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Essential fatty acid-rich diets protect against striatal oxidative damage induced by quinolinic acid in rats

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Essential fatty acids have an important effect on oxidative stress-related diseases. The Huntington's disease (HD) is a hereditary neurologic disorder in which oxidative stress caused by free radicals is an important damage mechanism. The HD experimental model induced by quinolinic acid (QUIN) has been widely used to evaluate therapeutic effects of antioxidant compounds. The aim of this study was to test whether the fatty acid content in olive- or fish-oil-rich diet prevents against QUIN-related oxidative damage in rats. Rats were fed during 20 days with an olive- or a fish-oil-rich diet (15% w/w). Posterior to diet period, rats were striatally microinjected with QUIN (240 nmol/μl) or saline solution. Then, we evaluated the neurological damage, oxidative status, and gamma isoform of the peroxisome proliferator-activated receptor (PPARγ) expression. Results showed that fatty acid-rich diet, mainly by fish oil, reduced circling behavior, prevented the fall in GABA levels, increased PPARγ expression, and prevented oxidative damage in striatal tissue. In addition none of the enriched diets exerted changes neither on triglycerides or cholesterol blood levels, nor or hepatic function. This study suggests that olive- and fish-oil-rich diets exert neuroprotective effects.

Keywords: Essential fatty acids, Olive oil, Fish oil, Oxidative damage, Huntington's disease

Introduction

Selective vulnerability of neuronal system is a remarkable characteristic of degenerative disorders including Parkinson's disease, Huntington's disease (HD), and amyotrophic lateral sclerosis.[1] In the last three decades, the study of nutritional factors and its influence on health, particularly in neurodegenerative disorders, has gained relevance. Mediterranean diet consists in high consumption of olive- and fish oils; even when it represents a high proportion of fat ingestion (40% of total calories), both olive- and fish-oil supplementation have been related with prevention against the loss of nerve conduction velocity in diabetic neuropathy,[2] and abrogation of the tau protein fibrillation.[3] Fish- and olive oils contain omega-3 [eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6)] and omega-9 (oleic acid; 18:1) fatty acids. Interestingly, reduction in DHA has been associated with cognitive and behavioral performance impairment, which is particularly relevant during brain development and protection against oxidative stress.[4]

The antioxidant effect of the oleic acid *per se* or contained in extra virgin olive oil diet has been

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demonstrated in different experimental models of HD. Tasset *et al.*[5] reported a significant lipid peroxidation (LP) reduction in rats injured by 3-nitropropionic acid (3-NP), under olive oil diet with high oleic acid content. Similarly, Alconchel-Gago *et al.*[6] demonstrated that oleic acid also prevented the depletion of reduced glutathione in the same 3-NP model.

Even when animals, including humans, are capable of synthesizing long-chain polyunsaturated fatty acids (LCPUFAs), dietary supplementation enhances their incorporation into membrane phospholipids, where fatty acids can be released from and lately can be metabolized and/or activate nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) to produce a wide array of effects.[7] LCPUFAs have been implicated in growth and function of healthy nervous tissue[8] and they are ligand-activated transcription factors, which belongs to the PPAR nuclear receptors superfamily.[9,10]

Clinical studies have reported that HD patients exhibited a significant improvement in motor execution and cognition after an oral supplementation with polyunsaturated fatty acid (PUFA), specifically linoleic acid and ethyl-EPA.[11,12] Evidence also showed that supplementation with essential fatty acids (EFAs) and PUFAs decreased the involuntary movements induced by antipsychotic drugs.[13,14] On the other hand, supplementation with EFAs in diet of HD transgenic mice prevented motor deficit.[15] Although the accurate mechanism for the neuroprotective action of ethyl-EPA and omega-3 fatty acids in HD remains unknown, it has been postulated that EFAs could be able to stabilize membranes and inhibit apoptosis.[16] Additionally EFAs could prevent oxidative stress, as it has been documented in both, experimental models (e.g. HD transgenic mice and quinolinic acid (QUIN); *N*-methyl-D-aspartate (NMDA) agonist-induced HD rats) and clinical studies.[17–20] Up to date, no effective treatment to ameliorate the mental and motor declination in HD patients exists. Based on this background, we aimed to test the effect of an olive- or fish-oil-enriched diet on the prevention of QUIN-induced oxidative damage in rats and to characterize the effect of such diet on PPARs expression.

Materials and methods

Materials

QUIN, quinine standard, apomorphine hydrochloride, 3-mercaptopropionic acid (3-MPA), and β-mercaptoethanol were obtained from Sigma Chemical Co. (St Louis, MO, USA); methanol and chloroform from J.T. Baker (Edo de México, Mexico), extra virgin olive oil from Filippo Berio™ (manufactured in the region of Massarosa in the province of Italy and imported by Marinter S.A. de C.V., Mexico City, Mexico), and fish oil from Farmacia 'París' S.A. de C.V. (México) were used. All other reagents were of the best available quality and obtained from known commercial sources. The solutions were prepared using deionized water obtained from a Milli-RQ purifier system (Millipore Co., MA, USA).

All animal care and experimental procedures were performed in compliance to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals[21] and were approved by our local Ethics Committee[22] (Instituto Nacional de Neurología 'Manuel Velasco Suárez' Mexico City, protocol 74/12). In addition, every effort was made to minimize animal pain and suffering. A minimal number of rats were used to obtain statistical significance.

Fatty acid composition analysis by gas chromatography

Lipids contained in olive- or fish oil and in synaptosomes were obtained as previously reported.[23] Fatty acids were extracted in a chloroform/methanol (2:1v/v) mixture containing 0.002% of butylated hydroxytoluene. Lipidic residue in chloroformic phase was evaporated by a nitrogen continuous flow. A derivation was performed by incubating lipidic residue with methanol and H_2SO_4 2% for 2 hours at 90°C and fatty acid methyl esters (FAMEs) were formed. FAMEs were analyzed in a Shimadzu gas chromatographic system with flame detection on a capillary column SP2330 (25 m × 0.25 mm i.d.). Oven temperature was isothermally maintained at 195°C and helium gas flow rate of 1 ml/min was used to carry the samples. The concentration of each fatty acid was calculated using a known concentration of heptadecanoic acid (C17:0) as an internal standard.[24,25]

Animals care and fatty acid-rich diet

One hundred and sixty male Wistar rats aged 7–8 weeks (weighing 140–160 g) breed and raised in our facilities were used. Animals' age and weight were selected based on the weight gain that 20 days of a fatty acid-rich diet produces, reaching at the end of the diet an appropriate weight (200–250 g) to perform the intrastriatal injection of QUIN using the Paxinos and Watson atlas reference coordinates.[26] Three days before the beginning of the study, five animals were placed *per cage* with free access to a standard commercial rat chow diet (laboratory rodent diet 5001, PMI Feeds Inc., Richmond, IN, USA) and drinking water from glass bottles. Housing room was maintained under constant conditions of temperature (25 ± 3°C), humidity (50 ± 10%), and lighting (12/12-hour light/dark cycle). Rats were randomly divided into three

experimental groups of six rats each. The first group received control standard diet, the second group received olive-oil-rich diet (15% w/w), and the third group received fish-oil-rich diet (15% w/w).

Fatty acid-rich diets were prepared by fractioning pellets of standard rat chow diet and mixing them with olive- or fish oil 15% (w/w) until full oil impregnation. Both diets were daily rationed, packaged in plastic bags flushed with nitrogen to minimize oxidation, sealed, and stored at 4°C. Diet was given for 20 days,[24] body weight from all animal groups was recorded every 5 days. At the end of diet period, blood biochemical parameters like glucose, cholesterol, and triglyceride levels were determined from a drop of blood obtained from the tail of each rat, using the One Touch TM II system (LifeScan, Inc., Milpitas, CA, USA). Additionally, activities of alanine aminotransferase (ALT),[25] gamma-glutamyl transpeptidase (GGT),[27] and alkaline phosphatase,[28] as specific markers of liver damage, were assayed in serum from blood samples obtained by cardiac puncture.

Synaptosomes isolation

Synaptosomal fractions were obtained from whole rat brain, after control and olive- and fish-oil supplementation group according to previously reported method.[29] We measured the fatty acid composition in synaptosomes from each different treated group by gas chromatography according the method described above.

QUIN intrastriatal injection

At the end of fatty acid-rich diet, corresponding to twenty-first day, rats were deep anesthetized with sodium pentobarbital (40 mg/kg i.p.), as previously reported,[19] to receive an intrastriatal injection of 1 µl of QUIN (240 nmol/µl) or 1 µl of sterile saline (SS). QUIN or SS was injected into the right striatum using the Paxinos and Watson atlas reference coordinates, 0.5 mm anterior to bregma, 2.7 mm lateral to the mid-line, and 4.5 mm ventral to *dura mater*.[27] Half of rats from each group were sacrificed by decapitation 2 hours after QUIN injection and their striata were dissected out on ice and stored at −75°C until assays were done. The remaining rats were preserved for circling behavior (CB) evaluation.

CB evaluation

CB was assessed 6 days after QUIN-induced striatal lesion by injecting apomorphine (APM). APM administration (1 mg/kg, s.c.) evokes CB toward the QUIN-induced injured side. The number of complete turns per hour was recorded for each animal. APM was diluted in L-(+) ascorbic acid solution (5.7 mM) to prevent oxidation. Control group animals were microinjected intrastriatally with SS.

Striatal gamma-aminobutyric acid measurement

Gamma-aminobutyric acid (GABA) content was determined by high-performance liquid chromatography (HPLC) coupled to a fluorescence detector (1100 series, Agilent), as previously described.[30] Seven days after QUIN injection (1 day after CB evaluation), rats were injected in the caudal vein (i.v.) with 3-MPA (1.2 mmol/kg), a glutamate decarboxylase inhibitor, to prevent *post mortem* changes in GABA levels. One minute later, animals were sacrificed and the striatum was obtained. Samples were stored at −70°C until analysis. The striatum tissue was homogenized in 1 ml 85% HPLC-grade methanol by using an ultrasonic processor (130 W, 40% amplitude, 10–15 seconds); samples were then centrifuged at 18 500 × g, 15 minutes, at 4°C, and supernatants were pre-column derivatized with *ortho*-phthalaldehyde reagent prior to analysis.

LP determination

LP was quantified in the striatum collected 2 hours after the QUIN or SS injection. To assess LP, the formation of lipid-soluble fluorescence compounds was measured as previously described by Pérez-Severiano *et al*.[31] Dissected lesioned striatum was homogenized in 3 ml of deionized water and 1-ml aliquots were added to 4 ml of chloroform/methanol (2:1 v/v). Samples were then stirred and placed on ice for 30 minutes in a dark room; the fluorescence of the chloroform phase was measured in a Perkin Elmer LS50B Luminescence Spectrophotometer using 370 nm of excitation and 430 nm of emission wavelengths. The sensitivity of the spectrophotometer was adjusted to 140 fluorescence units with a calibrating solution (0.001 mg/ml of quinine standard prepared in 0.05 M sulfuric acid). Final results are expressed as relative fluorescence units (RFU) per milligram of protein. Striatal protein content was measured by the Lowry assay method.[32]

PPARs detection by western blot

We carried out the western blot as previously described.[33] Briefly, 2 hours after QUIN- or SS-injection striata were dissected and homogenized in 250 µl of lysis buffer (50 mM Tris–HCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% β-mercaptoethanol, pH 7.5) containing a cocktail of protease inhibitors (100 µM leupeptin, 1 mM phenylmethylsulfonyl fluoride, 2 µg/ml aprotinin, 10 µg/ml soybean trypsin inhibitor) and 0.1% v/v Nonidet NP-40. Western blots were carried out using 50 µg of protein per lane on an 8% SDS–polyacrylamide gel and transferred onto polyvinylidene fluoride membrane (PVDF; Millipore Corporation, Billerica, MA, USA). After that, PVDF membrane was blocked with PBS containing

5% skim milk and 0.05% Tween for 1 hour at room temperature. Then, it was incubated overnight at 4°C with a monoclonal antibody against PPARγ (Santa Cruz Biotechnology, Dallas, TX, USA) at a 1:250 dilution. The membrane was then washed and incubated with the secondary goat anti-mouse peroxidase-labeled antibody (Zymed, Grand Island, NY, USA), diluted to 1:6000 in the blocking solution for 1 hour at room temperature. Blots were washed and protein was developed using enhanced chemiluminescence detection system according to the manufacturer's instructions (Western Lightning Ultra, NEL112001EA, PerkinElmer, Waltham, MA, USA). The blots were stripped and, as a control, β-actin levels were determined using a monoclonal antibody. Film images were digitally acquired with a BioDoc-It System (UVP) and a densitometry analysis was performed using the LabWorks (UVP Inc., Upland, CA, USA). Data are expressed as normalized optical density arbitrary units.

Statistical analysis

Results are presented as mean ± SEM. The statistical comparison in the blood analysis and serum hepatic enzyme activities after EFAs were evaluated by analysis of variance (ANOVA), followed by the Dunnett's *post hoc* test. The statistical comparison of fatty acids composition, lipid fluorescent products, and expression of PPARγ was made by using an ANOVA, followed by the Tukey's test. Data from CB were analyzed by Kruskal–Wallis test followed by Mann–Whitney U test. Values of $P < 0.05$ were considered as statistically significant.

Results

Fatty acids composition of olive- and fish oils determined by gas chromatography is summarized in Table 1.

Body weight and blood biochemical parameters

To assess any possible aversion, body weight gain, or biochemical changes induced by oil-rich diets ingestion, we evaluated the food consumption, body weight, and blood biochemical parameters in all groups tested. Twenty days of olive- or fish-oil-enriched diet did not elicit differences among the experimental groups in food consumption, body weight (data not shown), or glucose and cholesterol blood levels as compared with control diet group. Interestingly triglyceride levels were significantly lower in the olive- and fish-oil-enriched diet groups (90.60 ± 5.45, 94.60 ± 4.02 mg/dl, respectively), compared to control levels (111.7 ± 5.22 mg/dl) ($P < 0.5$ vs. control. One-way ANOVA followed by Dunnett's *post hoc* test).

Table 1 Fatty acids composition of olive- and fish oils

Fatty acid	Olive oil	Fish oil
C16:0	9.29	25.34
C16:1	0.69	0.822
C18:0	3.00	4.619
C18:1n-9	81.69	35.06
C18:2n-6	4.38	3.74
C18:3n-3	0.54	2.34
C18:3n-6	0.15	0.73
C20:4n-6	0.12	1.53
C20:5n-3	0.06	0.73
C22:6n-3	0.04	25.06

Data expressed on % of total fatty acids.

Fatty acids profile in synaptosomes

Fatty acid composition in whole brain synaptosome fractions obtained from control, olive-, and fish-oil diet groups were quantified, in order to verify that fatty acids reached the brain after the respective diets (Table 2).

Effect of fatty acid diet on liver function

As it is known, chronic oil-rich diet consumption could generate liver dysfunction. Considering this, we decided to analyze the liver function by measuring the activity of hepatic enzymes, ALT (marker of hepatocyte necrosis), GGT (associated with hepatocyte membrane damage), and alkaline phosphatase in control, olive-, and fish-oil diet groups. Data analysis showed that olive- or fish-oil-enriched diet did not induce alterations on any of the hepatic enzymes evaluated as compared to control group. There was no significant difference between the groups (data not shown).

CB

The QUIN-induced HD model is known to generate a characteristic CB due to a neurological damage. To evaluate a possible high fatty acid diet neuroprotection, we decided to assess the effect of olive- or fish-oil-enriched diets on QUIN-induced APM-mediated

Table 2 Fatty acid composition in synaptosome fractions from whole rat brain of control, olive-, and fish-oil diet groups

Fatty acid	Control	Olive oil	Fish oil
C16:0	28.35 ± 1.04	27.4 ± 1.91	25.2 ± 0.87
C16:1	0.35 ± 0.03	0.48 ± 0.04	0.29 ± 0.05*
C18:0	23.89 ± 0.69	24.15 ± 0.47	24.84 ± 0.67
C18:1n-9	18.55 ± 0.56	21.31 ± 0.34**	21.9 ± 0.28***
C18:2n-6	0.78 ± 0.04	0.9 ± 0.04	0.99 ± 0.15
C18:3n-3	0.97 ± 0.1	1.37 ± 0.27	1.32 ± 0.16
C18:3n-6	0.26 ± 0.11	0.16 ± 0.03	0.04 ± .01
C20:4n-6	11.64 ± 0.57	11.0 ± 0.64	10.09 ± 0.34
C20:5n-3	0.01 ± 0.007	0.02 ± 0.008^{+}	0.12 ± 0.03***
C22:6n-3	15.13 ± 1.0	13.3 ± 1.21	15.14 ± 0.88

Data expressed on % of total fatty acids composition in synaptosomes fractions.
*$P < 0.05$ fish oil diet versus olive oil diet, **$P < 0.005$, ***$P <$ 0.001 different to control group and $^{+}P < 0.005$ olive oil versus fish oil diet.
Mean values ± SEM of five rats per group are shown. One-way ANOVA followed by Tukey's *post hoc* test.

CB in rats. Intrastriatal SS-injected rats did not exhibit any CB while the QUIN-lesioned rats receiving the control diet showed an increased CB (206 ± 21 ipsilateral turns). In marked contrast, both olive- and fish-oil-rich diets significantly reduced the QUIN-induced APM-mediated CB (11 ± 4 and 22 ± 10 ipsilateral turns, respectively). In fact, there were no differences between the non-lesioned (SS-injected) group and the QUIN-lesioned groups receiving high fatty acid diet (Fig. 1).

Striatal GABA levels

Intrastriatal microinjection of QUIN but not SS decreased GABA levels in the striatum. Both, olive- and fish-oil-rich diet prevented the reduction in GABA levels induced by QUIN injection (Fig. 2).

Striatal oxidative damage

The oxidative stress status was evaluated by LP in control, olive-oil-, and fish-oil diet groups after QUIN intrastriatal injection. Obtained results show that QUIN administration increased LP significantly in control diet rats (1.093 ± 0.08 RFU/mg of protein), such effect was partially prevented in olive oil and fish oil diet rats (Fig. 3). Our results suggest that these diets exert an antioxidant protective effect.

Striatal PPARγ protein expression

To test if fatty acids contained in olive-oil- and fish-oil-rich diets exert influence on striatal PPARγ expression, we evaluated its expression by western blot. Results showed basal PPARγ expression in the control group (Fig. 4). Striatum from QUIN-lesioned rats exhibited lower PPARγ expression than SS control diet rats. However, olive oil and mainly fish oil diets were able to reduce QUIN-induced effect (Fig. 4A), yielding a higher expression of PPARγ. As a positive control we tested the striatum from a group injected with clofibrate, an agonist of PPARs able to stimulate PPARγ overexpression (Fig. 4B).

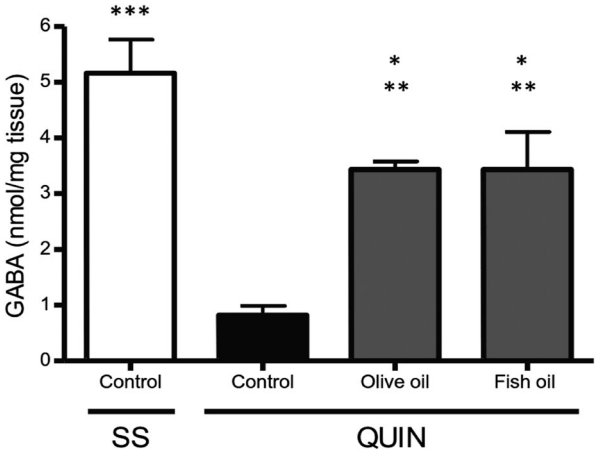

Figure 2 Effect of high fatty acid diets on QUIN-injured rats on the striatal GABA content. Animals received either control, olive-, or fish oil diet 20 days before a single intrastriatal injection of 1 μl of QUIN (240 nmol) or sterile saline (SS). Seven days after the lesions were done, rats were administered with 3-MPA (1.22 mmol/kg, i.v.) an inhibitor of glutamate descarboxylase, 2 minutes before sacrifice. *$P < 0.005$, ***$P < 0.001$ different to QUIN control group and **$P < 0.005$ different to control SS group. Mean values ± SEM of 8–10 rats per group are shown. One-way ANOVA followed by Tukey's *post hoc* test.

Discussion

In the present study, we evaluated the neuroprotective effect elicited by fatty acids included in olive- and fish

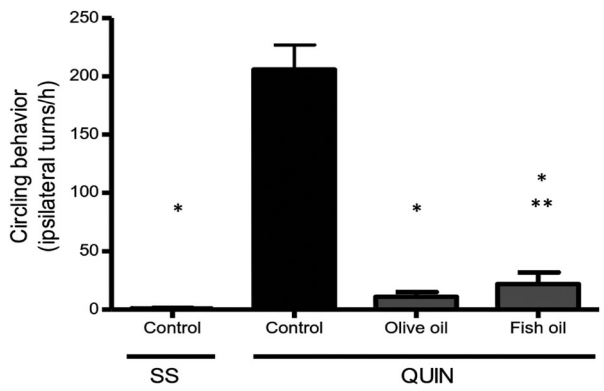

Figure 1 Effect of high fatty acid diets on quinolinate (QUIN)-induced CB in rats. Animals received either olive- or fish oil diet or control diet 20 days before a single intrastriatal injection of 1 μl of QUIN (240 nmol) or sterile saline (SS). Six days after injury, rats were administered with apomorphine (APM, 1 mg/kg s.c.) and CB was recorded during 60 minutes. Mean values ± SEM of 8–10 rats per group are shown. *$P < 0.05$ differences from control group and **$P < 0.001$, differences from control QUIN group. Kruskal–Wallis followed by Mann–Whitney *U* test.

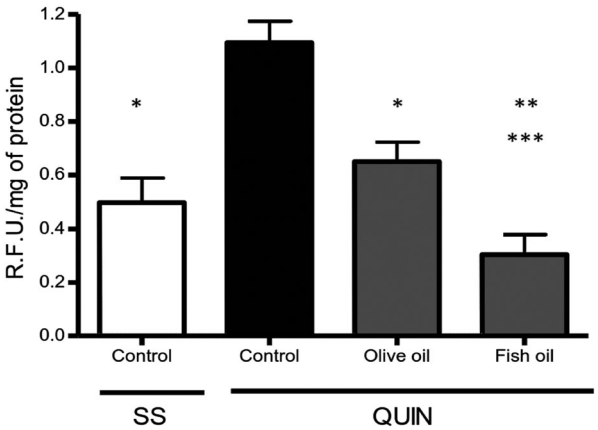

Figure 3 LP levels in striatal tissue after high fatty acid diets and injured with QUIN. Animals received control, olive-, or fish oil diet 20 days before a single intrastriatal injection of 1 μl of QUIN (240 nmol) or sterile saline (SS). Two hours later, the striatum were dissected and homogenized, LP was measured in the homogenate. *$P < 0.05$, **$P < 0.005$ different to QUIN control group and ***$P < 0.05$ different to SS control group. Mean values ± SEM from six rats per group are shown. One-way ANOVA followed by Tukey's *post hoc* test.

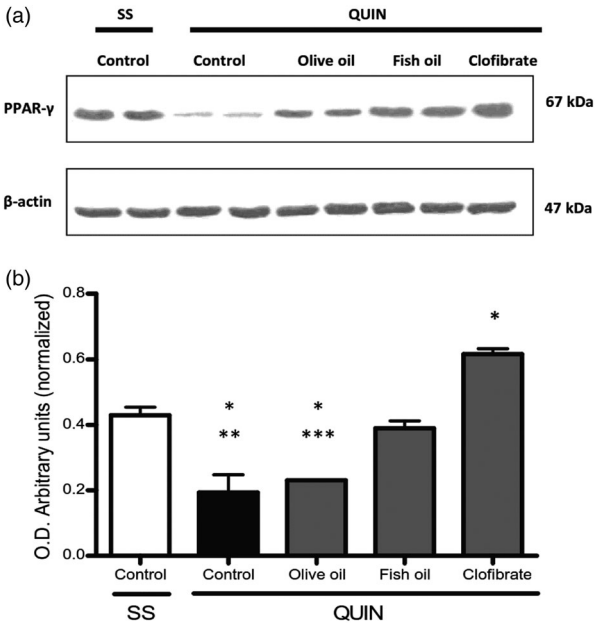

Figure 4 Expression of PPARγ in striatum of rats fed with control, olive-, or fish oil diet 20 days before a single intrastriatal injection of 1 μl of QUIN (240 nmol/μl), sterile saline (SS), or clofibrate, an inductor of the expression of PPARγ. Striata were dissected out 2 hours after the QUIN or SS injection. (A) Representative blots of the PPARγ and β-actin expression. (B) The expression of PPARγ normalized by β-actin. The bars represent mean values ± SEM of six rats per group. *$P < 0.05$, different to control group and **$P < 0.05$, ***$P < 0.001$ difference compared to clofibrate group. One-way ANOVA followed by Tukey's *post hoc* test.

oil diet on the QUIN-induced HD experimental model. Supplementation of olive- and fish-oil-rich diet during 20 days did not reflect significant changes in body weight, blood glucose, or cholesterol blood levels. Interestingly, triglyceride values in olive-oil- and fish-oil-rich diet exhibited a significant decrease when compared to the control diet, suggesting a hypotriglyceridemic effect and bioavailability of fatty acids included in oils. High-fat diets are associated with liver disease. To rule out a possible hepatotoxic effect due to 20 days of high-fat diets, we explored ALT, GGT, and alkaline phosphatase activities. The analysis did not show any differences among the groups. Our observation supports those of Abdou and Hassan,[34] confirming that omega-3 fatty acids exert a free radical scavenger activity as well as the ability to improve liver and kidney functions and hematological parameters against the toxicity induced by lead in female rats. Therefore Ndem *et al.*[35] show no changes in the hematological parameters after the omega-3 fatty acid-enriched diet.

The use of QUIN, an endogenous excitotoxin acting on NMDA receptor, experimentally mimics the alterations observed in HD.[36] The QUIN injection into the striatum of experimental rats produces loss of the GABAergic neurons characterized by the decreasing of GABA levels, and eliciting apomorphine-induced CB; all those parameters are considered neurotoxicity markers.[37] In the present study, we demonstrated that consumption of fatty acids contained in olive- and fish oils exerts neuroprotective actions against some of the end-point markers induced by QUIN neurotoxicity.

Rios and Santamaria[38] first suggested that the neurotoxic characteristics of QUIN might also be related to its ability to stimulate LP under physiological conditions; they found that administration of QUIN induced the formation of hydroxyl radical.[39] Previously our group demonstrated that LP and the nitric oxide are part of the damaging process involved in QUIN neurotoxicity and oxidative stress.[19] We have also reported that there are important changes in antioxidant systems.[31] Furthermore, oxygen radicals may cause damage attacking proteins, DNA, and lipid membranes, thereby disrupting cellular functions and integrity. Brain is vulnerable to oxidative damage because of its high content of PUFAs, which are particularly prone to free radicals attack, due to the presence of double bonds that allow easy removal of hydrogen atoms by reactive oxygen species such as $^{\bullet}OH$.[40] Regarding this, early reports suggest that EFAs counteract the excessive production of ROS in several human diseases such as hypertension, endothelial dysfunction, and hepatic disorder,[15] but the mechanism has not been clearly described. Using the QUIN-induced lesion as HD model and the supplementation of fish- and olive oil diets during 20 days, we found that the fatty acid-enriched diet significantly diminished LP, mainly in the group fed with fish oil as compared with the control diet group. Due to the reported antioxidant effect of EPA and DHA, it is highly probable that protective effect observed was due to free radical scavenging activity protecting the brain from QUIN-induced oxidative stress. Also previous reports have demonstrated the antioxidant effect of extra virgin olive oil diet in rats injured by 3-NP.[5,6]

It is possible that these EFAs have a predictable synergy with other cell membrane nutrients, specifically phospholipids and antioxidants. Depending on the requirements of the tissue, the phospholipids like phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine can carry substantial amounts of DHA in their 'tail' positions, especially on position 2.[40] These phospholipids 'parent molecules' also anchor EPA within the membrane.[41] Healthy cells have an inner antioxidant system, which help protecting them from destruction induced by intrinsic oxidants (produced by obligatory oxidative metabolism) or extrinsic oxidants imposed by lifestyle or environment.[40] Likewise, it is well known that DHA and EPA combinations showed a benefit in attention deficit/hyperactivity disorder, autism, dyspraxia, dyslexia, aggression, and cardiovascular diseases.[40]

A deeper knowledge of the complexity of lipid signaling will elevate our understanding of the role of lipid metabolism in various CNS disorders opening new opportunities for drug development and therapies for neurological diseases.[42] In the present work we demonstrated that together the other fatty acid, the oleic acid, DHA, and EPA are reaching the brain and we could suggest that also these fatty acids are arriving into the striatum to exert the neuroprotection. Our results together with Lukiw and Bazan findings lead us to suggest that EPA and DHA are critical contributors to cell structure and function in the CNS, and their deficit is associated with cognitive decline during aging and neurodegenerative disease.[43]

It is well known that fatty acids, eicosanoid derivatives, and lipid-lowering drugs (fibrates) regulate lipid and lipoprotein metabolism[8] through activation of PPARs.[9] However, the activation of PPAR by EFAs promotes several more actions like reducing the oxidative damage in striatum, most probably by activating the antioxidant defense mechanism, as reported previously.[15,43–45] In our study, PPARγ expression increased in those rats fed with olive- and fish-oil-rich diets, suggesting that its activation could stimulate the participation of the antioxidant enzymes such as superoxide dismutase or glutathione peroxidase. Interestingly, higher expression of PPARγ was found in fish oil-fed than in olive oil-fed rats; a result consistent with the reduction in oxidative damage. Our results support those previously described in literature,[9,46–48] which reported that PPAR activation also helps to regulate neuronal death in ischemic, neurodegenerative, and inflammatory cerebral diseases. *In vitro* studies have demonstrated that PPARγ agonists modulate brain inflammatory responses to bacterial endotoxin and also prevent endotoxin-induced neuronal death.[49] It has also been reported that PPARγ can inhibit macrophage and microglial activation, which contributes to degenerative, ischemic, or inflammatory processes leading to neuronal death.[50]

We suggest that EFAs contained in fish- and olive oil diets exert its protective effect through (a) the action of EFAs as agonist of the nuclear receptor PPARγ and (b) favoring the re-arrangement in the membrane phospholipids; this assumption is based on the fact that the ingestion of fatty acid-rich diets from different sources exerted different effects over lipid composition of cellular membranes and their function.[51] Additionally, PPARγ agonists significantly reduced neuronal death in response to glutamate and NMDA-mediated toxicity.[52]

In conclusion, olive-oil- and fish-oil-rich diets exert a neuroprotective effect in rats with QUIN-induced HD experimental model, evidenced as preserved behavioral function, preventing the fall of GABA striatal levels and reducing LP in the striatum. These neuroprotective effects may be due to the increased expression of PPARγ induced by EFAs and could stimulate the participation of antioxidant enzymes.

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Disclaimer statements

Contributors

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. A.M.-M., A.S.-M., and F.P.-S. designed the research; J.B.P.-F., J.C.M.-L., P.E.M.-G., M.E.-H., I.P.-N., N.C., and L.T.-L. conducted the research; S.M., A.Z.-C., and C.R. performed statistical analysis. F.P.-S. wrote the manuscript and had primary responsibility for the final content.

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Conflicts of interest None of the authors had any financial or personal conflict of interest to declare.

Ethics approval

All animal care and experimental procedures were performed in compliance to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals and were approved by our local Ethics Committee (Instituto Nacional de Neurología 'Manuel Velasco Suárez' Mexico City, protocol 74/12). In addition, every effort was made to minimize animal pain and suffering. A minimal number of rats were used to obtain statistical significance.

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