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Structural and ultrastructural evaluation of fibre muscles after exposure to Bisphenol-A, and a study of their possible recovery after treatment with platelet-rich plasma

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ABSTRACT

Bisphenol-A (BPA) is one of the chemical products most produced in large volumes world-wide. Among other items, it forms part of plastics and food containers, from which there is a migration of BPA into food, thus it enters our organism via the digestive tract, which in fact is one of the main sources of exposure in humans. In this study, BPA action has been investigated: at a muscular level with continuous exposure; after its withdrawal in order to evaluate the possible recovery of the muscle; and the potential effect of platelet-rich plasma (PRP) on a muscle previously modified by the action of BPA. For this purpose, and as a fundamental tool, histopathology was used, from which it was observed that muscle modifications were produced. These were compatible with the action of hormones administered exogenously to animals to fatten them up. It was also noted that, after the withdrawal of BPA, there was some muscle structure recovery, and, after treatment with PRP, this was practically total. Further research should investigate the mechanisms through which BPA affects muscle tissue and PRP succeeds in restoring this type of muscular lesion.

Key words: bisphenol-A, muscle, histopathology, collagen, platelet-rich plasma

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Introduction

Bisphenol-A (BPA) is a component of, among other items, plastics and food containers, and it is one of the chemicals produced in the largest volume in the whole world (LANG et al., 2008). It has been demonstrated that migration of BPA occurs from food containers, by its entering the digestive tract, this being one of the main sources of exposure in humans (LYONS, 2000; BREDE et al., 2003). It is also present in rivers and in drinking water, probably due to the displacement of plastic containers from industrial residue dumps (KOLPIN et al., 2003; COORS et al., 2003). BPA has an endocrine activity which, due to its structural similarity with steroid hormones, is capable of triggering, in target cells, a response resembling that of endogenous hormones, or inhibiting that response by exercising an antagonistic effect.

The use of BPA in containers in contact with foods is permitted in the European Union by ANONYM. (2002). This regulation covers plastic materials and objects designed for the protection of food products, in which a maximum migration limit is established for this substance of 0.6 mg/kg, to maintain the Tolerable Daily Intake of BPA for humans at 0.05 mg/kg/day (ANONYM., 2008). The European Union ANONYM. (2011) decided, as from March, 2011, to prohibit the production of babies` feeding bottles with BPA, and its commercialization as from 1 June, 2011 (Implementing Regulation EU 321/2011).

Endocrine disrupting chemicals (EDCs), by altering the endocrine function, disrupt the metabolism of carbohydrates and lipids, leading in turn to insulin resistance and diabetes and obesity, increasing the risk of cardiovascular complications. This particular implication of EDCs has made it generate great concern in the consumer due to its possible repercussions on public health.

At the fourth meeting of the OECD Task Force on Endocrine Disruptor Testing and Assessment (EDTA) it was agreed that histopathology should be adopted as a core endpoint in the assessment of oestrogen-active compounds (SEGNER et al., 2003).

With the aim of investigating the toxicity of BPA in depth, our objective was to undertake a histopathological study of the muscle with respect to the action of BPA in this tissue, and its recovery capacity after the withdrawal of exposure to this compound; the response was also investigated of the muscle after PRP application to animals previously exposed to BPA.

Materials and methods

*Animals.*16 two-month-old "Minipigs" were used from the Centralized Experimental Animal Service at the University of Córdoba, where they were housed during the whole experiment, following the conditions specified in the guidelines relative to the housing and care of the animals (RD 1201/2005). All the experiment protocols were approved by the Córdoba University Committee of Bioethics. The animals were fed once a day

during the study months (Nantaunic, Nantaporc PI®) and distributed randomly to one of the four experiment groups: Control Group (GC), (n = 4); the group treated with BPA (n = 4) orally 1 mg/kg bw/day (Sigma Aldrich®, St. Luis, EE.UU.) for four months; a third group BPA-withdrawal (n = 4) which, after two months of treatment, was taken off BPA for two more months up until their biopsy; and a fourth group, platelet-rich plasma (PRP) (n = 4). The animals of this group were treated with BPA for 2 months, then it was withdrawn and they began treatment with PRP. A weekly dose of platelets was injected into their longissimus lumbaris (LL) for three weeks. At the end of the different periods of exposure, treatment and/or withdrawal, depending on the study group, the animals were anesthetized by intramuscular injection (medetomidine 0.05 mg/kg bw combined with Zoletil® (Tiletamine/Zolacepam) 3 mg/kg bw) and the samples were collected by means of a biopsy of the LL muscle, for subsequent study.

PRP preparation. Two mL of blood were taken from the jugular vein using PRP kit tubes, containing 0.3 mL of anticoagulant and separator. After transferring the tube contents to another tube containing platelet activation-preventing substances and centrifugation of the tube at 1700 rpm for 12 min, its upper component, which contained platelets and plasma, was transferred to another tube containing preservative substances. After that, the tube was centrifuged at 3500 rpm for 7 min. Finally, the upper portion of the tube contents was discarded and after a 30 min delay, the remaining portion was injected into the animal.

Light and electron microscopy. For the structural evaluation, the samples were routinely processed for paraffin sections by fixing in 10 % formaldehyde, dehydrating in graded series of ethanol, immersing in xylol and embedding in paraffin wax. Every tenth section (4 μm thick) of each block was stained with haematoxylin and eosin and used for the morphological study.

For the ultrastructural study, small randomly selected samples were primarily fixed in a 2 % glutaldehyde solution in 0.1 M phosphate buffer (pH 7.4) overnight at 4 ºC and then refixed in 1 % osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 30 min. After dehydration in graded ethanol series and embedded in Araldite, semithin and ultra-thin sections were cut on an LKB ultramicrotome. Semithin sections were stained with toluidine blue, whereas ultra-thin sections were double-stained with uranyl acetate and lead citrate. Ultra-thin sections were viewed and photographed using a Philips CM10 transmission electron microscope.

Morphometric study

Quantitative evaluation of fibre. The fibre percentages were obtained after counting between 500 and 1,000 fibres from superficial and deep fields selected randomly, included in whole fasciculi, employing photographic sequences, which reconstructed the complete section of the different muscles, using dyed H&E preparations.

A Leitz Dialux 20 microscope was used, with an automatic camera incorporated into it. The preparations were photographed at 4x and 10x to carry out the most complete reconstruction possible of the muscle sections obtained. For the morphometric studies, the contours of the muscle fibres were marked.

Collagen quantification. Muscle sections were subjected to Masson's trichrome stain, which stains collagen blue, providing an excellent color contrast that differentiates it from other structures. Six pictures at high magnification were randomly taken from each slide and analyzed using ImageJ software version 1.46f; by thresholding for blue color, collagen was selected and measured for each picture, and the results were expressed as the mean percentage of collagen.

Statistical analysis. Data were analysed using the statistical program Statgraphic (Centurion XVI®) to determine BPA effects in every exposition group. ANOVA (test-F) was used to demonstrate whether significant differences existed between the averages. The Fisher LSD post hoc test was employed to perform multiple comparisons between groups and $P<0.05$ was considered to be significant.

Results

The muscle structure in the LL of the control group (Fig. 1) showed, under both the light and the electronic microscopes, the two essential components of the muscle. The striated muscle fibre, with all its components, in which first the muscle fibre stood out, displayed bands A and I, with a normal development of the sarcoplasmic reticulum and abundant mitochondria, which turned out to be multinucleated with peripheric nuclei. Second, satellite cells corresponding to muscle germ cells were prominent. These cells were so arranged as to be adhered to the muscle fibres, and both of them were surrounded by a basal membrane which separated them from the endomysium. These cell components were small in size, of a spherical to triangular shape, with a central nucleus and scant cytoplasm, a few cytoplasmic organoids and no trace of myofibrils. In the endomysium, with the trichrome stain, it was possible to observe that there were few collagen fibres.

In the BPA exposure group, up to the time they were euthanized, it was constantly observed that most of their LL fibres exhibited alterations in their morphology, which ranged from necrosis to degenerative processes (Fig. 2). The fibres developing necrosis stood out due to being scarce and showing dense nuclei with sinuous and uneven edges with dilatations of the nuclear envelope. In the cytoplasm, both the contractile and the coagulated cytoplasmic proteins were seen, they lost their morphology, and their myofibrils were scarcely identified. The mitochondria appeared as being swollen and the sarcoplasmic reticulum vacuolized.

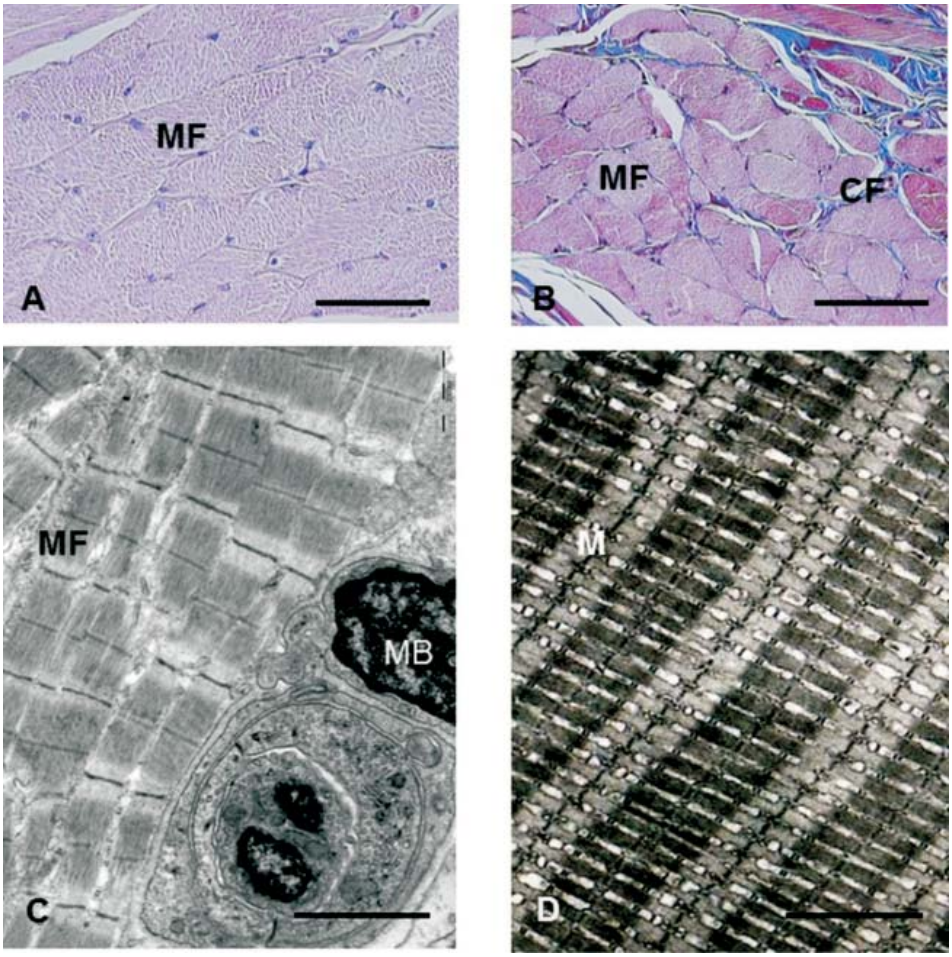

Fig. 1. Control group. Light microscope. Scale bars 50 µm (A), (B) ultrastructural observations. Scale bars 10 µm (C), (D). A - Inset of apparently normal muscle fibre (MF). H&E. B - Inset of the apparently normal muscle fibre (MF) in which scant collagen fibres (CF) are observed. Masson's trichrome stain. C - Normal muscle fibre (MF), associated with myoblasts (MB). D - Inset of apparently normal muscle fibre with myofibrils (M).

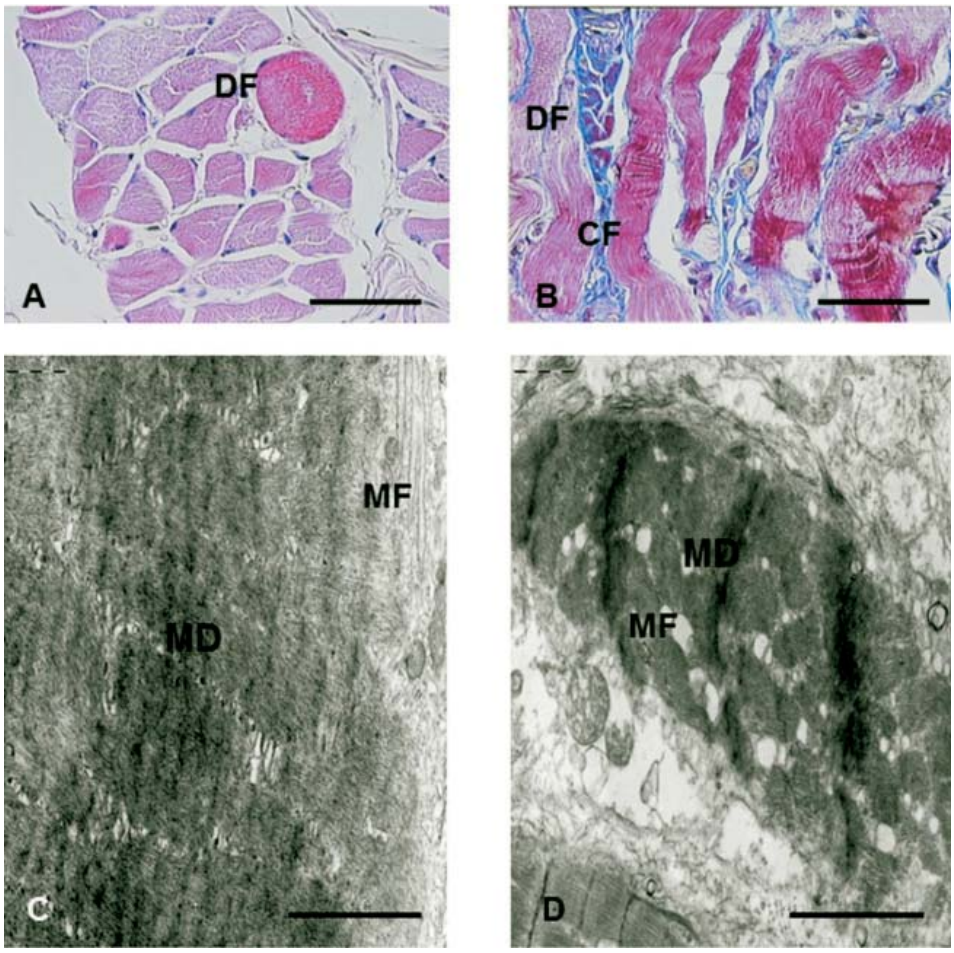

Fig. 2. Histological changes in BPA group. Light microscope. Scale bars 50 μm (A), (B) ultrastructural observations. Scale bars 10 μm (C), (D)

A. Inset of degenerated fibres (DF). H&E; B. Inset of the muscle with fibre degeneration (DF) in which abundant collagen fibres (CF) are noted. Masson's trichrome stain.; C. Muscle fibre (MF) with myofibril destruction (MD) with protein coagulation; D. Inset of fibres (MF), with a disorganization in their myofibrils (MD) and vacuolization.

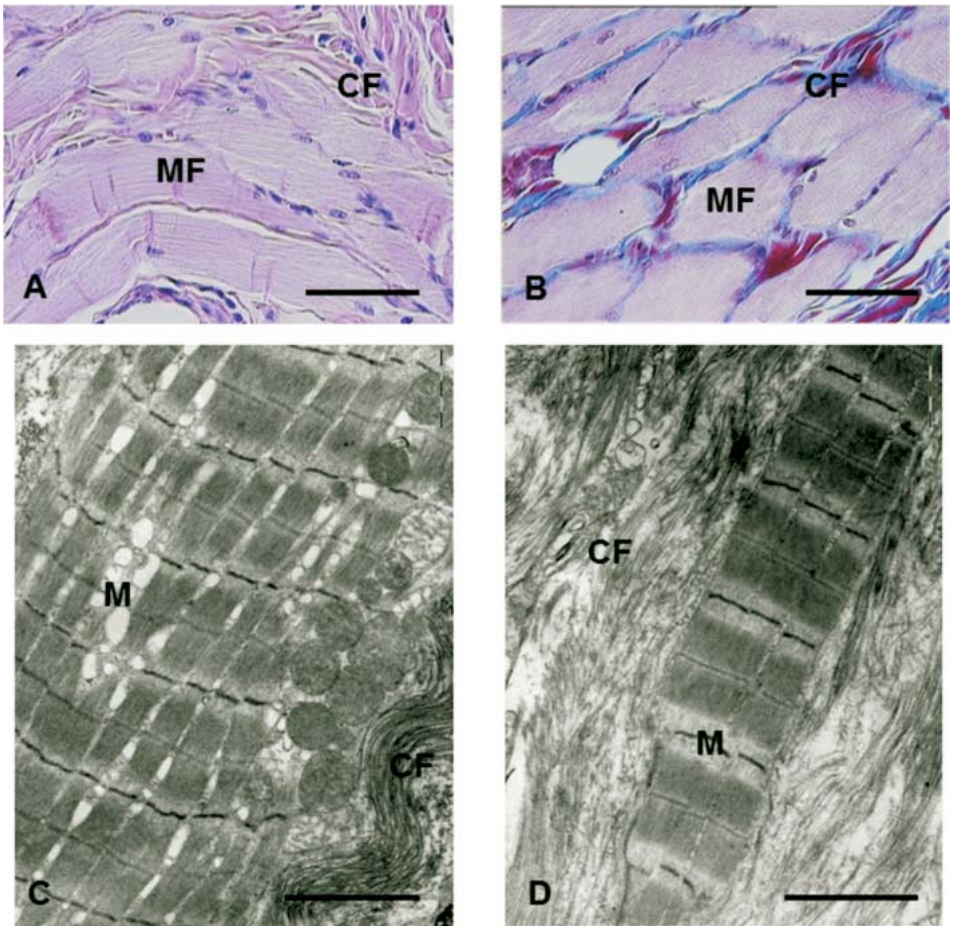

Fig. 3. Histological changes in BPA-withdrawal group. Light microscope. Scale bars 50 μm (A), (B) ultrastructural observations. Scale bars 10 μm (C), (D)

A. Inset of muscle with collagen fibres (CF) and normal muscle fibres (MF). H&E; B. Inset of the apparently normal muscle fibres (MF) A large amount of collagen fibres (CF) can be seen. Masson's trichrome stain; C. Muscle fibre with organized myofibrils (M) associated with a large amount of collagen fibres (CF) and vacuolizations; D. Inset of apparently normal myofibrils (M), and a large amount of collagen fibres (CF).

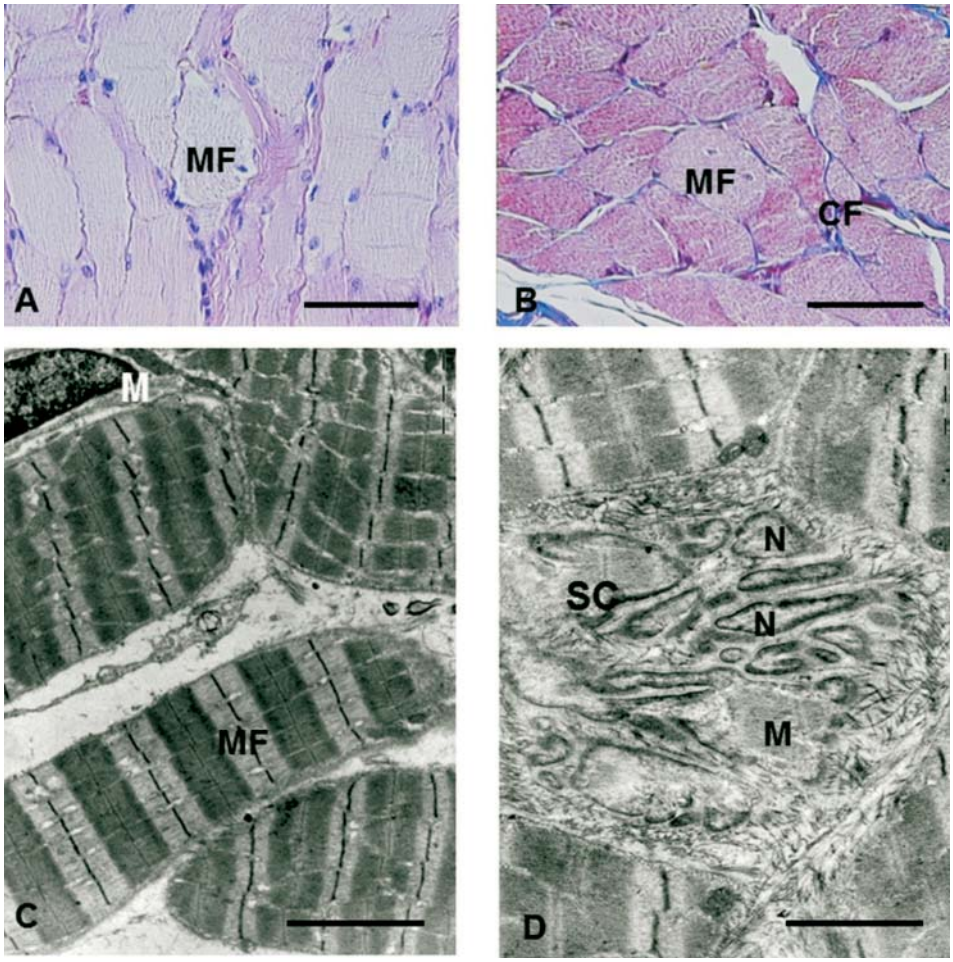

Fig. 4. Histological changes in PRP group. Light microscope. Scale bars 50 µm (A), (B) ultrastructural observations. Scale bars 10 µm (C), (D)

A. Inset of apparently normal muscle (MF). H&E; B. Inset of apparently normal muscle (MF) with few collagen fibres (CF). Masson`s trichrome stain; C. Muscle fibre (MF) with organized myofibrils (M); D. Inset of satellite cells (SC) with an increase in nuclei (N) and apparently normal myofibrilar contractile components (M).

The fibres in the degeneration process stood out because they showed strong vacuolization of all the membranous organoids, especially those of the sarcoplasmic reticulum. In the contractile components, densification of the myofilaments was noted, although all their bands were differentiated and their nuclei were uneven and dense. Also observed were groups of striated fibres, which partly lost some components of their myofibrils. With the trichrome stain and the electronic microscope, it was possible to discern an increase in the endomysium of collagen fibres, and although satellite cells were seen, the latter appeared in a small number, and with no apparent signs of reactivation.

In the group of animals to which BPA was administered but withdrawn over several months up to the time when they were euthanized, a recovery of their muscle fibres was observed, but with activation of the connective tissue of the endomysium, which was detected both with the trichrome stain and with the electronic microscope (Fig. 3). The striated fibres generally exhibited apparently normal myofibrils, with correctly arranged myofilaments, normal A and I bands, and abundant mitochondria were placed between them. In this group, the sarcoplasmic reticulum partly maintained a certain degree of dilatation. It could be observed how the connective tissue, in contact with the fibres (the endomysium), was enlarged and its collagen fibres had increased enormously, in parallel fasciculi or arranged unevenly, with the size of these collagen fibres increasing both parallel to the upper axis of the muscle fibre and in the fascicular areas. The satellite cells did not apparently show any obvious signs of activation.

In the group treated with PRP, recovery of the striated fibres was observed, with multinucleated fibres and peripheric nuclei being generated. The myofibrils displayed all their bands with a correct organization, with abundant mitochondria (Fig. 4). On the microscope images, reactivation in the satellite cells appeared. Under the light microscope an increase in their number was observed, ending up in the presentation of areas of hyperplasia, with condensations in these cells. The electronic microscope showed activation processes in which an increase in the number and volume of satellite cells appeared, and there was even evidence in their cytoplasm of the beginnings of the formation of myofibrils with organized striae. The muscle fibres appeared as being apparently normal, with organized contractile material, with their A and I bands, and peripheric nuclei. In the connective tissue corresponding to the endomysium, there was hardly any evidence of collagen fibres, with a certain increment in neoformed capillaries, with limited oedema.

On carrying out the quantification of the muscle fibres (Table), it was noted how, in the BPA exposure group, there was reduction in their number, although without any significant differences with respect to the control. In the study group in which BPA was withdrawn, a smaller number of muscle fibres was observed than in the groups treated constantly with BPA and the control, with statistically significant differences appearing, $P<0.05$, in the control group, but none in the group constantly exposed to BPA. However,

in the PRP-treated group, an increase could be seen in the number of muscle fibres, which was higher than the rest of the study groups, exhibiting significant differences, $P<0.05$, with respect to the BPA withdrawal group.

Table 1. Size (μm) of the muscle fibre in terms of the study group expressed by the mean ± SD

	Control	BPA	BPA-withdrawn	PRP
Muscle fibres	72.76 ± 7.81	69.93 ± 7.16	65.97 ± 6.77*	75.27 ± 5.55*

*Significantly different from the control with $P<0.05$

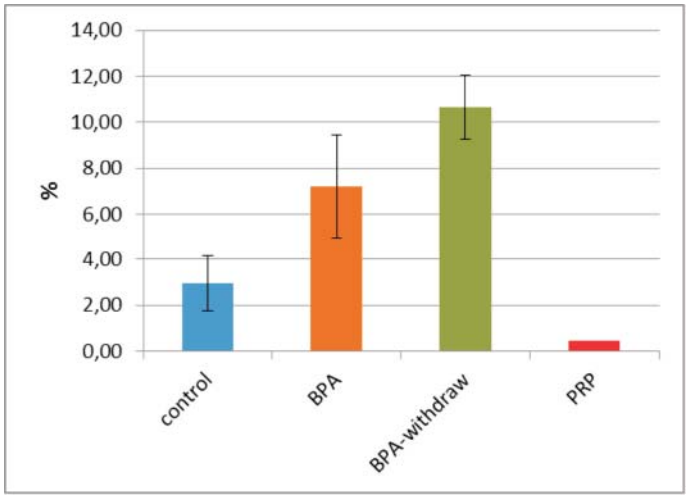

Fig. 5. Amount of collagen (%) versus muscle (+/-SD) which appeared in the different study groups

On quantifying the collagen (Fig. 5), it was seen that there was an increase in its production in the group continuously exposed to BPA and in that of the withdrawal group, with significant differences with respect to the control, $P<0.05$. The PRP-treated group displayed a reduction in the percentage of collagen, with significant differences, $P<0.05$, compared to the remaining study groups.

Discussion

BPA is produced industrially in large amounts and is found in food containers. Due to environmental pollution and the presence of BPA in food, both animals and humans are exposed daily to the action of this endocrine disruptor. There are many studies on the

action of BPA for its neuroendocrine properties at the gonad level (LINDHOLST et al., 2000; MANDICH et al., 2007; RODRIGUEZ et al., 2010; HATEF et al., 2012; LÓPEZ-CASAS et al., 2012). Although the action of this xenoestrogen has been studied in depth at different levels, and in different animal models, its effect on muscle tissue has not been evaluated in the same way (INDUMATHI et al., 2013; MORTAZAVI et al., 2013; WANG et al., 2013).

As a study objective, we proposed to evaluate the effect of BPA on the muscle after long-term exposure, and of its possible recovery after BPA withdrawal, as well as the effects of PRP treatment on a muscle previously treated with BPA, using histological tools for this purpose. To monitor the evolution of the muscle both light and electronic microscopes were used. However, an electron microscope was employed in our experiment because of the possible importance of satellite cells in muscle regeneration, which are small-sized and with an unspecific morphology as corresponds to germ cells. Therefore, the electron microscope was able to supply information on any intimate change in these cells in the recovery process of the muscle fibres.

In the control group, normal morphology of the muscle fibres was witnessed, with their corresponding bands, their relationship with the smooth or sarcoplasmic reticulum, and, what was more important, with their invaginations in the cytoplasmic membrane.

Endocrine disruptors in general, and particularly with the use of BPA, have been seen to alter the hypothalamic-hypophisary-gonadal axis, causing a modification of ovary and testicle functionality (WOLF et al., 2004; MANDICH et al., 2007; RODRÍGUEZ et al., 2010; HATEF et al., 2012). These alterations are fundamentally seen in a loss of the germ cells and, in relation to our work, qualitative and quantitative modifications. Although there are few reports in the literature on alterations in the muscle system of the connective tissue of the endomysium (ASANO et al., 2010; GAO et al., 2010; INDUMATHI et al., 2013; WANG et al., 2013), we found significant ones in the muscle fibres and connective tissue. BPA action evaluation as an endocrine disruptor has mainly been carried out in rodents and fish as animal models (HATEF et al., 2012; LOPEZ-CASAS et al., 2012; WANG et al., 2013), and there are few references to work employing other experimental animal species (GAO et al., 2010; CHOI and JEUNG, 2003; INDUMATHI et al., 2013). The modifications triggered in the muscle fibre could be caused by the direct or indirect action of BPA and/or endogenous hormones, which could act, as do other compounds, as anabolic agents (PELLEGRINO et al., 2004; DOUILLARD et al., 2011). As could be verified, the muscle fibre alterations in these treatments were varied, which is why we have described hypertrophies due to their possible anabolizing action, to degenerative processes and even to necrosis, coinciding with what was observed by other authors, who evaluated the action of other compounds with anabolizing actions at the muscle level (RAJAB et al., 2000; LAVOIE and BELIVEAU, 2002; BRICOUT et al., 2004). There are many studies indicating that, because of the estrogenizing action of BPA, there is an increase in the endogenous production of 17-β-estradiol and other hormones, which are naturally synthesized in the organism, but

when they are exposed to the disruptor, their production is increased (YANG et al., 2014). This fact could indicate to us that the effects generated in the muscle could be produced as secondary to the action of BPA, because these endogenous hormones could act in an anabolizing manner (HUANG and SILLENCE, 2000). Of all the lesions we detected, the most numerous were those from degenerative processes, which, since their components, the myofibrils and the smooth reiticulum, were only partially affected, could be restored (BRICOUT et al., 2004; DOUILLARD et al., 2011). Just as in the hypertrophy processes, necrotic fibres would be produced because of the protein coagulation process of the contractile microfilaments, which could final disintegrate. This recovery is because the effect of BPA is reversible, and the fact that no modification in the satellite cells was detected would infer that the recovery took place in the muscle cell itself.

From the studies made, it may be considered that BPA causes necrotic-degenerative myositis, but the lesions presented were clearly recoverable using the model proposed for their possible recovery. It could be noted that the sarcoplasmic reticulum partly maintained a certain degree of dilatation.

In the studies made after the withdrawal of BPA, recovery of the muscle fibres was found, with the reorganization of the myofilaments. The myofibrils clearly exhibited all their transversal bands, with mitochondria being incorporated (RAJAB et al., 2000), although some dilatations of the smooth reticulum could partly affect its functionality. What was highly representative in the muscle's recovery after BPA withdrawal was the reaction of the collagen fibre-producing cells in the endomysium, which produced a massive amount of collagen, whose fibres enveloped the muscle cells, causing fibrosis (HUANG et al., 2000; BRICOUT et al., 2004; PELLEGRINO et al., 2004). This phenomenon could be clearly detrimental to the total recovery of the muscle since fibrosis could generate sclerosis, which would harden the muscle and easily promote muscle tears and lesion recurrence, a problem which could greatly affect sportsmen and women. What stood out in the studies in this group was that the connective tissue in contact with the fibres (endomysium) was seen to be enlarged and with highly increased collagen fibres, in parallel or unevenly arranged fasciculi, and these collagen fibres incremented both in parallel to the upper axis of the muscle fibre, and in the fascicular areas. However, the satellite cells did not appear to show any evident signs of activation.

The use of platelet-derived growth factors enabled the muscles to recover very acceptably. In this group, the action of the satellite cells was fundamental; not only was activation produced, but also hyperplasia, with distribution in accumulations or dispersion throughout the fibre, with recently formed myofibrils being observed (CUNHA et al., 2014).

In addition, it could be said that the muscle fibres recovered totally in relation to their myofibrils, nuclei and remaining cytoplasmic organoids. This recovery of the muscle fibre would indicate that two paths were followed: the first by means of activation of the

satellite cells, and the second by the reaction of the muscle fibre itself, propitiated by the action of the platelet-derived growth factors. Recovery would occur within the actual striated fibre affected. Furthermore, it was observed that, at the level of the satellite cells which became myoblasts, there was an increase in their nuclei for generating new muscle fibres and contractile myofibrils.

Unlike the fibrosis processes mentioned above, in this group hardly any collagen fibres were observed in the endomysium, but abundant capillaries, which would favour recovery, stood out. In this case, the absence of fibrosis and of scar hardening would result in the better recovery of the muscle fibre and would, therefore, prevent any subsequent muscle lesion recurrence.

Conclusions

Our results confirm that BPA produces modifications in the histology of the muscle and fibrosis in the endomysium after long-term exposure, which could affect tissue functionality. However, if BPA exposure is terminated, the muscle is capable of regenerating part of the histological modification of its fibres due to the non-participation of the satellite cells, as well as there being an increment in the fibrosis of the endomysium. It was also demonstrated that, after PRP treatment, the muscle fibre was restored by the fibres themselves and by means of the activation of the satellite cells, eliminating the fibrosis generated by the BPA, with good recovery reported, since there was no fibrosis in the endomysium. These results have shown us that it is necessary to continue investigating the mechanism by which BPA affects muscle tissue after long-term exposure, and the different paths through which PRPs could act by modifying this type of muscle lesion.

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References

ANONYMOUS (2002): Directive 2002/72/EC from the Commission of 6 August, 2002, relative to plastic materials and objects destined to enter into contact with food products.

ANONYMOUS (2005): Royal Decree 1201/2005 of 10 October on the protection of animals used for experimentation and other scientific purposes.

ANONYMOUS (2008): European Food Safety Authority. (2008): Statement of EFSA on a study associating bisphenol A with medical disorders. The EFSA Journal 838, 1-3.

ANONYMOUS (2011): Directive 2011/8/EU from the Commission of 28 January, 2011, which modified Directive 2002/72/CE referring to the restriction of the use of bisphenol-A in plastic feeding bottles for infants.

ANONYMOUS (2011): Implementing regulation (EU) No. 321/2011 from the Commission of 1 April, 2011, modifying Regulation (EU) 10/2011 restricting the use of bispheno-A in infant feeding bottles.

ASANO, S., J. TUNE, G. DICK (2010): Bisphenol A activates Maxi-K (K_{ca} 1.1) channels in coronary smooth muscle. Brit. J. Pharmacol. 160, 160-170.

BREDE, C., P. FJELDAL, I. SKJEVRAK, H. HERIKSTAD (2003): Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Addit. Contam. 20, 684-689.

BRICOUT, V. A., B. D. SERRURIER, X. BIGARD (2004): Clenbuterol treatment affects myosin heavy chain isoforms and MyoD content similarly in intact and regenerated soleus muscles. Acta Physiol. 180, 271-280.

CHOI, K., E. JEUNG (2003): The biomarker and endocrine disruptors in mammals. J. Reprod. Dev. 49, 337-345.

COORS, A., P. D. JONES, J. P. GIESY, H. T. RATTE (2003): Removal of estrogenic activity from municipal waste landfill leachate assessed with a bioassay based on reporter gene expression. Environ. Sci.. Technol.. 37, 3430-3434.

CUNHA, R. C., J. C. FRANCISCO, M. A. CARDOSO, M. A. MATOS, L. F. MATOS, D. LINO, R. B. SIMEONI, G. PEREIRA, A. C. IROIDA, P. R. SIMEONI, L. C. GUANITA-SOUZA, K. A. CARVALHO (2014): Effect of platelet rich plasma therapy associated with exercise training in musculoskeletal healing in rats. Transplant Proc. 46, 1879-1881.

DOUILLARD, A., O. GALBES, B. ROSSANO, B. VERMUS, A. BONNIEU, R. CANDAU (2011): Time course in calpain activity and autolysis in slow and fast skeletal muscle during clenbuterol treatment. Can. J. Physiol. Pharmacol. 89, 117-125.

GAO, X., L. YU, L. CASTRO, A. MOORE, T. HERMON, C. BORTNER, M. SIFRE, D. DIXON (2010): An endocrine-disrupting chemical, fenvalerate, induces cell cycle progression and collagen type I expression in human uterine leiomyoma and myometrial cells. Toxicol. Lett. 196, 133-141.

HATEF, A., S. M. HADI ALAVI, A. ABDULFATAH, P. FONTAINE, M. RODINA, O. LINHERT (2012): Adverse effects of bisphenol A on reproductive physiology in male goldfish at environmentally relevant concentrations. Ecotox. Environ. Safe. 76, 56-62.

HUANG, H., N. SILLENCE (2000): Differential effect of dexamethasone and clenbuterol on rat growth and on beta 2-adrenoceptors in lung and skeletal muscle. J. Anim. Sci. 78, 604-608.

INDUMATHI, D., S. JAYASHREE, J. SELVARAJ, C. MAVILVANAN, N. AKILAVALLI, K. BALASUBRAMANIAN (2013): Effect of bisphenol A on insulin signal transduction and glucose oxidation in skeletal muscle of adult male albino rat. Hum. Exp. Toxicol. 32, 960-971.

KOLPIN, D. W., E. T. FURLONG, M. T. MEYER, E. M. THURMAN, S. D. ZAUGG, L. B. BARBER, H. T. BUXTON (2003): Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams. Environ. Sci. Technol. 36, 1202-1211.

LANG, I. A., T. S. GALLOWAY, A. SCARLETT, W. E. HENLEY, M. DEPLEDGE, R. B. WALLACE, D. MELZER (2008): Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 300, 1303-1310.

LAVOIE, J., L. BELIVEAU (2002): A farnesyltransferase inhibitor attenuatted bata-adrenergico receptor downregulation in rat skeletal muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282, 317-322.

LINDHOLST, C., K. L. PEDERSEN, S. N. PEDERSEN (2000): Estrogenic response of bisphenol A in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 48, 87-94.

LÓPEZ-CASAS, P., S. C. MIZRAK, L. A. LÓPEZ-FERNÁNDEZ, M. PAZ, D. G. DE ROOIJ, J. DEL MAZO (2012): The effects of different endocrine disruptors defining compound-specific alterations of gene expression profiles in the developing testis. Reprod. Toxicol. 33, 106-115.

LYONS, G. (2000): Bisphenol A: A known endocrine disruptor. WWF European Toxics Programme Report, UK.

MANDICH, A., S. BOTTERO, E. BENFENATI, A. CEVASCO, C. ERRATICO, S. MAGGIONI, A. MASSARI, F. PEDEMONTE, L. VIGANO (2007): *In vivo* exposure of carps to graded concentrations of bisphenol A. Gen. Comp. Endocrinol. 153, 15-24.

MORTAZAVI, S., A. BAKHTIARI, A. SARI, N. BAHRAMIFAR, F. RAHBARIZADEH (2013): Occurrence of endocrine disruption chemicals (Bisphenol A, 4-nonylphenol, and Octylphenol) in muscle and liver of, *Cyprinus Carpino Common*, from Anzali wetland, Iran. Bull. Environ. Contam. Toxicol. 90, 578-584.

PELLEGRINO, M. A., G. D´ANTONA, S. BORTOLOTTO, F. BOSCHI, O. PASTORIS, R. BOTINELLI, B. POLLA, C. REGGIANI (2004): Clenbuterol antagonizes glucocorticoid-induced atrophy and fibre type transformation in mice. Exp. Physiol. 89, 89-100.

RAJAB, P., J. FOX, S. RIAZ, D. TOMLINSON, D. BALL, P. L. GREENHAFF (2000): Skeletal muscle myosin heavy chain isoforms and energy metabolism after clenbuterol treatment in the rat. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, 1076-1081.

RODRÍGUEZ, H. A., N. SANTAMBROSIO, C. G. SANTAMARÍA, M. MUÑOZ-DE-TORO, E. H. LUQUE (2010): Neonatal exposure to bisphenol A reduces the pool of primordial follicles in the rat ovary. Reprod. Toxicol. 30, 550-557.

SEGNER, H., K. CAROLL, M. FENSKE, C. R. JANSSEN, G. MAACK, D. PASCOE, C. SCHÄFERS, G. F. VANDENBERG, M. WATTS, A. WENZEL (2003): Identification of endocrine- disrupting effects in aquatic vertebrales and invertebrates: report from the European IDEA project. Ecotoxicol. Environ. Saf. 54, 302-314.

WANG, X., Q. DONG, Y. CHEN, H. JIANG, Q. XIAO, Y. WANG, W. LI, C. BAI, C. HUANG, D. YANG (2013): Bisphenol A affects axonal growth, musculature and motor behaviour in developing zebrafish. Aquat. Toxicol. 142, 104-113.

WOLF, J. C., D. R. DIETRICH, U. FRIEDERICH, J. CAUNTER, A. BROWN (2004): Qualitative and quantitative histomorphologic assessment of fathead minnow *Pimephales promelas* gonads as an endpoint for evaluating endocrine-active compounds: A Pilot Methodology Study. Toxicol. Pathol. 32, 600-612.

YANG, X., Y. LIU, J. LI, M. CHEN, D. PENG, Y. LIANG, M. SONG, J. ZHANG, G. JIANG (2014): Exposure to Bisphenol AF Disrupts Sex Hormone Levels and Vitellogenin Expression in Zebrafish. Environ. Toxicol. 31, 285-294.

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SAŽETAK

Bisfenol-A (BPA) je kemijska tvar koja se u velikim količinama proizvodi širom svijeta. Između ostaloga, bisfenol-A je sastavni dio plastike i ambalaže za prehrambene proizvode, odakle se otpušta u hranu te tako dospijeva u probavni sustav čovjeka pa hrana time postaje jedan od glavnih izvora izloženosti ljudi toj supstanciji. U ovom istraživanju učinci BPA promatrani su u mišićju nakon trajne izloženosti, u mišićju nakon prestanka davanja BPA kako bi se procijenio njihov mogući oporavak, te u mišićju s primijenjenom plazmom obogaćenom trombocitima (PRP) kako bi se utvrdio njezin utjecaj na BPA uzrokovana oštećenja. Opažene patohistološke promjene u mišićima bile se slične onima kod djelovanja hormona rabljenih za bolji prirast tovnih životinja. Nakon prestanka davanja BPA uočen je određeni oporavak mišićne građe, a nakon primjene PRP oporavak je bio potpun. Daljnjim istraživanjima treba utvrditi kojim mehanizmima BPA utječe na mišićno tkivo i kako PRP uspijeva oporaviti nastala mišićna oštećenja.

Ključne riječi: bisfenol-A, mišići, histopatologija, kolagen, plazma obogaćena trombocitima