ADMINISTRATION OF A LEPTIN ANTAGONIST DURING THE NEONATAL LEPTIN SURGE INDUCES ALTERATIONS IN THE REDOX AND INFLAMMATORY STATE OF THE IMMUNE SYSTEM IN PERIPUBERTAL /ADOLESCENT RATS

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Abstract

The importance of the neonatal leptin surge in rodent on neurodevelopmental processes has aroused curiosity in its implication in other physiological systems. Given the role of leptin in neuro-immune interactions we hypothesized that the neonatal leptin surge could have an effect on the oxidative and inflammatory stress situations of immune system. We blocked the neonatal leptin surge by a leptin antagonist and measured several parameters of oxidative and inflammatory stress in the spleen of peripubertal/adolescent female and male rats. The treated rats showed lower activity of several antioxidant enzymes in the spleen and their leukocytes released lower levels of mitogen-stimulated IL-10 and IL-13 and higher of TNF-alpha. In conclusion, the neonatal leptin surge may have a key role in the establishment of an adequate redox and inflammatory state in the immune system, which is important to allow an adequate immune response and to reach a good health.

Key words: Neonatal leptin; leptin antagonist; antioxidant defenses; proinflammatory and anti-inflammatory cytokines; peripubertal rats

Running head: Neonatal leptin, oxidative stress & cytokine production

1. Introduction

Leptin, an adipokine produced mainly by adipocytes, is a pleiotropic hormone involved in many physiological processes such as food intake and energy balance, immune system functions, reproduction, reward, stress and neurodevelopment (Bellefontaine et al., 2014; Folch et al., 2012; Meyer et al., 2014). In rodents there is a postnatal leptin surge, that in male mice begins around postnatal day (PND) 5 and peaks between PND9 and 10 (Ahima and Hileman, 2000). In rats, leptin increases between PND4 and 7, is higher between PND7 and 10 and then declines by PND14 (Delahaye et al., 2008). This leptin surge has been implicated in hypothalamic development by modifying neuronal outgrowth and synaptic connectivity, as well as neurogenesis and neuronal and glial survival (Bouret, 2013). The timing and magnitude of this surge appears to be important for normal metabolic control and weight gain in the adult animal (Ahima et al., 1998; Attig et al., 2008; Cottrell et al., 2009; Delahaye et al., 2008; Yura et al., 2005). Most studies on the neurotrophic role of leptin during its neonatal peak have been focused on the hypothalamus (Bouret, 2013; Bouret et al., 2004; Garza et al., 2008; Mela et al., 2012; Pinto et al., 2004; Udagawa et al., 2006a, 2006b; Viveros et al., 2010). In fact, this brain structure has traditionally been the focus of studies related to central leptin actions. For instance, we have recently found that administration of a pegylated super leptin antagonist from PND 5 to 9, coincident with the neonatal leptin surge, affects the gene expression of growth factors, glial proteins, and neuropeptides involved in the control of metabolism and reproduction in the hypothalamus of peri-pubertal male and female rats (Mela et al., 2015).

Currently, there are abundant studies that confirm the bidirectional communication between the homeostatic systems, namely the nervous, endocrine and immune systems, which is mediated by neurotransmitters, hormones and cytokines

through the presence of their receptors on the cells of the three systems. Thus, any influence exerted on one of these systems will have an effect on the others and *viceversa* (De la Fuente, 2014; Wrona, 2006). The leptin receptors are highly expressed in immune cells such as monocytes, macrophages, neutrophils and lymphocytes (Goldenberg, 2014) and, thus, leptin affects almost all cells of the immune system (Zabeau et al., 2014), modulating immune cell functions and cytokine networks, which may contribute to the protection from infections (Conde et al., 2014; Procaccini et al., 2013). Consequently, a decrease in leptin release or a disruption of its action could be deleterious for the immune response. Indeed, Farooqi et al., (Sadaf Farooqi et al., 2002) showed that individuals with leptin deficiency were more likely to suffer infections due to the impairment in T cell proliferation and cytokine release. In leptin deficient mice (ob/ob) dendritic cells are less effective in the stimulation of allogenic T cells (Macia et al., 2006), and the balance of Th1/ Th2 cytokines is deregulated, changing the sensitivity to autoimmune diseases (Zabeau et al., 2014).

Leptin can also promote the release of reactive oxygen species (ROS) and ROS have a strong role as signaling molecules in the central nervous system, and more particularly in the hypothalamus. In POMC neurons, ROS levels that may be regulated among other factors by leptin, appear to play a crucial role in modulating cellular responses involved in the regulation of energy metabolism. In fact, the short-term hypothalamic ROS peak generated by metabolic signals (nutrients and hormones) appears to be fundamental to elicit a proper behavioral, endocrine and autonomic response to nutrient intake (Diano, 2013; Drougard et al., 2015). In an experimental model of L. donovani infection leptin induced the macrophage phagocytic activity by enhancing the intracellular ROS generation which helps in phagolysosome formation and oxidative killing of the parasite (Dayakar et al., 2016). ROS production is important for an appropriate body function,

including the immune response. However, when there is an excessive increase in ROS levels, due to an enhancement in their production or a decrease in the antioxidant defenses that neutralize the excess of ROS, an oxidative stress situation appears, and these ROS can be deleterious for the organism causing oxidation of macromolecules (Gyengesi et al., 2012; Vida et al., 2014). It is known that oxidation and inflammation are related processes and that an excess of oxidant and inflammatory compounds maintained through time, produces an oxidative and inflammatory stress situation, which deregulates immune function and is the cause of aging, illness and death (Vida et al., 2014). Alterations in immune and inflammatory responses are present in leptin- or leptin-receptor deficient animals, as well as during starvation and malnutrition, two conditions characterized by low levels of circulating leptin (Fantuzzi and Faggioni, 2000).

In spite of the findings described above, the specific role of the neonatal leptin surge on the development and establishment of a correct redox state and a regulated inflammatory response has not been investigated. To address this question, diverse parameters related to oxidative and inflammatory stress in the spleen of peripubertal/adolescents male and female rats that received daily injections of a rat pegylated super leptin antagonist from PND5 until PND9, the time period of the neonatal leptin surge, were analyzed. This allows to know if this leptin has a role in the redox and inflammatory state of peripubertal/adolescent animals.

2. Materials and Methods

2.1 Animals

Adult Wistar rats were purchased from Harlan Interfauna Ibérica S.A. (Barcelona, Spain) and allowed to acclimatize for 2 weeks before mating. One male was

placed in a cage with two females for 10 days. On the day of birth (PND0), litters were culled to eight pups per dam (four males and four females). No cross-fostering was employed. Thus, only litters with at least 4 pups of each sex at birth were used. Rats were maintained at a constant temperature (22 ± 1 °C) and humidity (50 ± 2%) in a reversed 12-h light-dark cycle (red light on at 08:00 and white light on at 20:00). Pregnant rats were given free access to rat chow (commercial diet for rodents; A03, Safe, Augy, France) and water. The animals used in this study were the same as those used in a recently published paper focused on the effects of the same leptin antagonist treatment on hypothalamic systems related to reproduction (Mela et al., 2015). As our aim in that study was to investigate the pubertal transition, right before the appearance of external phenotypic signs of puberty, and because the timing of puberty differs between the sexes, females were euthanized on PND33 and males on PND43. These studies were approved by the local ethics committee and complied with the Royal Decree 1201/2005 (BOEn° 252) pertaining to the protection of experimental animals and with the European Communities Council Directive (86/609/EEC). The experiments were conducted in accordance with the guidelines and protocols of the Royal Decree 53/2013 regarding the care and use of laboratory animals for experimental procedures, and were approved by the Committee for Animal Experimentation of the Complutense University of Madrid.

2.2 Leptin antagonist treatment

From PND5 until PND9 rats were injected sc with 5 mg/kg bodyweight (bw) of rat pegylated super leptin antagonist (mutant D23L/L39A/D40A/F41A), a gift of Protein Laboratories (Rehovot, Israel), which was prepared as described in Jamroz-Wiśniewska

et al. (Jamroz-Wiśniewska et al., 2014). In order to avoid the stress effect caused to the animals by marking them at this early age, we treated each litter with either vehicle (controls) or antagonist. The animals received one injection per day at 9:00. Control rats were injected with the same volume of distilled water as vehicle (2.5 ml/kg). After each injection the animals were immediately returned to their mothers. Each experimental group consisted of 12 animals. To avoid/minimize a possible litter effect all experimental groups contained animals from at least three different litters.

2.3 Tissue collection

Female (PND33) and male (PND43) rats were sacrificed after a 12h fast by decapitation. The spleen was rapidly and aseptically removed, freed of fat and divided into two parts. One of them was stored at -80ºC until the assays were performed. The other was minced with scissors, and gently pressed through a mesh screen (Sigma, St. Louis, and USA) to obtain the cell suspension.

2.4 Antioxidant enzymes:

2.4.1 Superoxide dismutase (SOD) activity

Total SOD activity was measured (n=8-11) by a modified method described by Marklund and Marklund (Marklund and Marklund, 1974), with slight modifications (Alvarado et al., 2006). The tissue samples (50 mg/ml) in Tris–HCl buffer (50 mM, pH 8.2) were homogenized and centrifuged at 3200g for 20 min at 4ºC. Supernatant (750 µl) was mixed with an icecold ethanol-chloroform mixture (5:3 v/v) (600 µl) and immediately shaken for 60 s and centrifuged at 2500g for 10 min at 4ºC. Supernatant (SOD extract) (25 µl) was mixed with Tris–HCl buffer (50 mM, pH 8.2) containing DTPA (1 mM) and pyrogallol (24 mM prepared in HCl 10 mM) (25 µl). The enzymatic

activity was measured spectrophotometrically at 420 nm through the oxidation of pyrogallol. One unit of activity is defined as the amount of enzyme causing a 50% inhibition of the autoxidation of pyrogallol at 25ºC. The protein contents of the same samples were evaluated following bicinchoninic acid protein assay kit protocol (Sigma-Aldrich, Spain). The results were expressed as arbitrary units (U) of enzymatic activity per milligram of protein (AU SOD/mg protein).

2.4.2 Catalase (CAT) activity

Catalase activity was determined (n=6-11) following the method described by Aebi (Aebi, 1984) with modifications previously introduced by Alvarado et al. (Alvarado et al., 2006), and based on the decrease of the absorbance at 240 nm because of the decomposition of H_2O_2 by the enzyme. The assays were performed using aliquots of the homogenized tissue samples (50 mg/ml in tampon phosphate 66 mM, pH 7.5) and centrifuged at 3200g for 20 min at 4ºC. The supernatant extract (30 µl) was mixed with 670 µl of H_2O_2 (14 mM in phosphate buffer 66 mM). The enzymatic activity was measured spectrophotometrically for 80 s at 240 nm through the decomposition of H_2O_2 into $H_2O + O_2$. One unit of CAT was defined as the amount of enzyme which liberates half of the peroxide oxygen from H_2O_2 solution in 80 s at 25ºC. The protein contents were evaluated following the some bicinchoninic acid protein assay kit protocol. The results were expressed as units (U) of enzymatic activity per milligram of proteins (U CAT/mg protein).

2.4.3 Glutathione peroxidase (GPx) activity

The glutathione peroxidase (GPx) activity was determined (n=7-11) according to the technique described by Lawrence and Burk (Lawrence and Burk, 1976). The assays

were performed with aliquots of the homogenate tissue samples (50 mg/ml in tampon phosphate 50 mM, pH 7.5) and centrifuged at 3200g for 20 min at 4ºC. The total activity was determined using cumene hydroperoxide (Sigma-Aldrich), which carried out the oxidation of the glutathione regenerated by the addition of B-nicotinamide adenine di-nucleotide phosphate, in its reduced form (β-NADPH, Sigma-Aldrich, Spain), in the presence of glutathione reductase (Sigma-Aldrich). The reaction was followed spectrophotometrically by the decrease of the absorbance at 340 nm. The protein contents were evaluated following the previously mentioned protocol. The results were expressed as miliunits of enzymatic activity per milligram of proteins (mUGPx/ mg protein).

2.4.4 Glutathione reductase (GR) activity

The glutathione reductase (GR) activity was measured (n=8-10) by the method described by Massey and Williams (Massey and Williams, 1965). The assays were performed with aliquots of the homogenate tissue samples (50 mg/ml in tampon phosphate 50 mM, pH 7.5 with 6, 3 mM EDTA) and centrifuged at 3200g for 20 min at 4ºC. The total activity was measured through the oxidation of NADPH spectrophotometrically at 340 nm. The protein contents of the same samples were again evaluated following the previously described protocol. The results were expressed as miliunits of enzymatic activity per milligram of proteins (mUGR/mg protein).

2.4.5 Total Glutathione, GSH and GSSG concentrations

The total glutathione (GSH plus GSSG) was measured (n=10-12) by fluorometry, according to the Hissin and Hilf method (Hissin and Hilf, 1976), with some modifications (Schultz et al., 2010), in which GSH was sequentially oxidized by o-

phthaldialdehyde and reduced by NADPH in the presence of GR. The oxidized glutathione level (GSSG) was determined by masking GSH with N-ethylmaleimide. The protein contents of the same samples were evaluated following the previously mentioned protocol. The amounts of GSH and GSSG were calculated using a standard curves, which were prepared using a glutathione solution (glutathione reductase and oxidized form 1µg/ml) (Sigma-Aldrich, Spain), and expressed in nmol per milligram of protein (nmol/mg protein). The redox index was calculated by dividing the concentration of GSSG by that of GSH.

2.4.6 Cytokine levels released by spleen leukocytes

Spleen cell suspensions were centrifuged in a gradient of Ficoll-Hypaque (Sigma-Aldrich, Spain) with a density of 1.070 g/ml. The cells were recollected from the interface and resuspended in RPMI 1640 medium enriched with L-glutamine (PAA, Pasching Austria) and supplemented with 10% heat-inactivated fetal calf serum (Gibco, Canada) and gentamicin (10 mg/ml, Gibco) (complete medium). Aliquots of 200 µl of mononuclear cell suspension adjusted to 1×10^5 cells/ml complete medium were cultured in 96-well plates in the presence of lipopolysaccharide (LPS) (Escherichia coli 055:B5, 1 µg/mL per well; Sigma-Aldrich, Spain) or concanavaline A (ConA, 1 µg/mL per well; Sigma Sigma-Aldrich, Spain). After 48 h of incubation at 37°C in a sterile and humidified atmosphere of 5% CO2, 100 µL of culture supernatant from each well was collected and stored at -80 C until used for cytokine analysis. Levels of proinflammatory (TNF-α) and anti-inflammatory cytokines (IL-10, IL-13) cytokines were measured (n=6-8) simultaneously by Luminex xPONENT (Rat Bio Plex Pro Citokine assays using magnetic beads, U.S, BIO-RAD). The results were expressed as pg/ mL.

2.5 Statistical analysis

Data were analyzed by SPSS for Windows, release 15.0. Normality was checked by Shapiro-Wilks′s test (p>0.05). Data were analysed by one-way analysis of variance (ANOVA). As males and females were of a different chronological age due to the differential timing of puberty onset, data were not directly compared between sexes.

3. Results

3.1 Spleen weight

Regarding spleen weights, a significant effect of the neonatal leptin antagonist treatment was found in both sexes [Males: $F(1,22)= 22012$; $p<0.001$. Females: $F(1,17)=22.380$; $p<0.001$] with treated males exhibiting lower spleen weights and treated females showing higher spleen weights when comparing with their respective control counterparts (Fig.2).

3.2 Parameters of oxidative stress

Both, males and females treated neonatally with the leptin antagonist showed significantly lower activity of the three enzymes analyzed, CAT [Males: $F(1,20)= 3.027$; $p<0.001$. Females: $F(1,15)= 19.732$; $p<0.001$] (Fig.3A), SOD [Males: $F(1,19)=8.625$; $p<0.01$. Females: $F(1,17)=4.752$; $p<0.05$] (Fig.3B) and GPx (Fig.4A), [Males: $F(1,19)= 42.964$; $p<0.001$. Females: $F(1,16)=11.034$; $p<0.01$]. In addition, male and female treated animals showed higher GR activity (Fig.4B) [Males: $F(1,18)= 73.738$; $p<0.001$. Females: $F(1,16)=29.017$; $p<0.001$]. No significant differences were found in the GSSG/GSH ratios (Fig.5).

3.3 Cytokine levels

The levels of the anti-inflammatory cytokine IL-10 released by spleen leukocytes from treated animals were significantly lower in the presence of LPS [Males: $F(1,10)= 682.412$; $p<0.001$. Females: $F(1,14)= 17.044$; $p<0.01$] and also in the presence of ConA [Males: $F(1,14)= 44.522$; $p<0.001$. Females: $F(1,14)= 8.256$; $p<0.05$] (Fig.6). The treatment with the leptin antagonist resulted in lower levels of the anti-inflammatory cytokine IL-13 in male leukocytes stimulated with LPS (Fig. 7A) [$F(1,14)=28.767$; $p<0.001$] and in female leukocytes stimulated with ConA (Fig. 7B) [$F(1,13)=13.506$; $p<0.01$].

As Figure 8 shows, the levels of the proinflammatory cytokine TNF-α were also modified by the neonatal treatment with the leptin antagonist. In presence of LPS leukocytes of treated males released lower levels, and those of females higher levels of this cytokine than their corresponding control counterparts [Males: $F(1,12)= 14.960$; $p<0.01$. Females: $F(1,14)= 22.464$; $p<0.001$]. In presence of ConA, treated males showed higher TNF-α levels [Males:$F(1,25)=6.639$; $p<0.05$], whereas no differences were found among females (Fig.8).

4. Discussion

The observation that the activity of diverse antioxidant enzymes and the levels of pro-inflammatory and anti-inflammatory cytokines are modified during the peripubertal/adolescent phase in the neonatally treated rats, provides the first evidence for a programming role of the physiological leptin surge in the redox and inflammatory state of the animal later in life.

Similar to that described in mice (Ahima et al., 1998), rats also experience a plasma surge of leptin during the neonatal period (Delahaye et al., 2008). In these animals, leptin increases between PND4 and 7, is higher between PND7 and 10,

declining by PND14. Therefore, the present leptin antagonist treatment was administered precisely during the time of the leptin surge. Leptin appears to play a key role in immune and inflammatory responses (Conde et al., 2014; Valleau and Sullivan, 2014). However, the implication of the neonatal leptin surge in the development and establishment of these responses later in life has not been previously analyzed. In fact, to the best of our knowledge, we provide here the first evidence showing that disruption of leptin signaling during the time period of the physiological neonatal leptin surge has delayed consequences affecting the oxidative and inflammatory state in peripubertal/adolescent male and female rats.

The spleen is the largest secondary immune organ in the body and is responsible for initiating immune reactions to blood-borne antigens and for filtering the blood of foreign material and old or damaged red blood cells. In rats, this organ reaches peak development at puberty, followed by gradual involution. It appears to be a key player in cytokine production when there is an infection or after trauma resulting in a systemic inflammation regulated by the autonomic nervous system (Gigliotti and Okusa, 2014). In this study, we show changes in the spleen weights of peripubertal animals treated neonatally with the leptin antagonist. Treated males showed lower weights whereas treated females showed higher weights of their spleen. Since leptin acts as an immunomodulator (Conde et al., 2014; Valleau and Sullivan, 2014), it seems plausible that the treatment has affected the correct development of spleen. In fact, leptin increases thymic cellularity in obese mice (Howard et al., 1999). The data suggest that this effect might be sexually dimorphic. In a previous analysis of these same animals, we showed that the treatment with the leptin antagonist increased estradiol levels in females (Mela et al., 2015). It might be that this change in estradiol levels is implicated in the specific direction of spleen weight change in this sex. Moreover, in males an

opposite relation between estradiol levels and body weight has been described (Si et al., 2011).

SOD acts as an enzymatic antioxidant defense that transforms the superoxide anion to H_2O_2 and works in parallel with CAT who is responsible to eliminate the excess of H_2O_2 (Vida et al., 2014). In the present study, we show lower activities of both enzymes in the animals treated in the neonatal period with the leptin antagonist. These results are indicative of decreased antioxidant defenses in the spleen. The glutathione system includes GPx and GR, two enzymes that play an important role in maintaining the correct balance between GSH and GSSG levels. In normal conditions glutathione is usually found in its reduced form since GR is constitutively active (Vida et al., 2014). In the present study, the treated animals showed higher GR activity and lower GPx activity than the corresponding controls. It is plausible that an increase in GR activity acts as a compensatory mechanism to mitigate the oxidative stress. In fact, when the ratio GSSG/GSH was analyzed as a marker of oxidative stress, no significant effect of the neonatal treatment was found.

It is known that appropriate levels of leptin are needed for a correct immune response (Stofkova, 2009). Leptin appears to stimulate both innate immunity, through the upregulation of TLR expression in monocytes, and adaptive immune response carried out by lymphocytes. This stimulation is related with the protection against infections and associated with production of pro-inflammatory cytokines and autoimmune diseases (Conde et al., 2014; Zabeau et al., 2014; Zarkesh-Esfahani et al., 2001). Moreover, leptin enhances T lymphocyte shift toward T helper 1 (Th1), decreasing the activation of T helper 2 (Th2) cells and consequently the production of cytokines such as IL-10 and IL-13 (Batra A1, Okur B, Glauben R, Erben U, Ihbe J, Stroh T, Fedke I, Chang HD, Zeitz M, 2010; Martín-Romero et al., 2000). Thus, both

obese ob/ob (leptin deficient) and db/db (with deficient leptin receptors) mice showed deteriorated immune response and increased infection susceptibility (Milner and Beck, 2012), and leptin administration counteracts this impaired immune response in animal models of leptin deficiency (Busso et al., 2002; Lord et al., 1998). In addition, leptin deficiency is associated with a decreased production of several cytokines. Nevertheless, the pattern of cytokines regulated by leptin is very different depending on several factors, notably the type of stimulus used to induce their release (R Faggioni et al., 2000; Raffaella Faggioni et al., 2000). Therefore, in the present study, in order to measure the effects of the leptin antagonist on pro-inflammatory and anti-inflammatory cytokine secretion by spleen leukocytes, these cells were stimulated with either LPS or ConA. LPS is known to directly activate innate immune cells to produce pro-inflammatory cytokines and several regulatory cytokines, chemokines and growth factors that promote inflammation, and it also activates the adaptive immune response conducted by B cells. ConA is a T cell mitogenic lectin that induces activation and proliferation of both $CD4^+$ y $CD8^+$ T cells (Luo et al., 2013). In the present study, we show lower IL10 levels in both sexes and with both types of spleen leukocytes stimulations in antagonist treated animals. Since IL10 is considered a clear anti-inflammatory cytokine, this fact suggests a decreased protection against a potential inflammatory state. Moreover, lower levels of anti-inflammatory cytokines such as IL-10 have been detected after LPS in ob/ob mice (Raffaella Faggioni et al., 2000). IL13, a typical Th2 cytokine, shows anti-inflammatory and immunoregulatory roles (Huang et al., 2015; Wynn, 2015). The present results show lower IL13 levels in LPS-stimulated leukocytes from males and in ConA-stimulated leukocytes from females. Thus, the neonatal treatment with the leptin antagonist seems to decrease this cytokine in a sex- and stimulus- dependent manner. The pro-inflammatory cytokine TNFα showed lower

levels in the LPS-stimulated leukocytes from males treated with the leptin antagonist, whereas the opposite was found in LPS-stimulated leukocytes from treated females, i.e. an increase in TNFα. In relation with this latter result, it is worth mentioning that leptin has been shown to inhibit TNFα induction by LPS in female mice Faggioni et al., (R Faggioni et al., 2000). Some results suggest that a defect in leptin production is associated with a shift of the immune response toward a proinflammatory phenotype as consequence of the up-regulation of proinflammatory and down-regulation of anti-inflammatory cytokines. However, no conclusive data are available on TNF levels after LPS. For example, they have been found to be either unchanged or decreased in ob/ob mice and, in macrophages from control mice, high doses of leptin up-regulate LPS-induced production of TNF (Faggioni et al., 2001). The present results also show that TNFα was increased in the ConA-stimulated leukocytes from males treated with the leptin antagonist, whereas no significant changes were observed in ConA-stimulated leukocytes from treated females. As a whole, the present data support the view that the pattern of cytokines regulated by leptin is very different depending on the type of stimulus used to induce their release (R Faggioni et al., 2000; Raffaella Faggioni et al., 2000). Moreover, we show here clear differences between males and females. It is worth re-emphasizing that, due to the reason indicated in the method section, the males and females used in this study were of different chronological ages and, therefore, the differences observed between males and females here cannot be exclusively attributed to their sex. This does not negate the fact that blockage of neonatal leptin signaling affects the future oxidative status of the animals and their ability to respond to inflammatory insults during the critical period close to pubertal onset in both males and females.

We have previously found that maternal deprivation (MD) during 24 hours on postnatal day 9 i.e., during the neonatal leptin surge period, produces a marked decrease in leptin levels (Viveros et al., 2010) as well as short and long term detrimental effects on the immune system of the animals (De La Fuente et al., 2009; Viveros et al., 2009). Thus, it is very likely that both, a social intervention such as MD and the present pharmacological treatment affect the development of the defense system of the organism through their interference of the neonatal leptin surge.

5. Conclusion

These findings point to a programming effect of the neonatal leptin surge on the establishment of an appropriate redox and inflammatory state of the immune system, which could be related with a correct immunity of the animals. Since all these aspects are relevant to maintain a healthy state in the adulthood and throughout the aging process (De la Fuente and Miquel, 2009; Hunsche et al., 2015), it follows that any situation that avoid/interfere the neonatal leptin surge may represent a risk factor for the future individual health.

Acknowledgements

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Figures

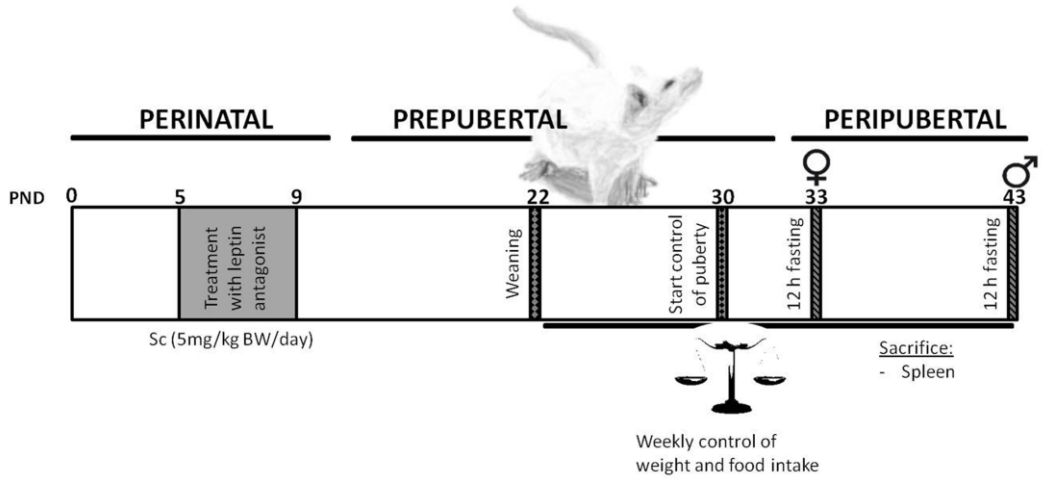

Fig.1. Experimental design diagram.

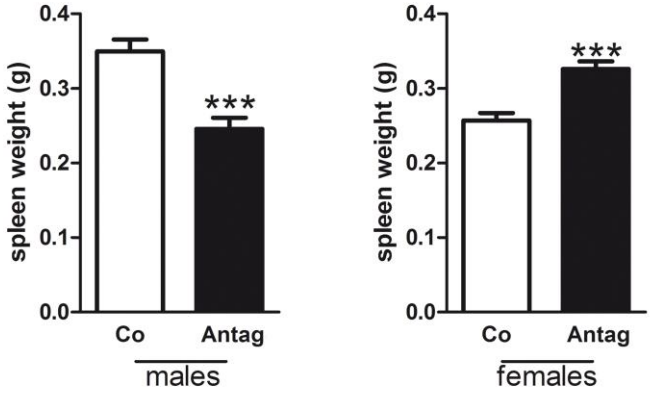

Fig.2. Changes in spleen weight (data expressed as mean ± S.E.M.) at the time of sacrifice in peripubertal male and female rats treated neonatally with a leptin antagonist (Antag) or vehicle (Co). n=8-12 per experimental group. One way ANOVA: *** $p<0.001$.

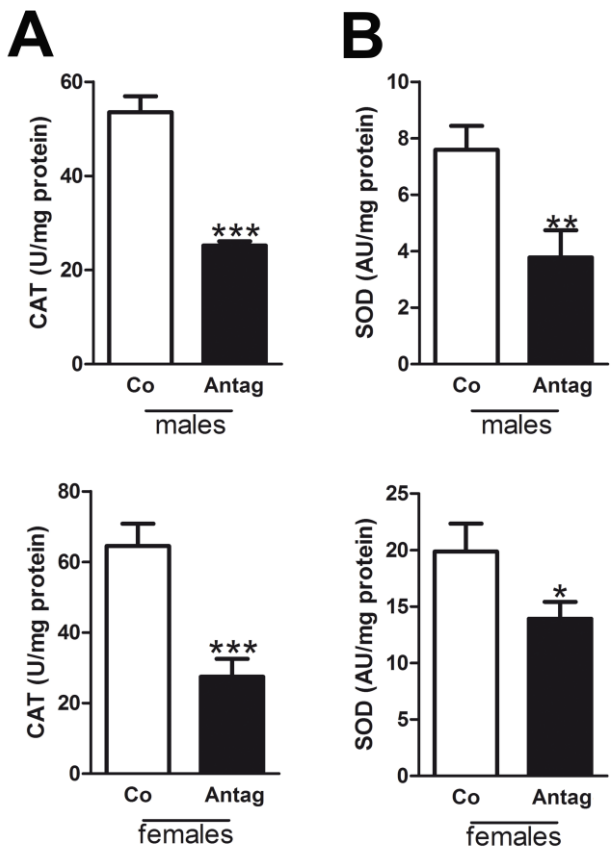

Fig.3. Changes in CAT (A) and SOD (B) activity in spleen homogenate (data expressed as mean ± S.E.M.) at the time of sacrifice in peripubertal male and female rats treated neonatally with a leptin antagonist (Antag) or vehicle (Co). n=6-11 per experimental group. One way ANOVA: *p<0.05, ** p<0.01,*** p<0.001.

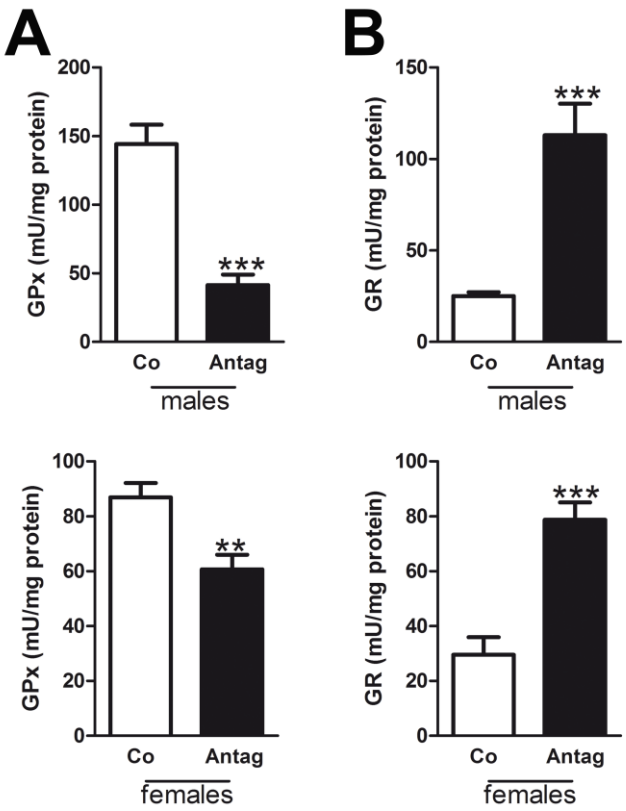

Fig.4. Changes in GPx (A) and GR (B) activity in spleen homogenate (data expressed as mean ± S.E.M.) at the time of sacrifice in peripubertal male and female rats treated neonatally with a leptin antagonist (Antag) or vehicle (Co). n=7-12 per experimental group. One way ANOVA: ** $p<0.01$,*** $p<0.001$.

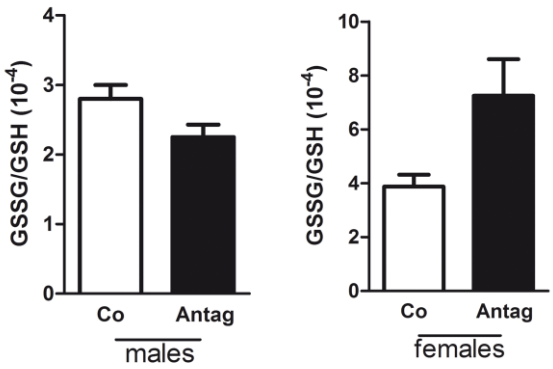

Fig.5. Changes in GSSG/GSH ratio in spleen homogenate (data expressed as mean ± S.E.M.) at the time of sacrifice in peripubertal male and female rats treated neonatally

with a leptin antagonist (Antag) or vehicle (Co). n=10-12 per experimental group. One way ANOVA: * p<0.05.

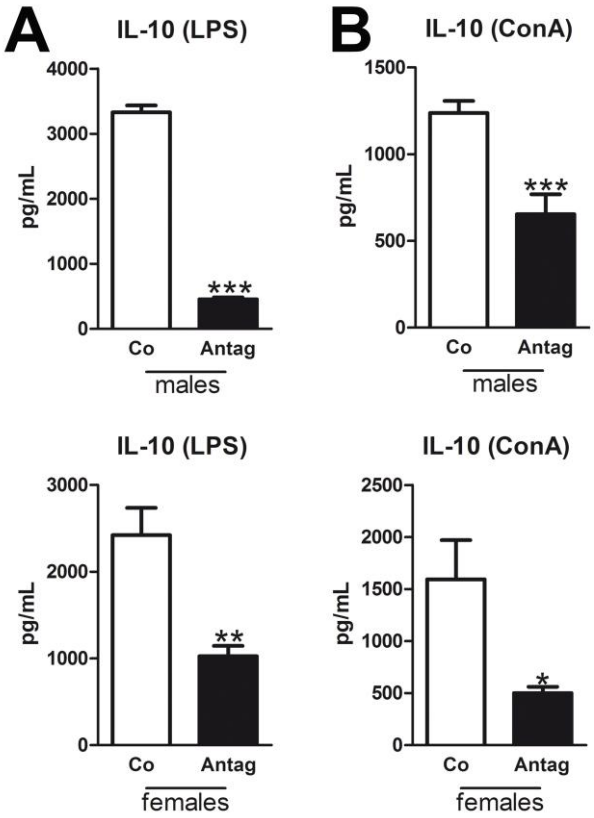

Fig.6. Changes in IL10 levels released (data expressed as mean ± S.E.M.) by spleen leucocytes treated with either LPS (A) or ConA (B) at the time of sacrifice in peripubertal male and female rats treated neonatally with a leptin antagonist (Antag) or vehicle (Co). n=6-8 per experimental group. ANOVA one way:*p<0.05, **p<0.01, ***p<0.001.

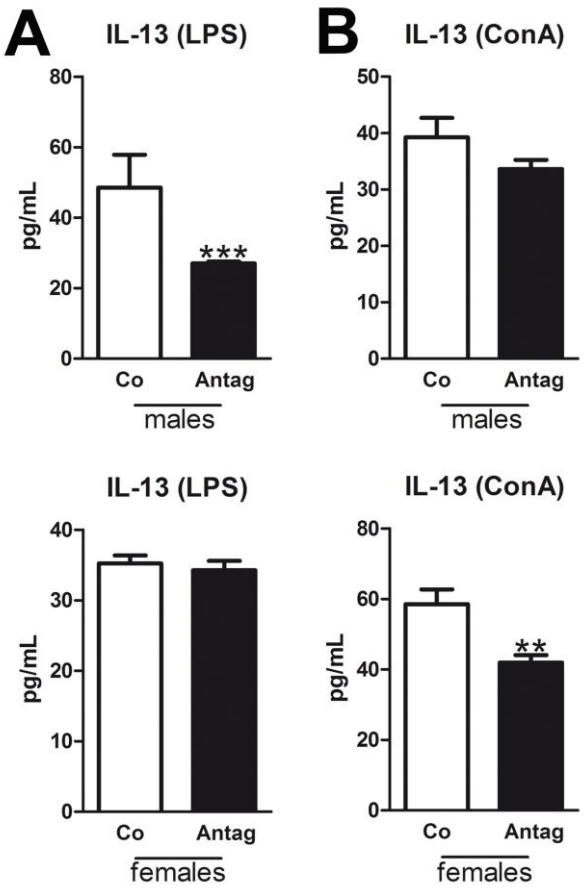

Fig.7. Changes in IL-13 levels released (data expressed as mean ± S.E.M.) by spleen leucocytes treated with either LPS (A) or ConA (B) at the time of sacrifice in peripubertal male and female rats treated neonatally with a leptin antagonist (Antag) or vehicle (Co). n=7-8 per experimental group. One way ANOVA: **p<0.01, ***p<0.001.

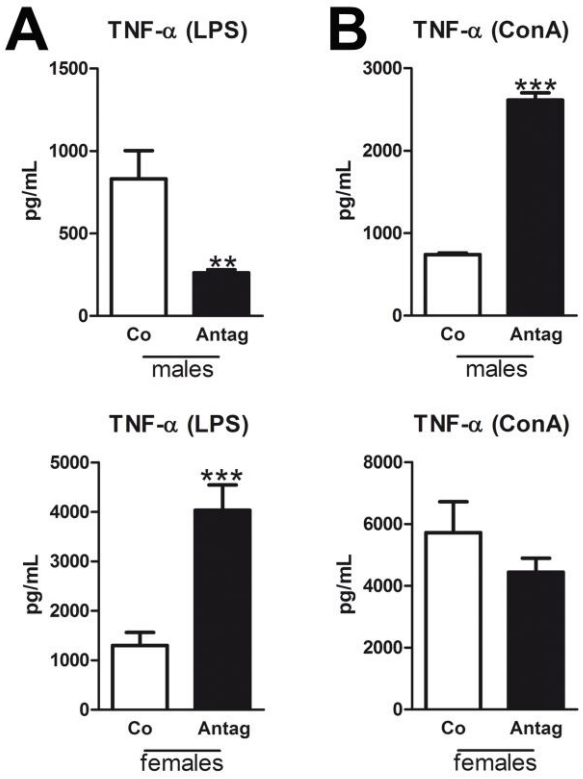

Fig.8. Changes in TNFα levels released (data expressed as mean ± S.E.M.) by spleen leucocytes treated with either LPS (A) or ConA (B) at the time of sacrifice in peripubertal male and female rats treated neonatally with a leptin antagonist (Antag) or vehicle (Co). n=6-8 per experimental group. One way ANOVA: *p<0.05, **p<0.01, ***p<0.001.

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