein group. These results were similar to those of Lavigne *et al.* [25], who showed that cod proteins lowered fasting and postprandial plasma glucose in normoglycemic rats. Indeed, fish proteins improved peripheral

Table 2: Serum glucose, glycated haemoglobin (HbA_1C), serum total cholesterol (TC),triacylglycerols(TG) and TBARSin diabetic rats fed casein or sardine proteins.

	CAS	SP
Glucose (mM)	1.73±0.12	1.19±0.07*
HbA_1C (%)	11.13±0.93	7.13±1.06*
TC (mM)	2.79±0.10	2.06±0.21*
TG (mM)	1.09±0.04	0.96±0.02*
TBARS (µmol.ml^{-1})	6.56±0.49	4.70±0.36*

Note: Data are mean±SEM of six rats per group. Statistical analysis was performed using the Student t-test. The differences were considered significantly different between sardine proteins (SP) and casein (CAS) at P<0.05.

© 2023 The Authors

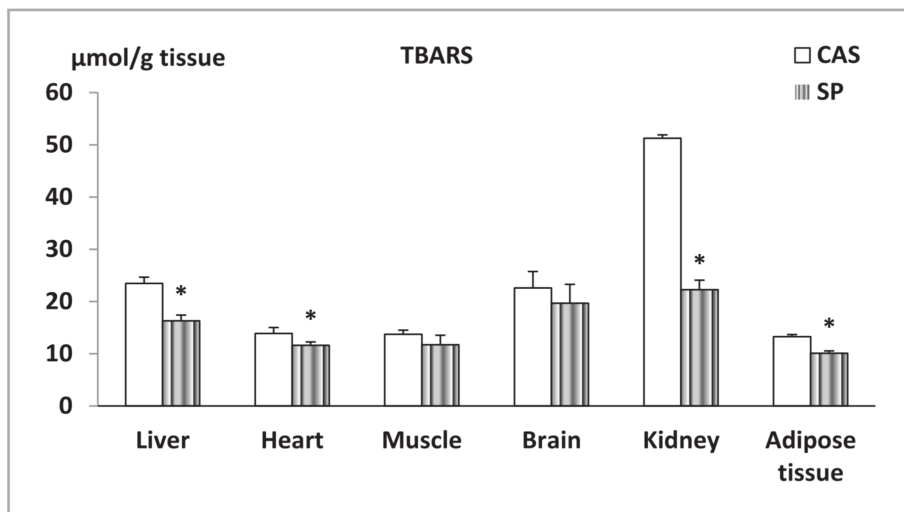

Figure 1: Tissue TBARS concentrations in diabetic rats. Data are mean±SEM of six rats per group. Statistical analysis was performed using the Student t-test. The differences were considered significantly different between sardine proteins(SP)and casein (CAS) at P<0.05.

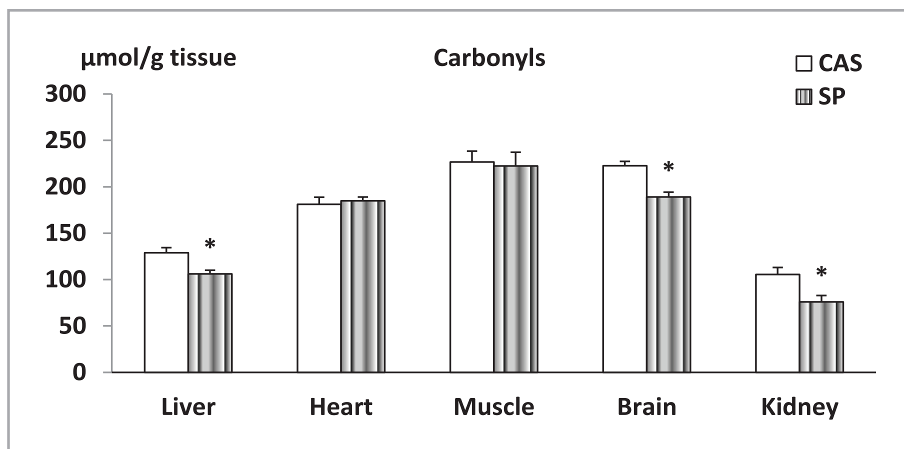

Figure 2: Tissues carbonyl contents in diabetic rats. Data are mean±SEM of six rats per group. Statistical analysis was performed using the Student t-test. The differences were considered significantly different between sardine proteins (SP)and casein (CAS) at P<0.05.

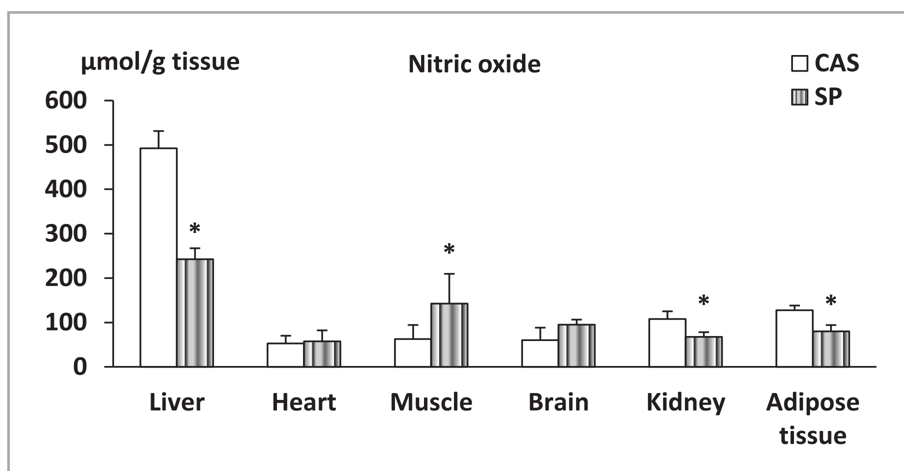

Figure 3: Tissues nitric oxide (NO) contents in diabetic rats. Data are mean±SEM of six rats per group. Statistical analysis was performed using the Student t-test. The differences were considered significantly different between sardine proteins (SP)and casein (CAS) at P<0.05.

https://doi.org/10.46389/rjd-2023-1195
© 2023 The Authors

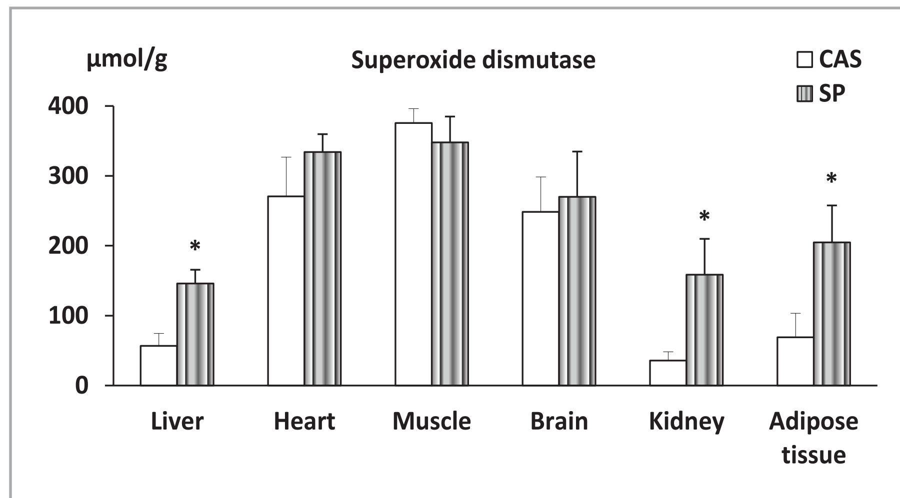

Figure 4: Tissues superoxide dismutase (SOD) activity in diabetic rats. Data are mean±SEM of six rats per group. Statistical analysis was performed using the Student t-test. The differences were considered significantly different between sardine proteins (SP)and casein (CAS) at P<0.05.

insulin sensitivity in obese rats [12] and humans [15]. This effect might result from the enzymatic breakdown of sardine proteins, yielding biologically active peptides stimulating insulin action by improving glucose peripheral uptake, particularly by skeletal muscle. Another hypothesis was that these peptides might regenerated pancreas cells destroyed by the streptozotocin. On the other hand, it has been shown that amino acids from ingested proteins might play a role in hormonal response to feeding. Thus, amino acids such as arginine, lysine, phenylalanine, ornithine, alanine, leucine and isoleucine stimulate insulin secretion [26].

Glycated hemoglobin quantification, principally its major fraction the hemoglobin A1c (HbA1c), is used for retrospective glycemic balance evaluation in diabetes mellitus. The glycation was accelerated in response to the hyperglycemia and was implicated in the pathogenesis of chronic complications. The results showed a significant decrease of HbA1c concomitant by the glycemia reduction in diabetic rats fed sardine proteins compared to those fed casein. These results corroborated with those obtained by authors who reported that amino acids such as lysine, glycine, alanine, glutamic and aspartic acids decreased the crystalline lens protein glycation in men, and likewise, it decreased glycemia [27]. In addition, *in vitro* studies on human vitreous collagen showed that glycation and glycoxidation were inhibited by lysine [28].

In diabetes, dyslipidemia was frequently observed with hyperglycemia and elevated HbA1c. Our study showed that feeding sardine proteins compared to casein lowered serum total cholesterol (TC). This result

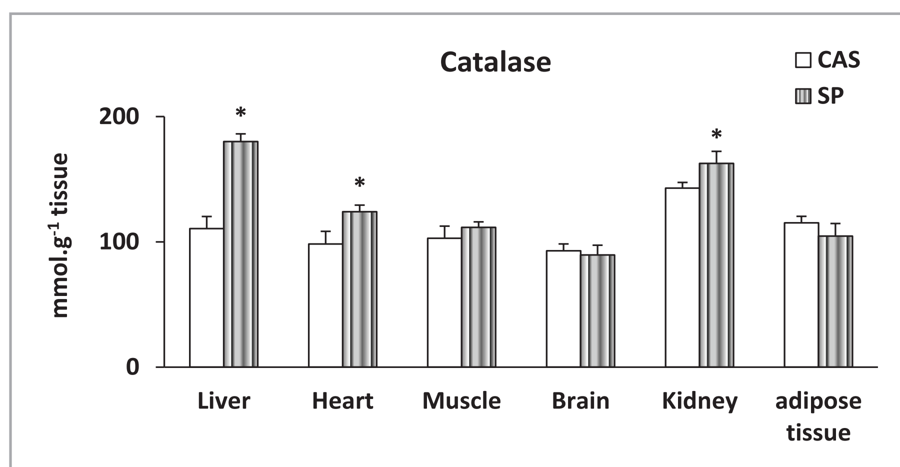

Figure 5: Tissues catalase (CAT) activity in diabetic rats. Data are mean±SEM of six rats per group. Statistical analysis was performed using the Student t-test. The differences were considered significantly different between sardine proteins (SP)and casein (CAS) at P<0.05.

was similar to those of Shukla *et al.* [29], who reported the hypocholesterolemic effect of fish proteins in rats and rabbits. Dietary proteins regulate lipid metabolism, slow lipids' absorption and synthesis, and promote lipid excretion [30]. This effect was due to the significant increase in fecal cholesterol excretion with the SP diet compared to the CAS diet [31]. Indeed, the hypocholesterolemic effect of SP was probably due to the hepatic cholesterol diminution, which was due to the biliary acids synthesis stimulation. The gene expression data suggested strongly that the cholesterol-lowering effect of fish proteins might be caused by an up-regulation of mRNA of cholesterol 7α-hydroxylase (CYP7A1), the key enzyme in the conversion of cholesterol to bile acids, in pigs [31]. This led to increased fecal excretion, the major cholesterol excretion route. Fish proteins are easily digestible and rich in essential amino acids, and animal studies suggest that fish protein may affect both plasma and liver lipids [29]. It has been suggested that individual amino acids are involved in the hypolipidemic effects of dietary proteins [29]. High levels of cysteine, high arginine: lysine and low methionine: glycine ratios are supposed to be responsible for the lipid-lowering effect [32]. Indeed, sardine proteins are characterized by high concentrations of cysteine and arginine compared to casein [33]. Serum TG concentrations were diminished in the SP group *vs*. the CAS group. This result was similar to those obtained by Demonty *et al.* [34]. Indeed, hepatic TG value was positively correlated with those of serum [34]. This reduction may be explained by the lowered circulating VLDL. The same result was reported by Boukortt *et al.* [14] in streptozotocin-induced diabetes in spontaneously hypertensive (SHR) rats.

In another way, it is well established that oxidative stress, resulted from the imbalance between free radical production and its scavenging by antioxidant defenses, contributes to cardiovascular disease initiation and progression. In diabetes mellitus, glucose auto-oxidation, protein glycation and advanced glycation end products interaction with its specific macrophagic receptors represent the principal mechanism leading to elevated free radical production and tissue damage. It has been demonstrated that lipid peroxidation products, among which thiobarbituric acid reactive substances (TBARS), were increased in plasma [35] in STZ-induced diabetes. Several enzymatic hydrolysates of fish proteins or prawns have antioxidant activity, which was demonstrated by *in vitro* and ex-cellular lines, and active peptides were identified [36]. In our study, the results obtained revealed that serum TBARS concentrations decreased with SP compared to casein. The same effect was observed in diabetic hypertensive rats [14]. This was related to the diminution of serum TC and TG concentrations, leading to less lipid peroxidation. Our results showed that TBARS concentrations lowered, particularly in the liver, heart, kidney and adipose tissue, in the SP group *vs*. CAS group.

Moreover, increased CAT activity was noted in the liver, heart and kidney. This indicated a compensatory response to oxidative stress due to elevated H_2O_2 endogen production. Mendis *et al.* [37] reported that in cultured cells, antioxidant enzyme activity was elevated in the presence of peptides from the enzymatic breakdown of fish protein and suggested that this peptide may be implicated in maintaining the cellular redox balance. In diabetes, free radicals enhancement was the cause of amino acid oxidation, leading to unstable carbonyl intermediates [38]. In the diabetic group fed sardine proteins compared to the group fed casein, carbonyl contents, which represented protein oxidation index, were significantly reduced in the liver, kidney, and brain. These results suggested that by lowering oxidative stress, fish proteins appeared to be effective in protecting organs, especially the kidney and brain, against diabetic complications.

NO is known to react with superoxide anion, forming a highly aggressive peroxynitrite radical, which can cause cytotoxicity and DNA damage through lipid peroxidation [39]. This NO alteration is due to its low bioavailability by free radicals, particularly superoxide anion [40]. Our results showed a significant reduction of NO concentration in liver, kidney and adipose tissue, whereas a high value was noted in skeletal muscle in diabetic rats fed SP compared to those fed CAS. Increased oxidative stress and inflammation are the major causes of increased NO quenching in obesity and diabetes. Uncoupled eNOS produces superoxide anion instead of NO, which rapidly combines with NO to produce peroxynitrite [41, 42]. In addition, the role of glucose levels in NO production is controversial; it has been reported that NOS activity and, subsequently, NO production gradually increase due to an elevation in glucose concentrations within the pancreatic islets [43]. This might be explained by certain antioxidant enzyme activities as well as SOD in the liver, kidney and adipose tissue. SOD is considered an essential primary antioxidant enzyme that reacts to the organism's defense against the cellular metabolism of toxic products. It can scavenge superoxide anion by dismutation. Its role is to transform, in mitochondria, the superoxide radical in hydrogen peroxide, itself reduced by catalase (CAT).

 https://doi.org/10.46389/rjd-2023-1195 © 2023 The Authors

Conclusion

Compared to casein, Sardine proteins have a hypoglycaemic effect and reduce the glycated hemoglobin value, indicating an improvement of abnormal glucose metabolism and reducing its toxicity. This effect constitutes one of the ways to control cardiovascular risk factors and the diabetes evolution to its complications.

The hypocholesterolemic and hypotriglyceridemic effects of fish proteins restore lipid profile impairment generated by STZ injection. They may protect against atherosclerosis risk. On the other hand, SP, compared to CAS, decreases lipid peroxidation (TBARS) in serum, liver, heart, kidney and adipose tissue. They reduce carbonyl content in the liver, kidney and brain. Moreover, nitric oxide concentrations are reduced in the liver, kidney and adipose tissue and enhanced in muscle. Concerning the antioxidant enzyme activities, the study showed that SOD activity increases in the liver, kidney and adipose tissue. CAT activity is elevated in the liver, kidney and heart in the SP group compared to the CAS group. Fish proteins exert antioxidant activities and protect the tissues specifically against free radicals generated by type 1 diabetes.

Sardine proteins, compared to casein, are hypoglycaemic, hypocholesterolemic and hypotriglyceridemic. Moreover, they effectively protect against radical attack by stimulating antioxidant systems. All these beneficial effects contribute to the decrease of metabolic and vascular complications related to diabetes mellitus.

It may be interesting, in the future, to use sardine protein as a functional food to prevent diabetic complications.

Conflict of interest

The authors declare no conflict of interest.

References

1. Lupascu, F.G., Giusca, S.E, Caruntu ID, Anton, A., Lupusoru, C.E and Profire, L. (2019). The safety profile of new antidiabetic xanthine derivatives and their chitosan based formulations. European Journal of Pharmaceutical Sciences. 127: 71-78.
2. Lin, C., Shih, Z., Ku, Y., Huang, G., Tu, P., Shih, C. (2018). Antidiabetic and antihyperlipidemic effects of the flower extract of Eriobotrya japonica in streptozotocin-induced diabetic mice and the potential bioactive constituents in vitro. Journal of Functional Foods. 49: 122-136.
3. Ansar, S., Koska, J., Reaven, PD. (2011). Postprandial hyperlipidemia, endothelial dysfunction and cardiovascular risk: focus on incretins. Cardiovascular Diabetology. 10-61
4. Sami, W, Ansari, T., Butt, N.S., Hamid, M.R.A. (2017). Effect of diet on type 2 diabetes mellitus: a review. International Journal of Health Sciences. 11: 65-71.
5. Sagoo, M.K., Gnudi, L. (2018). Diabetic nephropathy: Is there a role for oxidative stress. Free Radical Biology and Medicine. 116: 50-63.
6. Zheng, J., Huang, T., Yu.Y., Hu, X., Yang, B., Li D. (2012). Fish consumption and CHD mortality: an updated meta-analysis of seventeen cohort studies. Public Health Nutrition. 15: 725-737.
7. Mozaffarian, D., Wu, J.H. (2011). Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. Journals of the American College of Cardiology. 58: 2047-2067.
8. Pilon, G., Ruzzin, J., Rioux, L.E., Lavigne, C., White, P.J., Frøyland, L., Jacques, H., Bryl, P., Beaulieu, L., Marette, A. (2011). Differential effects of various fish proteins in altering body weight, inflammatory status, and insulin sensitivity in high-fat-fed rats. Metabolism. 60: 1122-1130.
9. Lavigne, C., Tremblay, F., Asselin, G., Jacques, H., Marette, A. (2001). Prevention of skeletal muscle insulin resistance by dietary cod protein in high fat-fed rats. American Journal of Physiology-Endocrinology and Metabolism. 28: 62-71.
10. Madani, Z., Louchami, K., Sener, A., Malaisse, W.J., AitYahia, D. (2012). Dietary sardine protein lowers insulin resistance, leptin and TNF-α and beneficially affects adipose tissue oxidative stress in rats with fructose-induced metabolic syndrome. International Journal of Molecular Medicine. 29: 311-318.
11. Benaicheta, N., Labbaci, F.Z., Bouchenak, M., Boukortt, F.O. (2016). Effect of sardine proteins on hyperglycaemia, hyperlipidaemia and Lecithin: cholesterol acyltransferase activity, in high-fat diet-induced type 2 diabetic rats. British Journal of Nutrition. 115: 6-13.
12. Tremblay, F., Lavigne, C., Jacques, H., Marette, A. (2003). Dietary cod protein restores insulin-induced activation of phosphatidylinositol 3-kinase/Akt and GLUT4 translocation to the T-tubules in skeletal muscle of high-fat-fed obese rats. Diabetes. 52: 29-37.
13. Mellouk, Z., Ait Yahia, D., Boukortt, F.O, Benaicha, N., Madani, Z., Bouchenak, M. (2009). Dietary sardine (Sardina pilchardus) protein attenuates hyperglycemia and hyperlipidemia and ameliorates tissue morphology changes in strptozotocin-induced diabetic rats. Metabolism Functional Research and Diabetes. 2: 45-54.
14. Boukortt, F., Girard, A., Prost, J., AitYahia, D., Bouchenak, M., Belleville, J. (2004). Fish protein improves the total antioxidant status of streptozotocin-induced diabetes in spontaneously hypertensive rat. Medical Science Monitor. 10: 397-404.
15. Ouellet, V., Weisnagel, S.J., Marois, J.J., Julien, P., Gougeon, R., Tchernof, A., Holub, B. J., Jacques, H. (2008). Dietary cod protein reduces plasma C-reactive protein in insulin-resistant men and women. Journal of Nutrition. 138: 2386-2391.
16. Mendis, E., Rajapakse, N., Kim, S.K. (2005). Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatine hydrolysate. Journal of Agricultural and Food Chemistry. 53: 581-587.
17. Udeland, I., Kelleher, S.D., Hultin, H.O. (2002). Recovery of functional proteins from herring (Clupeaharengus) light muscle by an acid or alkaline solubilization process. J Agric Food Chem. 50: 7371-7379.

© 2023 The Authors

18. Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiological Research. 50: 537-546.
19. Council of European Communities. "Council instructions about the protection of living animals used in scientific investigations". Official J 1987 L358 of 18-12-1986. Corrigendum Official J L117 of 05-05-1987. 1987.
20. Quintanilha, A.T., Packer, L., Szyszlo, J.M., Racanelly, T.L., Davies, K.J. (1982). Membrane effects of vitamine E deficiency bioenergetic and surface charge density studies of skeletal muscle and liver mitochondria. Annals of the New York Academy of Sciences. 393: 32-47.
21. Salih, A.M., Smith, D.M0, Price, J.F., Dawson, L.E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation. Poultry Science. 66: 1483-1488.
22. Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W., Shaltiel, S. (1990). Determination of carbonyl content in oxidatively modified proteins. Methods in Enzymology. 186: 464-478.
23. Cortas, N., Wakid, N. (1990). Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clinical Chemistry. 36: 1440-1443.
24. Aebi, H. (1974). Catalase. In: Methods of Enzymatic Analysis (Ed H.U. Bergmeyer). 2nd edition. Verlagchemie GmbH. Weinheim. 2: 673-684.
25. Lavigne, C., Marette, A., Jacques, H. (2000). Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. Am J Physiol Endocrinol Metab. 278: 491-500.
26. Van Loon, L.J., Saris, W.H., Verhagen, H., Wagenmakers, A.J. (2000). Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. American Journal of Clinical Nutrition. 72: 96-105.
27. Sulochana, K.N., Lakshmi, S., Punitham, R., Arokiasamy, T., Sukuman, B., Ramakrishman, S. (2002). Effect of oral supplementation of free amino acid in type 2 diabetic patients. A pilot clinical trial. Medical Science Monitor. 8: 131-137.
28. Sulochana, K.N., Ramprasad, S., Coral, K., Lakshmi, S., Punitham, R., Narayanasamy, A., Sivaramakrishna, R. (2003). Glycation and glycoxidation studies in vitro on isolated human vitreous collagen. Medical Science Monitor. 9: 220-224.
29. Shukla, A., Bettzieche, A., Hirche, F., Brandsch, C., Stang,l G.I., Eder, K. (2006). Dietary fish protein alters blood lipid concentrations and hepatic genes involved in cholesterol homeostasis in the rat model. British Journal of Nutrition. 96: 674-682.
30. El Khoury, D., Anderson, G.H. (2013). Recent advances in dietary proteins and lipid metabolism. Current Opinion Lipidology. 24: 207-213.
31. Spielmann, J., Kluge, H., Stang, l. GI., Eder, K. (2008). Hypolipidaemic effect of potato protein and fish protein in pig. Journal of Animal Physiology and Animal Nutrition. 93: 400-409.
32. Lin, C.C., Yin, M.C. (2007). Effect of cystein-containing compounds on biosynthesis of triacylglycerols and cholesterol and anti-oxidative protection in liver from mice consuming a high-fat diet. British Journal of Nutrition. 99: 37-43.
33. Souci, S.W., Fachmann, W., Krant, H. (2000). La composition des aliments. Tableaux des valeurs nutritives. 6ième édition CRC Press. Medpharm Scientific Publishers. 5-1182.
34. Demonty, I., Deshaies, Y., Lamarche, B., Jacques, H. (2003). Cod protein lowers the hepatic triglyceride secretion rate in rats. Journaf of Nutrition. 33: 1398-1402.
35. Stephens, J.W., Khanolkan, M.P., Bain, S.C. The biological relevance and measurement of plasma markers of oxidative stress in diabetes and cardiovascular disease. (2009). Atherosclerosis. 202: 321-329.
36. Bouglé, D. (2007). Peptides fonctionnels marins. Phytothérapie. 2: 1-5.
37. Mendis, E., Rajapakse, N., Kim, S.K. (2005). Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. Journal of agricultural and food chemistry. 53: 581-587
38. Levine, R.L. (2002). Carbonyl modified proteins in cellular regulation. Aging and disease. Free Radical Biology. 32: 790-796.
39. Tipoe, G..L, Leung, T.M., Liong, E.C, Lau, T.Y.H., Fung, M.L., Nanji, A.A. (2010). Epigallocatechin-3-gallate (EGCG) reduces liver inflammation, oxidative stress and fibrosis in carbon tetrachloride (CCl4)-induced liver injury in mice. Toxicology. 273: 45-52.
40. Bagi, Z., Koller, A., Kaley, G. (2003). Superoxide-NO interaction decreases flow- and agonist-induced dilations of coronary arterioles in Type 2 diabetes mellitus. American Journal of Physiology-Heart and Circulatory Physiologyl. 285: 1404-1410.
41. Murad, F. (2006). Shattuck Lecture: Nitric oxide and cyclic GMP in cell signaling and drug development, New England Journal of Medicine. 355: 11.
42. Kobayashi, J. (2015). Nitric oxide and insulin resistance. Immunoendocrinology. 2: 10.
43. Henningsson, R., Salehi, A, Lundquist, I. (2002). Role of nitric oxide synthase isoforms in glucose-stimulated insulin release. American Journal of Physiology Cell Physiology. 283: pp. 296-304.

 https://doi.org/10.46389/rjd-2023-1195 © 2023 The Authors