

Original Research

Expression and immunolocalisation of PPAR γ in reproductive tissues of rabbits fed n-3 PUFA-enriched diets

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Submitted: 26 November 2021 Revised: 9 January 2022 Accepted: 17 January 2022 Published: 2 March 2022

Abstract

Background: Since fatty acids are natural ligands of peroxisome proliferator-activated receptors (PPARs), involved in a wide spectrum of metabolic activities, we explored the protein expression and localisation of PPAR γ , as well as susceptibility to lipoperoxidation (LPO), in reproductive tissues of rabbits fed n-3 polyunsaturated fatty acid (PUFA)-enriched diets. **Methods:** Nine rabbit bucks were divided into three groups and fed different diets: FLAX diet supplemented with 10% extruded flaxseed, FISH diet containing 3.5% fish oil and standard diet as control. Protein expression by western blot analysis, localisation of PPAR γ by immunofluorescence and susceptibility to LPO by measuring F2-Isoprostanes (F2-IsoPs) were explored. **Results:** Down-regulation of PPAR γ expression was observed in control rabbit testes compared with FLAX ($p < 0.01$) and FISH ($p < 0.05$) dietary groups. On the contrary, an up-regulation in rabbit epididymis was observed in both n-3 PUFAs dietary groups compared with the control ($p < 0.001$). Immunofluorescent staining of PPAR γ showed a strong expression in the interstitial tissue and seminiferous tubules of testes from groups fed n-3 PUFA-enriched diets compared to controls; the PPAR γ signal was brighter in the epididymis of controls than in those of rabbits fed n-3 PUFA-enriched diets. Interestingly, F2-IsoP levels were significantly higher in testis and epididymal tissue of control compared to n-3 PUFAs dietary groups ($p < 0.001$). **Conclusion:** PPAR γ is present in male reproductive tissues, in particular in the epididymis and interstitial testicular tissue. PPAR γ expression appears to be influenced by n-3 PUFAs dietary sources and to play a role in supporting sperm maturation. n-3 PUFAs diets, reducing F2-IsoP levels, commonly considered proinflammatory molecules linked to oxidative injuries, may also support PPAR γ activity.

Keywords: Peroxisome proliferator-activated receptors; Polyunsaturated fatty acids; N-3 fatty acid; Diet; Reproductive tissues; Immunofluorescence; Western blot; F2-isoprostanes; Animal model

1. Introduction

The main regulators of fatty acid metabolism are the peroxisome proliferator-activated receptors (PPARs), transcription factors activated by metabolic ligands such as hydroxylated eicosanoids, prostaglandins and leukotrienes [1]. Three isotypes of PPARs (PPAR α , PPAR β /PPAR δ and PPAR γ) have been identified in mammals and are involved in the regulation of lipid and glucose metabolism, inflammatory processes, tissue repair, apoptosis and cancer progression. Although PPAR γ has been particularly studied in adipose tissue, its expression has also been investigated and detected in different compartments of the hypothalamic-pituitary-gonadal axis, suggesting a role in sexual maturation, embryo implantation and foetus development [2–4]. In the female reproductive system, PPARs are widely expressed and involved in energy homeostasis [5]; in addition, loss of the PPAR γ gene in oocytes and granulosa cells leads to impaired fertility in mice. Concerning male reproductive tissues, PPAR γ was detected in Sertoli cells and germ cells [2].

Essential fatty acids cannot be synthesised endogenously and hence must be provided by food. This category includes linoleic acid (LA) and α -linolenic acid (ALA), from which a series of long-chain fatty acids (≥ 20 carbon atoms), such as n-3 and n-6 polyunsaturated fatty acids (PUFAs), are originated. LA, belonging to n-6 series, is a precursor of arachidonic acid (ARA), whereas eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) originate from ALA (n-3 series). From PUFAs, lipid mediator signalling molecules can be derived, which play important roles in regulating inflammation [6]. Previous studies have shown the importance of providing 20-carbon (C-20, eicosanoids) or 22-carbon (C-22, docosanoids) PUFAs and maintaining a dietary n-6/n-3 PUFAs ratio at 1–4:1 to reduce the incidence of inflammatory diseases [7]. Increased intake of n-6 PUFAs at the expense of n-3 PUFAs enhances inflammatory processes and consequently exacerbates various diseases [7,8]. With reference to male reproduction, PUFAs accumulate in mammalian testes during puberty and support sperm maturation, motility and acrosome reaction; in particular, docos-



apentaenoic acid (DPA, 22:5n-6) is present at a high percentage in rodents and DHA in humans. Dietary administration of fish oil, rich in ALA derivatives, and flaxseed, mainly composed of ALA, increased the n-3 PUFA content in the cell membranes of rabbit testes [9]. On the other hand, PUFAs are particularly subjected to lipid peroxidation (LPO). The F2-Isoprostanes (F2-IsoPs), originating from non-enzymatic metabolism of arachidonic acid, are initially formed *in situ* on phospholipids (i.e., esterified F2-IsoPs) and subsequently released into biological fluids as unesterified F2-IsoPs (i.e., free F2-IsoPs) [10]; they may therefore represent a gold standard measurable in all biological fluids and tissues, thus allowing the evaluation of the oxidative status in several human pathologies and in male infertility conditions [11,12].

This study investigated the expression and localisation of PPAR γ and the susceptibility to LPO by measuring F2-IsoP levels in testes and epididymides of rabbits fed different n-3 PUFA-enriched diets to point out the behaviour of PPAR γ as a regulator of lipid metabolism in reproductive tissue.

2. Materials and methods

2.1 Rabbits and type of diet

New Zealand White rabbit bucks, 140 days old, underwent a training period during which libido (i.e., the period between meeting a female rabbit and ejaculation time) and seminal parameters (volume, concentration, motility rate and vitality) were recorded to form homogeneous groups. Nine rabbits were selected, divided into three experimental groups (n = 3/group) and fed different diets (Table 1):

- CONTROL group fed *ad libitum* with a standard diet.
- FLAX group fed a standard diet supplemented with 10% extruded flaxseed.
- FISH group fed standard diet contained 3.5% fish oil (NORDIC NATURALS Omega-3®, Supplementa Corporation B.V., Winschoten, Netherlands).

The dietary protocols have previously been tested and standardised by Castellini *et al.* [9]. Animals were housed in individual cages subjected to a light/dark cycle of 16L:8D at temperatures ranging from 16 to 25 °C; fresh water was constantly available, and forage was supplied *ad libitum*. The dietary protocol involved 110 days of feeding: 50 days of adaptation, during which rabbits were monitored, and a subsequent 60-day period (a full spermatogenic cycle) in which sperm were collected and evaluated as reported elsewhere [9].

2.2 Rabbit organ sampling

After the diet administration period, rabbits were sacrificed by the responsible veterinarian using an overdose of pentobarbital sodium (intravenously 200 mg/kg l.w., Sigma-Aldrich, Milan, Italy), and testes and epididymides (both sides) were carefully removed. Two samples were placed in sterile tubes and stored at -80 °C until F2-IsoP

determination and western blot analysis, and one part was processed for the immunocytochemistry technique. Three samples per organ were collected and analysed.

2.3 Western blot analysis

Testes and epididymides of rabbit bucks fed with control and n-3 PUFA-enriched diets were solubilised in RIPA lysis buffer (Sigma-Aldrich, Milan, Italy) with the addition of 1% (v/v) of Halt™ Protease and Phosphatase Inhibitor Single-Use cocktail (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, 250 μ L of RIPA lysis buffer was added to each vial, and the samples to be solubilised were pounded by hand with a potter on ice in three hot-cold cycles and sonicated in three cycles of 15 seconds each. Finally, they were centrifuged at 12,000 g for 15 minutes at 4 °C and frozen at -80 °C until use.

The protein content was determined through the Bradford assay [13], and 40 μ g of total protein was loaded on 10% acrylamide/bis-acrylamide (Sigma-Aldrich, Milan, Italy) gel electrophoresis at a constant voltage 135 V per 80 minutes in Tris-Glycine-SDS (TGS) buffer (BioRad Laboratories Inc., Cambridge, MA, USA).

After protein electrophoresis, transfer was performed on nitrocellulose membrane (Amersham Hybond) into the Mini TransBlot System (BioRad Laboratories Inc., Cambridge, MA, USA) transfer cell filled with Transfer Buffer (25 mM Tris, 190 mM glycine, 20% (v/v) methanol). An electric field at 100 Volt constant was applied for 2 hours, and the membranes were blocked in 5% (w/v) non-fat dry milk dissolved in Tris-buffered saline pH 7.3 (TBS) containing 0.1% (w/v) Tween20 for 1 hour at room temperature. Membranes were incubated with mouse monoclonal anti-PPAR γ (E-8) antibody (Santa Cruz Biotechnology, Inc., CA, USA) diluted at 1:250 in 5% (w/v) non-fat dry milk dissolved in TBS-Tween20 and with α -actin (Santa Cruz Biotechnology, Inc., CA, USA) diluted at 1:2000 in 5% (w/v) non-fat dry milk dissolved in TBS-Tween20 overnight at 4 °C. Subsequently, the membranes were washed and incubated for 1 hour at room temperature with appropriate horseradish peroxidase-conjugated IgG (BioRad, Laboratories Inc., Cambridge, MA, USA). The reaction was revealed using chemiluminescent reagents (BioRad Laboratories Inc., Cambridge, MA, USA), and the membranes were digitalised with the CHEMIDOC Quantity One 1D Analysis Software Version 4.6.6 (BioRad Laboratories Inc., Cambridge, MA, USA). Western blot densitometries were performed through the ImageJ software (NIH). The bands of the blots were measured as mean-density grey values for the area.

2.4 Immunofluorescence analysis

Testes and epididymides of rabbit bucks fed with control and n-3 PUFA-enriched diets were cut into small pieces, treated with 10% buffered formalin (Sigma-Aldrich, Milan, Italy) for 24 hours at 4 °C and washed in water for 1

Table 1. Formulation and proximate analysis of the control, FLAX and FISH diets.

Ingredients	Fresh matter g/kg	CONTROL	FLAX	FISH
Dehydrated alfalfa meal	“	300	380	380
Soybean meal 44%	“	150	100	150
Barley meal	“	410	310	335
Wheat bran	“	52	52	52
Soybean oil	“	30	-	-
Extruded flaxseed	“	-	100	-
Fish oil*	“	-	-	35
Beet molasses	“	20	10	10
Calcium carbonate	“	7	7	7
Calcium diphosphate	“	13.5	13.5	13.5
Salt	“	7	7	7
DL-methionine	“	0.5	0.5	0.5
Vitamin-mineral premix [†]	“	10	10	10
Crude protein	g/kg	175	174	175
Ether extract	“	480	472	425
Crude fibre	“	124	137	130
Ash	“	89	84	90

*NORDIC NATURALS Omega-3® = purified deep-sea fish oil (from anchovies and sardines) containing eicosapentaenoic acid (EPA) 330 mg/100 g, docosahexaenoic acid (DHA) 220 mg/100 g, other n-3 long chain polyunsaturated fatty acids (n-3 LC PUFAs) 140 mg/100 g + α -tocopherol for preservation.

[†]Per kg diet: vitamin A 11.000 IU; vitamin D₃ 2000 IU; vitamin B₁ 2.5 mg; vitamin B₂ 4 mg; vitamin B₆ 1.25 mg; vitamin B₁₂ 0.01 mg; alpha-tocopheryl acetate 200 mg; biotin 0.06 mg; vitamin K 2.5 mg; niacin 15 mg; folic acid 0.30 mg; D-pantothenic acid 10 mg; choline 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.

hour. After fixation, tissues were dehydrated in a series of ethanol (50%, 75%, 95% and 100%, AppliChem GmbH, Darmstadt, Germany) and cleared with xylene (AppliChem GmbH, Darmstadt, Germany). Samples were treated with three infiltrations of molten paraffin at 60°C for 1 hour and then solidified at room temperature. The obtained blocks were sectioned using a Leica RM2125 RTS microtome (Leica Biosystem, Germany); sections of 4 μ m were placed on slides and stained using the haematoxylin-eosin method for routine histology. Paraffin sections were deparaffinised with xylene, treated in a decreasing series of ethanol (100%, 90%, 80% and 70%) for 5 minutes and, finally, with distilled water to rehydrate the tissues. For antigen retrieval, sections were treated with Sodium Citrate Buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0, Sigma-Aldrich, Milan, Italy) for 20 minutes at 95 °C. After treatment with 5% Bovine Serum Albumin (BSA, Sigma-Aldrich, Milan, Italy) solution for 30 minutes at room temperature, samples were incubated with mouse anti-PPAR γ (E-8) antibody FITC-conjugated (Santa Cruz Biotechnology, Inc., CA, USA) at a dilution of 1:50 overnight at 4 °C. Slides were washed three times with phosphate-buffered saline (PBS, Sigma-Aldrich, Milan, Italy) for 10 minutes and treated

with 4,6-diamidino-2-phenylindole (DAPI) solution (Vysis, Downers Grove, IL, USA) for nuclei staining. After washes in PBS, slides were mounted with 1,4-diazabicyclo (2.2.2) octane (DABCO, Sigma-Aldrich, Milan, Italy). The antibody specificity was verified by performing a negative control without the primary antibody. Observations were made with a Leica DMI 6000 Fluorescence Microscope (Leica Microsystems, Germany), and images were acquired by a Leica AF6500 Integrated System for Imaging and Analysis (Leica Microsystems, Germany).

2.5 Determination of F₂-isoprostane levels

According to F₂-IsoP determination in tissue samples [14], here, to evaluate the total F₂-IsoP (free plus esterified form) levels in testes and epididymides, collected tissues were thawed and, at the time of assay, homogenised (10% w/v) in PBS (pH 7.4) with the addition of butylhydroxytoluene (Sigma-Aldrich, Milan, Italy, BHT, 100 μ M prepared in absolute ethanol) to prevent artifactual LPO. In homogenised tissues, basic hydrolysis was carried out by incubation (45°C for 45 minutes) in the presence of 1N KOH (Sigma-Aldrich, Milan, Italy). Afterwards, samples were acidified with 1N HCl (PanReacAppliChem, ITW

Reagents, Italian distributors) and tetradeuterated derivative of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha-d4}$; 500 pg, Cayman Chemical, Ann Arbor, MI, USA) was added as an internal standard. Sample purification was performed by two extraction steps. First, ethyl acetate (10 mL, PanReacAppliChem, ITW Reagents, Italian distributors) was added to each sample to extract total lipids by vortex mixing and centrifugation at 1000 g for 5 min at room temperature. In the second step, the obtained lipid extract was applied to an aminopropyl (NH_2) cartridge (500 mg Sorbent Cartridge, 55–105 μm particle size, 6 cc, Waters) to extract and collect isoprostanoids. All collected eluates were derivatised to convert the carboxylic group of isoprostanoids into pentafluorobenzyl ester and the hydroxyl group into trimethylsilyl ethers. The F_2 -IsoP levels were determined by gas chromatography/negative-ion chemical ionisation tandem mass spectrometry. The mass ion determined was the product of ions at m/z 299, which was derived from the $[M-181]^-$ precursor ions (m/z 569) [13,15]. For the calibration curve, F_2 -IsoPs (15- F_{2t} -IsoP, also known as 8-epi $PGF_{2\alpha}$ or 8-isoprostane) as reference molecule was used (Cayman Chemical, Ann Arbor, MI, USA). The F_2 -IsoP values are reported as ng per g of tissue.

2.6 Statistical analysis

Graphs were generated and statistical analyses were performed with the GraphPad Prism 6.0 software (GraphPad Software, Inc., San Diego, CA, USA). Data are presented as mean \pm standard error of the mean (SEM). All variables were tested for normal distribution (Shapiro-Wilk test). To identify significant differences between groups, one-way analysis of variance (ANOVA) was carried out, followed by Tukey's post hoc multiple comparison test. Statistical significance was set at $p < 0.05$.

3. Results

3.1 PPAR γ expression in testes and epididymides of three dietary groups

We investigated, by western blot analysis, whether diets enriched with n-3 PUFAs could lead to alterations in PPAR protein expression in testis and epididymis tissues of rabbits. Western blot data, reported in Fig. 1, showed a protein band of 50–55 kDa, corresponding to PPAR γ both in the control and in the n-3 dietary groups. Densitometric analysis normalised to the β -actin content revealed a statistically significant up-regulation of PPAR γ expression in testes of rabbits fed with FLAX ($p < 0.01$) and FISH ($p < 0.05$) diets in comparison to the control group.

The western blot data reported in Fig. 2, related to epididymides, showed a protein band of 50–55 kDa, corresponding to PPAR γ both in the control and in the n-3 dietary groups. Densitometric analysis against β -actin revealed a statistically significant down-regulation of PPAR γ expression in epididymides of rabbits fed with FISH and flax diet in comparison to the control group ($p < 0.001$).

3.2 PPAR γ immunolocalisation in testes and epididymides of three dietary groups

We performed PPAR γ immunolocalisation in testicular and epididymal tissues of rabbit bucks fed control or n-3 PUFA-enriched diets. The PPAR γ labelling was strongly evident in the interstitial tissue of testes from groups fed n-3 PUFA-enriched diets (Fig. 3C–F), particularly in the FLAX group (Fig. 3E,F), compared to the controls (Fig. 3A,B). A weak fluorescence was evident in seminiferous tubules of control rabbits (Fig. 3A,B), whereas a bright signal was evident in germ cell stages of the spermatogenic process and Sertoli cells of rabbit bucks fed PUFA n-3-enriched diets (Fig. 3C–F).

The immunofluorescent staining of PPAR γ was brighter in epididymal tissues of the control group than in those of rabbits fed n-3 PUFA-enriched diets (Fig. 4B,C); in particular, epididymal tissue from control rabbits revealed a high intense signal in the nucleus of principal cells (Fig. 4A). An intense fluorescent signal was also detected in epididymal spermatozoa of the three different dietary groups.

3.3 F_2 -isoprostane determination in testes and epididymides of three dietary groups

The F_2 -IsoPs were measured in testes and epididymides of rabbit bucks fed control and n-3 PUFA-enriched diets. The levels were significantly higher in the testes of control rabbits with respect to FISH and FLAX groups (both $p < 0.001$, Fig. 5A). In epididymal tissue, F_2 -IsoP levels were significantly higher in the dietary control group than in the FISH ($p < 0.001$) and FLAX ($p < 0.01$) groups (Fig. 5B).

4. Discussion

Because of the presence of a polar binding domain, PPARs are activated by fatty acids and their derivatives [16,17]. We evaluated the protein expression and immunolocalisation of PPAR γ in reproductive tissues of rabbits, as an animal model, fed different n-3 PUFA dietary sources. The FLAX diet was rich in ALA, whereas the FISH diet directly provided ALA derivatives (EPA, DPA and DHA). In this study, PPAR γ expression was increased in testis tissues of rabbits fed n-3 PUFA-enriched diets, particularly in the FLAX group. Consistently, the immunofluorescence revealed an intense PPAR γ labelling in the interstitial tissue and germ cells in seminiferous tubules of both n-3 PUFAs dietary groups with respect to the controls. Considering the bright signal in the interstitial tissue, we suppose an increase in lipid metabolism, based on the presence of Leydig cells, which produce testosterone from cholesterol, whose influx is regulated by PPAR γ [18]. The intense signal of PPAR γ in germ cells of seminiferous tubules of n-3 PUFAs dietary groups was probably related to an increase in Sertoli cell metabolism via activation of PPAR γ . The PPAR γ forms heterodimers with retinoid X receptors,

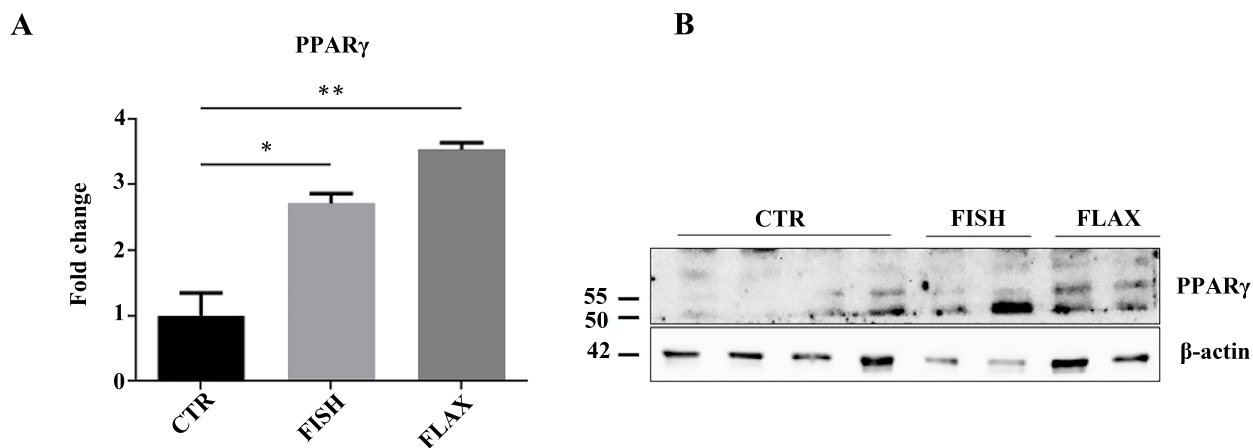


Fig. 1. Effect of n-3 PUFA-enriched diets on PPAR γ in rabbit testis samples. (A) Densitometric analysis and (B) representative immunoblot, performed in testes collected from rabbits fed with control (CTR), FISH (FISH) and FLAX (FLAX) diets. β -actin was used as the loading control. Bars, mean; error bars, SEM. Results are expressed as fold change compared with the control diet (ANOVA * $p < 0.05$; ** $p < 0.01$).

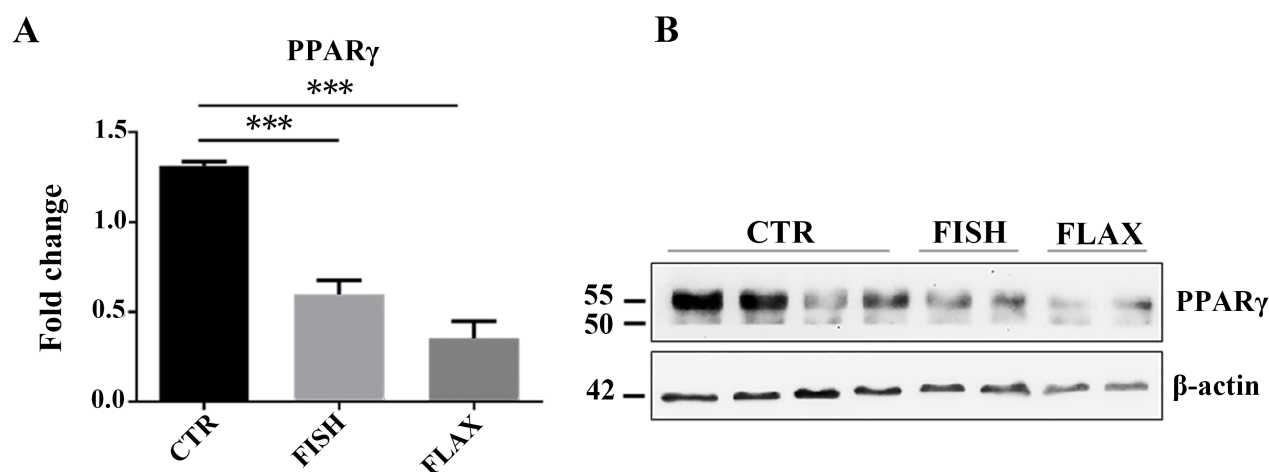


Fig. 2. Effect of diet on PPAR γ in rabbit epididymis samples. Densitometric analysis and (B) representative immunoblot, performed in epididymides collected from rabbits fed with CONTROL (CTR), FISH (FISH) and FLAX (FLAX) diets. β -actin was used as the loading control. Bars, mean; error bars, SEM. Results are expressed as fold change compared with the control diet (ANOVA *** $p < 0.001$).

which regulate target genes of lipid metabolism, thereby providing energy for spermatogenesis [2,4,19]. Gorga *et al.* [20] have reported that PPAR γ activation increases lactate production, an energy source for germ cells, glucose uptake and GLUT2 expression. The relationship between reduced expression of fatty acid metabolism genes and low activation of PPAR γ promoters in human testes with impaired spermatogenesis has also been described [21]. Interestingly, on the other hand, a down-regulation of PPAR γ expression in epididymides of rabbits fed n-3 PUFA-enriched diets compared to the control group was observed. We assume that this is due to a balancing of the up-regulation occurring at the testicular level. The increased presence of PPAR γ in the testes of n-3 PUFAs dietary groups may be

linked to an increased lipid metabolism that enriches sperm membranes early, compared to the control group, leading to a reduction in metabolism at the epididymal level. According to this hypothesis, Castellini *et al.* [9] have reported that sperm from rabbits fed n-3 PUFA-enriched diets showed a higher presence of DHA and EPA than that from rabbits fed standard diets. Surprisingly, spermatozoa in the epididymis showed a higher PPAR γ labelling than in the testes. We are aware of the fact that the different PPAR γ expression levels in epididymal sperm compared to those of testes need to be further investigated. To our knowledge, the absence of a label in sperm of testes could be justified by the increasing chromatin compaction, occurring during spermatogenesis, which makes spermatozoa transcriptionally silent. In the

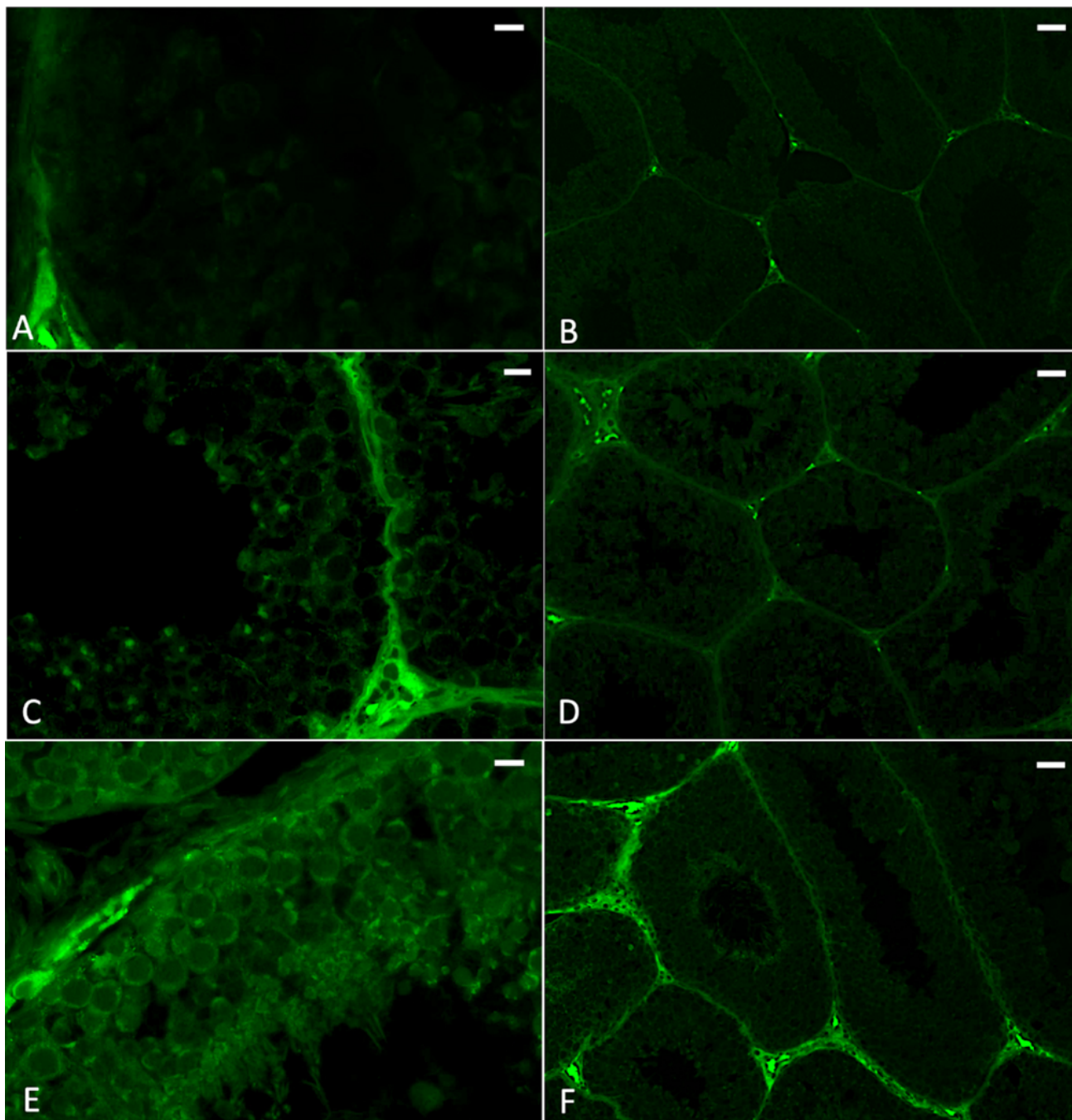


Fig. 3. Immunolocalisation of PPAR γ (green) in testis tissues of rabbit bucks fed CONTROL (A,B), FISH (C,D) and FLAX (E,F) diets. PPAR γ fluorescence is more intense in interstitial tissue of testis of FISH (C,D) and FLAX (E,F) groups than in the controls (A,B). An intense signal is evident in the germ cells of seminiferous tubules of FLAX (E,F) and FISH (C,D) groups. Bars: 10 μ m A, C, E; 50 μ m B, D, F.

epididymis, spermatozoa mature and undergo changes to acquire capacitation ability; these modifications essentially consist of the remodelling of membrane proteins and lipids [22]. Changes in the cholesterol content during epididymal transit increase membrane fluidity, essential for capacitation and membrane fusion during fertilisation. It is conceivable that PPAR γ is involved in the metabolic pathway for modelling membrane components. Whitfield *et al.* [23]

have reported that the expression of MiR-27b, a miRNA that is present in epididymosomes, results in the blocking of the expression of PPAR γ and C/EBP α , the main regulators of adipogenesis, and could represent a link between lipid metabolism, epididymis and sperm maturation. The role of PPAR γ in spermatogenesis could be confirmed by the high expression in the case of normozoospermia compared to asthenozoospermia [24].

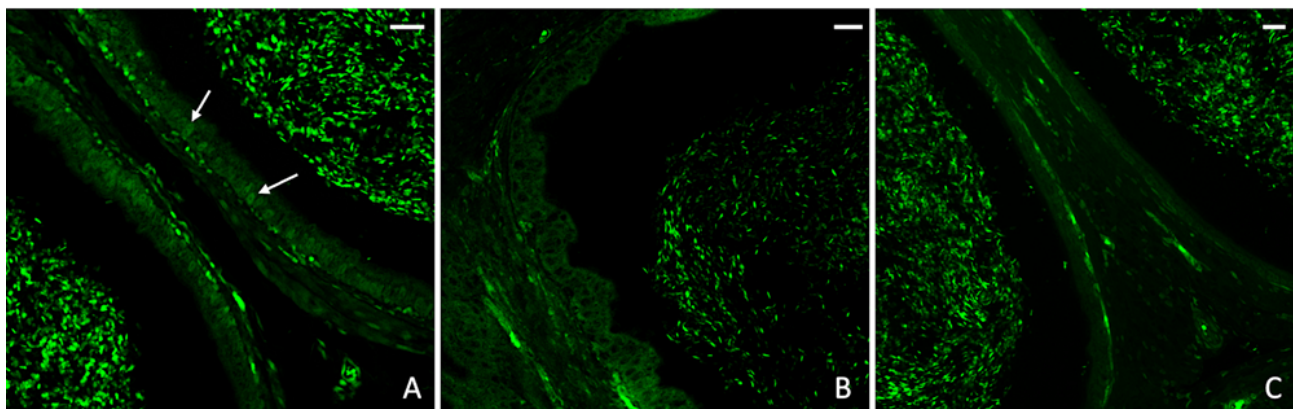


Fig. 4. Immunolocalisation of PPAR γ (green) in epididymal tissues of rabbit bucks fed control (A), FISH (B) and FLAX (C) diets. Brighter labelling in principal cells of the control group (A) is shown (arrows). Bars: 50 μ m.

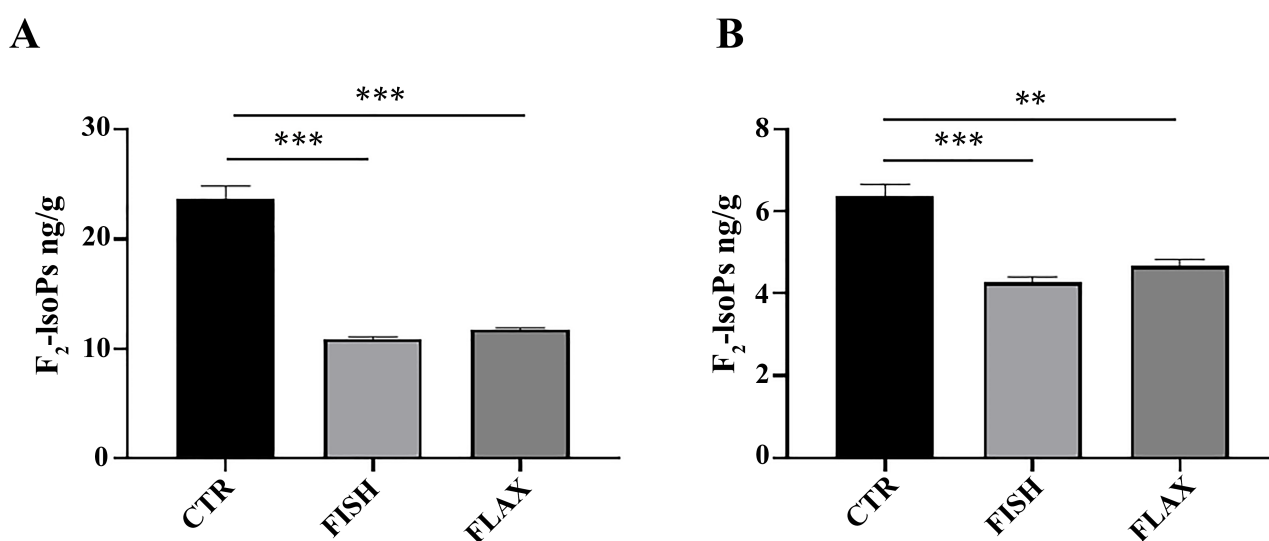


Fig. 5. F₂-isoprostane (F₂-IsoPs ng/g) determination in rabbit testes (A) and epididymides (B) collected from rabbits fed with CONTROL (CTR), FISH (FISH) and FLAX (FLAX) diets. Bars, mean; error bars, SEM (ANOVA ** p < 0.01, *** p < 0.001).

The F₂-IsoPs, produced by the oxidative metabolism of arachidonic acid, are mediators in many inflammatory pathologies, including those associated with male infertility [11]. Their levels were higher in epididymides and testes of controls than in those of n-3 PUFAs dietary groups. It is known that n-3 PUFAs modulate the fatty acid composition of the cell membrane, leading to a decrease in the contents of arachidonic acid and eicosanoid derivatives, such as prostaglandin E₂ (PGE₂) or leukotriene B₄ (LTB₄), involved in inflammatory mechanisms [25]. Thus, the reduced F₂-IsoP levels in both epididymides and testes of n-3 PUFAs dietary groups could be due to a lower presence of arachidonic acid, from which F₂-IsoPs are produced, or to a decrease of an inflammatory state following n-3 PUFA-enriched diets. It has been supposed that PUFAs activate PPAR γ , which inhibits the signalling pathway of nuclear factor kappa B (NF- κ B) [25,26], an inflammatory response mediator, determining a concomitant improvement

in sperm quality [27]. Moreover, n-3 PUFAs increase the production of compounds with strong anti-inflammatory activity, such as resolvins, derived from EPA [25], highly present in the FISH diet.

5. Conclusions

The results of this study show that PPAR γ is present in male reproductive tissues, in particular at the epididymal level and in the interstitial tissue of the testis. Diets rich in n-3 PUFAs stimulate an increased expression of PPAR γ , mainly at the testicular level, suggesting a positive role in supporting the spermatogenic process and preserving PPAR γ activity by reducing F₂-IsoP levels, commonly considered proinflammatory molecules and linked to oxidative injuries.

Author contributions

GC, CS, EM, DN, SP designed the research study. SP, DN, GB, SM performed the research. SP and DN analysed the data. SP, DN and CS wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was conducted in accordance with the Guiding Principles in the Use of Animals and approved by the Animal Ethics Monitoring Committee of the University of Siena, Italy (CEL AOUS; authorisation no. 265/2018-PR, ISOPRO 7DF19.23).

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

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