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ORIGINAL ARTICLE

OBESITY DURING PREGNANCY AND LACTATION INCREASES THE INFLAMMATORY RESPONSE AND REDUCES THE EFFICACY OF NIMESULIDE IN THE OFFSPRING OF WISTAR RATS

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Highlight:
(1) Pregnant rats on a Western diet intensify the acute-phase inflammatory response in offspring.
(2) Maternal Western diet consumption alters nimesulide's pharmacological efficacy in offspring.
(3) Lipid peroxidation increases in offspring of rats exposed to a Western diet during gestation.

ABSTRACT

The type of diet consumed during pregnancy and early life increases the possibility of developing metabolic syndrome, which can result in altered efficacy of some drugs. The aim of this study was to evaluate the inflammatory response in the offspring of *Wistar* rats fed a Western diet (WFD) and the anti-inflammatory effect of nimesulide. Twenty *Wistar* rats and their male offspring were used. The dams were divided on the 1st day of gestation into two groups: 1) DPgl: received standard diet (SD) and 2) DOgl: received DO, both from gestation to the end of lactation. On the 21st day of lactation, the male offspring were divided into six groups (n=5 animals/group) and assigned to different experimental protocols, in order to assess on the 60th day of life the influence of the type of diet on the inflammatory response (through carrageenan-induced paw oedema) and the anti-inflammatory action of nimesulide (5 mg/kg, i.p), by quantifying the levels of IL-6, TNF-α and myeloperoxidase (MPO) in the plantar tissue of the rats. The volume of edema was greater in the offspring of DOgl dams, regardless of whether they received PD or DO post-weaning; in those that received DO, the activity of nimesulide was reduced and the levels of IL-6, TNF-α and MPO were elevated. In the offspring of DPgl dams, which received PD post-weaning, the anti-inflammatory activity of nimesulide, verified in the paw volume, was maintained at all three evaluation times. Thus, the inflammatory response was more significant in the DOgl offspring that received DO, and these animals showed a reduction in the anti-inflammatory effect of nimesulide.

Keywords: western diet; acute inflammation; nimesulide.

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INTRODUCTION

Obesity is a chronic metabolic disease characterized by the excessive accumulation of body fat1 and is considered a risk factor for the development of other morbidities such as diabetes, dyslipidemia and heart disease2. Obesity is currently referred to as a chronic non-communicable disease and according to data from the World Health Organization (WHO) in 2021[3], more than 1.9 million adults were overweight, 600 millions of whom were obese. The habits of today's society, such as the high consumption of junk food, the expansion of fast-food chains and the reduction in physical activity, have been associated with the prevalence of obesity in the West[4]. These behaviors are associated with the availability and high palatability of the foods consumed, in parallel with the intake of processed and/or ultra-processed foods, which contribute to the increase in obesity rates in the Western world[5].

The association between obesity and inflammation is related to high levels of cytokines and acute phase proteins. Adipocytes synthesize a variety of cytokines and other acute phase inflammatory proteins which ultimately increase the synthesis and circulation of these chemical mediators[6,7]. Excess fat induces an increase in the production of adipokines, resulting in impacts on body physiology, such as changes in blood pressure, energy balance, the immune system, insulin sensitivity and angiogenesis[8]. Adipokines synthesized by adipose tissue include adiponectin, leptin, interleukin 1 (IL- 1), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), C-C motif chemokine ligand 2 (CCL2) and serine protease inhibitors[9,10].

To mitigate the imbalance between pro-inflammatory mediators, anti-inflammatory drugs are used in therapy. One example is nimesulide, which is a drug in the non-steroidal anti-inflammatory drug (NSAID) class and has analgesic and antipyretic properties.

Studies have shown the therapeutic potential of nimesulide on non-alcoholic fatty liver disease (NAFLD) and insulin resistance in mouse models submitted to a high-fat diet[11]. In addition, other studies have verified the beneficial effect of nimesulide in inhibiting the growth of various tumors (cervix, pancreas, breast, lung) and in potentiating the activities of specific antioxidant enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), as well as total antioxidants (Antox)[12-14].

In this context, our hypothesis is that the consumption of Western diet during the period of fetal development until weaning can cause exacerbation of the acute inflammatory response induced by an edematogenic agent and decrease the pharmacological efficacy of nimesulide. Thus, we sought to evaluate the acute phase of the inflammatory response, as well as the anti-inflammatory activity of nimesulide in the adult offspring of *Wistar* rats from dams fed a Western diet during gestation and lactation and/or post-weaning. This was done using the carrageenan-induced paw edema model and quantification of cytokines (IL-6 and TNF-α) and myeloperoxidase (MPO).

MATERIAL AND METHODS

Place of study

This work was carried out at the Bioactive Products Laboratory (LFPB) in the Department of Physiology and Pharmacology and the Experimental Nutrition and Dietetics Laboratory in the Department of Nutrition, located at the Federal University of Pernambuco – UFPE, Recife *Campus*.

Animal Model

Twenty primiparous albino rats of the Wistar strain and their offspring made up of 30 male rats were used. The animals were obtained from the breeding colony of the Nutrition Department of the Federal University of Pernambuco. Mating took place at a ratio of one male (n=10) to two females

(n=20). All the animals were kept in an experimental vivarium and housed in polypropylene cages measuring 46x31x21 cm (LxWxH) in an environment with a temperature of 22 ± 1° C, an inverted light-dark cycle of 12/12 h and filtered water and diets ad libitum.

The experimental protocol was approved by the Animal Experimentation Ethics Committee of the Biosciences Center of the Federal University of Pernambuco (Protocol No. 23076.003.845/2015-63).

Diet

The Western diet (WD), characterized by having a higher amount of saturated lipids than the standard diet (SD), was produced from a variety of natural and purified foods, crushed and homogenized until pellets were obtained. The composition of the DO is described by Ferro-Cavalcanti et al.[15,16]. The composition of the macronutrients of the diets used according to their total energy value is described in Table 1.

Table 1 – Macronutrients according to the Total Energy Value (TEV) of the standard (Presence®) or Western diet

Diet	(% kcal VET)	(% kcal VET)	(% kcal VET)	VET (kcal/g)
Western*	**20**	**49**	**31**	**4.2**
Presence **	**26**	**63**	**11**	**3.6**

*Calculations of the centesimal composition of macronutrients were based on the nutritional information sent by the company supplying the products and on the Brazilian Table of Food Composition (TACO). VET= total energy value

**Determined by the Adolfo Lutz Institute, 1985.

Nutritional manipulation

From the 1st day of pregnancy (confirmed by the presence of sperm in the vaginal swab), the breeding rats were randomly divided into two groups: 1) DPgl who received vivarium PD (Presence®) during gestation and lactation and 2) DOgl who received DO during gestation and lactation.

On the 21st day of lactation, which corresponded to the day of weaning, only the male offspring remained in the experiment and were allocated to six groups (n=5 animals/group) divided as follows:

i. DPgl-DP – breeding rats that received DP during gestation and lactation, with the male offspring being kept on DP from post-weaning until the 60th day of life;

ii. DOgl-DO – breeding rats that received DO during gestation and lactation, with the male offspring being kept on DO from post-weaning until the 60th day of life;

iii. DPgl-DO – breeding rats that received DP during gestation and lactation, with the male offspring being fed DO from post-weaning until the 60th day of life;

iv. DOgl-DP – dams that received DO during gestation and lactation, with the male offspring being fed DP from post-weaning until the 60th day of life.

v. DPgl-DPn – dams that received PD during gestation and lactation, with the male offspring being fed PD from post-weaning until the 60th day of life and to evaluate the efficacy of nimesulide, they received (5 mg/kg, i.p. single application);

vi. DOgl-DOn – dams that received DO during gestation and lactation, with the male offspring being fed DO from post-weaning until the 60th day of life and to assess the efficacy of nimesulide (5 mg/kg, i.p. single application).

Weight development of breeding rats fed DP or DO and their offspring

Body weight measurements were taken weekly on the breeding rats during gestation and lactation and on their respective male offspring at birth and from the 21st to the 60th day of life.

Determining food consumption

The feed consumption of the breeding rats during gestation and lactation and of their offspring in the post-weaning period up to 60 days of age was assessed every two days and calculated by subtracting the initial weight of the feed offered from the leftovers in the cage over a 48-hour period.

Determination of biochemical and hematological profile

At 60 days of age, the animals were fasted for 10 hours and subjected to induction of paw edema. After the end of the experiments, the animals were anesthetized with thiopental (30 mg/kg) to obtain blood samples (5mL) during decapitation by guillotine to determine biochemical (serum albumin and C-reactive protein (CRP)) and hematological (total and differential leukocyte count) variables. The sample was centrifuged to obtain serum and stored in a -20º C freezer for later analysis of acute phase proteins. Albumin was determined at the Central Laboratory of the Hospital das Clínicas of the Federal University of Pernambuco and a specific commercial kit for rats was used for C-Reactive Protein (Boster Biological Technology Co., Ltd., Fremont, California, USA).

The automated method used to determine the total number of leukocytes, and the differential count was carried out at the Central Laboratory of the Hospital das Clínicas of the Federal University of Pernambuco.

Influence of DO on the intensity of the acute inflammatory response and the anti-inflammatory activity of nimesulide in rats

To study the acute inflammatory response and the anti-inflammatory effect of nimesulide, the carrageenan-induced edema model was used[17]. For this purpose, six groups were structured (n=5 animals/group), all the animals were 60 days old, and were fasted for 10 hours before being submitted to the induction of paw edema. The groups were composed as follows: I) control – DPgl-DP, DOgl-DO, DPgl-DO and DOgl-DP) and II) treated with nimesulide, (DPgl-DPn, DOgl-DOn) and subjected to the following experiments:

Experiment A: the animals in the DPgl-DP, DOgl-DO, DPgl-DO and DOgl-DP groups were treated with distilled water (5 mg/kg, i.p) 45 min before receiving a subplantar injection of carrageenan (0.1 mL - 1% v/v in saline) in the left hind paw.

Experiment B, the DPgl-DPn and DOgl-DOn groups were previously treated with nimesulide (5 mg/kg, i.p) 45 min before the subplantar injection of the oedematogenic agent (carrageenan) under the same conditions described in experiment A.

The volume of the injected paw was measured using a digital plethysmometer (Panlab - Harvard Apparatus, Model LE 7500) before (0 min) and 30, 60, 120, 180 and 240 min after the injection of carrageenan. The change in paw volume was expressed in milliliters by the difference recorded in paw volume before (0 min.) and after the injection of the oedematogenic agent at the end of each time interval. The intensity of the inflammatory response (edema volume) exhibited by the DOgl-DO, DOgl-DP and DPgl- DO groups was compared with the DPgl-DP group

Percentage of inhibition = 1- Vt/Vc x 100, where Vt corresponds to the mean of the differences resulting from the paw measurements in the groups treated with nimesulide (DPgl-DPn and DOgl-DOn) and Vc corresponds to the mean of the differences resulting from the paw measurements in the control groups (DPgl-DP and DOgl-DO)

At the end of the experiments, blood samples were taken to assess biochemical and hematological variables and the entire subplantar region of the animals' left paw was used to quantify cytokines (IL-6 and TNF-α) and MPO.

Dosage of IL-6 and TNF-α in homogenate from the subplantar region

Four hours after the injection of the edematogenic agent (carrageenan), the animals were euthanized to remove tissue from the plantar region of the paws and these were homogenized in 500 µL of buffer solution. The Elisa method was used to determine TNF-α and IL-6 levels using specific commercial kits (Boster Biological Technology Co., Ltd., Fremont, California, USA). The samples were tested in triplicate and the results generated by comparing the absorbance with the standard curves.

Myeloperoxidase activity

Myeloperoxidase (MPO) is an enzyme present in the azurophilic granules of neutrophils and has been used as a marker to quantify neutrophil migration in inflammatory processes in various tissues. To assess cell infiltration to the inflammatory site by quantifying myeloperoxidase (MPO), samples from the subplantar region of the swollen paw of the animals were used, collected and stored in 500 µL of 0.5% CTAB buffer (cetyltrimethylammonium bromide pH 6.0, 1 g/ml) in a -80° C freezer. For analysis, the samples were homogenized in a Politron (Teclab®) with CTAB buffer. The homogenate was then centrifuged at 14,000 rpm for 10 min at 4º C and the supernatant collected.

Myeloperoxidase activity per mL of tissue was measured using the technique described by Bradley; Christensen; Rothstein[18]. 7 µL of supernatant was poured into a 96-well plate. Next, 200 µL of o-dianisidine solution was added per well (16.7 mg of o- dianisidine; 90 mL of MilliQ water; 10 mL of potassium phosphate buffer). 1% hydrogen peroxide was used as a substrate for MPO. The unit of MPO activity was defined as that capable of converting 1µmol of hydrogen peroxide into water in 1 min at 22º C. As hydrogen peroxide is degraded, superoxide anion is produced, which converts o-dianisidine into a brown compound. The results were expressed as MPO/mL activity.

Statistical analysis

The results were expressed as mean ± standard deviation and the normality of the data was checked using the Kolmogorov Smirnoff test. The two-way Anova test, followed by the Bonferroni test, was used to assess body weight, edema volume, the intensity of the acute inflammatory response and the percentage of edema inhibition by nimesulide. The Student's t-test was used to evaluate the mothers' weight during pregnancy and lactation. The one-way Anova test followed by the Tukey test were used to compare the groups and analyze the hematological and biochemical parameters, cytokine quantification and myeloperoxidase activity. Statistical analysis was carried out using Graphpad Prism software® 8.0. The significance level $P< 0.05$ (5%) was considered for statistical purposes.

RESULTS

Weight development of breeding rats fed DP or DO and their offspring

During gestation and lactation, there were no differences between the weight of the mothers, DPgl or DOgl groups or in the weight of the offspring at birth or at weaning (Table 2). However, at 60 days of age, the DOgl-DO, DOgl-DP and DPgl-DO groups showed statistically significant differences ($P<0.05$) when compared to the DP-fed group (DPgl-DP).

Table 2: Body weight (grams) of dams fed the standard diet (SD) or the western diet (OD) during gestation and lactation and their respective offspring

MATRIXES	**DP**		**DO**	
Late pregnancy	297.60 ± 18.36		305.60 ± 29.74	
End of Lactation	288.60 ± 46.81		281.80 ± 20.90	
PROLES	DPgl-DP	DPgl-DO	DOgl-DO	DOgl-DP
At birth	6.50 ± 0.70	7.30 ± 0.60	6.80 ± 0.50	7.10 ± 0.30
At weaning (22 days)	53.40 ± 8.99	57.80 ± 2.48	51.90 ± 4.04	49.10 ± 4.77
At 60 days	229.70 ± 16.34	276.00 ± 26.44*	291.80 ± 24.29*	275.30 ± 12.61*

Values expressed as mean ± SD. Significance obtained from ***two-way*** ANOVAfollowed by the ***Bonferroni***

test (***P<0***.05) when comparing DP ***versus*** DO or DPgl-DP ***versus*** DOgl-DO, DOgl-DP or DPgl- DO or (***n=5*** animals/group).

The weekly weight evolution (g) of the groups fed DP or DO during gestation, lactation and/or post-weaning is shown in Figure 1. The animals in the DOgl-DO group showed a 16% (*P<0*.05) greater gain in body weight in the 3rd, 6th and 7th weeks of diet consumption, when compared to the animals in the DPgl-DP group (Figure 1). While the DOgl-DP group showed greater weight gain than the DPgl-DP from the 4th week to the 7th week (Figure 1). As for the body weight of the DPgl-DO, there was a difference in weight gain, which was greater than DPgl-DP from the 5th week to the 7th week (Figure 1).

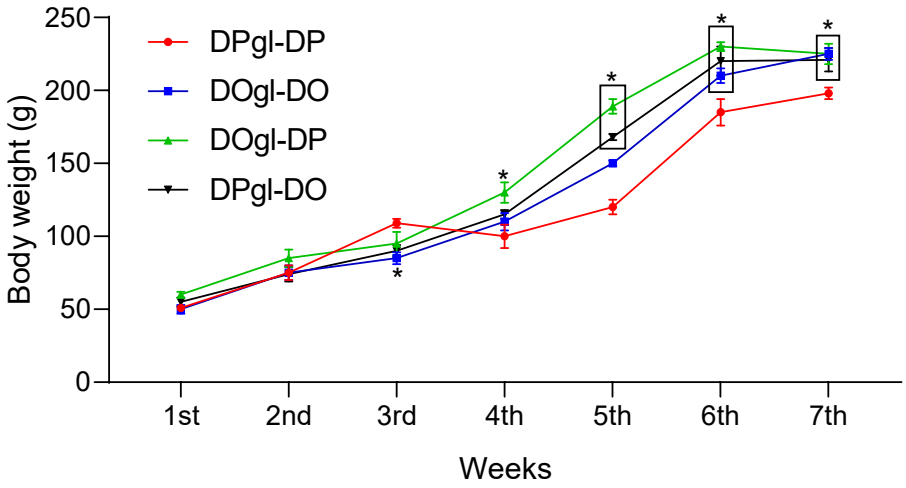


Figure 1 – Average body weight (g) of Wistar rats (n=5 animals/group) fed a standard diet (SD) or western diet (OD) during gestation, lactation and/or post-weaning. Values expressed as mean

± SD. Significance obtained from two-way Anova followed by the Bonferroni test (*P<0.05). The * refers to the difference between the experimental groups (DOgl-DO or DPgl-DO or DOgl-DP) compared to the control group (DPgl-DP).

Food consumption

The daily food consumption (g) of the groups fed DP or DO during gestation, lactation and/or post-weaning is shown in Figure 2. As can be seen, the animals in the DOgl-DO group showed a

reduction in food consumption in the 3rd and 4th week of the diet compared to the DPgl-DP group. The groups that had their diet changed during gestation, lactation and/or post-weaning (DPgl-DO and DOgl-DP) were also compared with the DPgl-DP group. It was found that the food consumption of the DOgl-DP group did not differ from that of the DPgl-DP group. On the other hand, when comparing DPgl-DO with DPgl-DP, it was observed that DPgl-DO reduced consumption in the 4th week post-weaning.

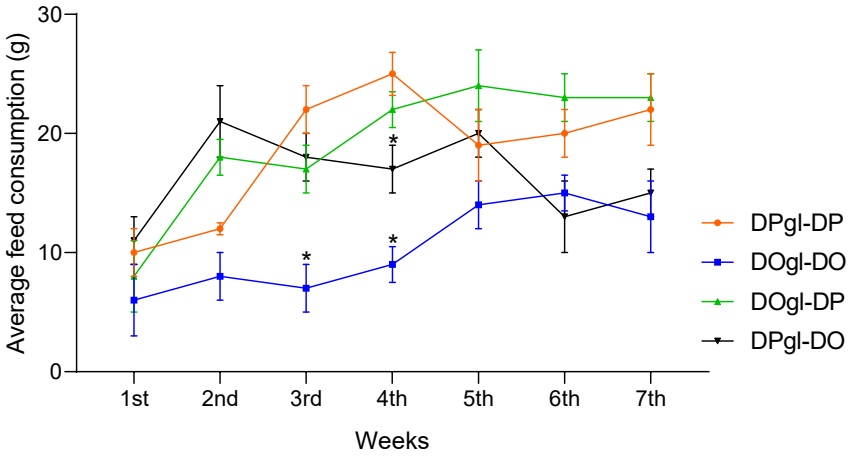


Figure 2 – Average daily food consumption (g) of Wistar rats (n=5 animals/group) fed a standard diet (SD) or western diet (OD) during gestation, lactation and/or post-weaning. Values expressed as mean ± SD. Significance obtained from two-way Anova followed by the Bonferroni test (*P<0.05). The * refers to the difference between the experimental groups (DOgl-DO or DPgl-DO or DOgl- DP) compared to the control group (DPgl-DP).

Biochemical and hematological parameters

Analysis of the biochemical and hematological parameters revealed no statistical difference between the groups (DPgl-DP and DOgl-DO; DOgl-DP and DPgl-DO) at 60 days of age (table 3)

Table 3 – Biochemical and hematological parameters of the offspring of adult *Wistar* rats (*n=5* animals/group) fed DP or DO during gestation, lactation and from post-weaning to the 60th day of life (DPgl-DP and DOgl-DO, respectively) or subjected to a diet swap (DOgl-DP and DPgl- DO) after the post-weaning period

Parameters	**DPgl-DP**	**DOgl-DO**	**DOgl-DP**	**DPgl-DO**
Albumin (g/dL)	2.38 ± 0.24	2.18 ± 0.49	2.22 ± 0.21	2.44 ± 0.08
Total protein (g/dL)	5.02 ± 0.34	4.72 ± 0.69	4.76 ± 0.32	4.98 ± 0.49
CRP (ng/dL)	6.74 ± 24.1	6.75 ± 4.70	6.75 ± 4.70	6.75 ± 3.80
Leukocytes (10^3mm^3)	9.53 ± 0.52	8.57 ± 1.16	9.06 ± 0.96	8.08 ± 2.01
Neutrophils (%)	2.96 ± 0.62	2.96 ± 1.12	1.99 ± 0.51	2.91 ± 1.23
Eosinophils (%)	0.12 ± 0.03	0.08 ± 0.05	0.08 ± 0.02	0.12 ± 0.05
Basophils (%)	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01

Lymphocytes (%)	4.46 ± 1.04	5.88 ± 1.21	5.52 ± 1.30	4.11 ± 8.06
Monocytes (%)	2.19 ± 2.85	1.70 ± 3.01	2.09 ± 1.30	2.15 ± 2.84

Values are presented as mean ± SD. Significance obtained from ***One-Way*** ANOVA followed by ***Tukey***'s test (***P<0**.05). CRP: C-reactive protein; DPgl-DP: offspring of breeding rats that received PD during gestation, lactation and post-weaning up to the 60th day of life; DOgl-DO: offspring of breeding rats that received DO during gestation, lactation and post-weaning up to the 60th day of life; DPgl-DO: offspring of breeding rats that received DP during gestation and lactation, and DO post- weaning up to the 60th day of life; and DOgl-DP: offspring of breeding rats that received DO during gestation and lactation, and DP post-weaning up to the 60th day of life.

Influence of DO on the intensity of the acute inflammatory response in Wistar rats

The acute inflammatory response induced by carrageenan was more severe in the DOgl-DO, DOgl-DP and DPgl-DO groups at 120, 180 and 240min when compared to the DPgl-DP group (table 4)

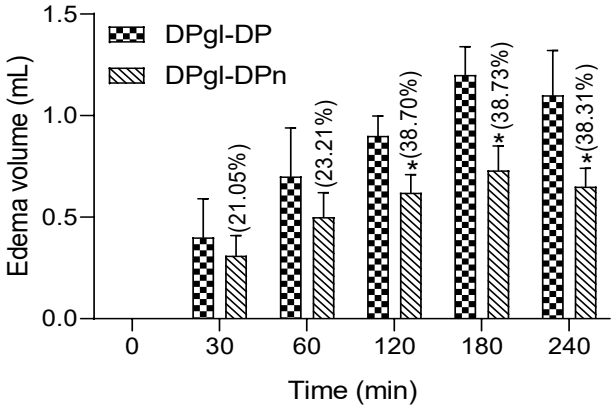
Table 4 – Average paw volume in the DPgl-DP, DOgl-DO, DPgl-DO and DOgl-DP groups (n=5 animals/ group) with paw edema induced by subplantar injection of carrageenan (0.1 mL).

	Paw edema (mL)				
	30 min	60 min	120 min	180 min	240 min
Groups					
DPgl–DP	0.38 ± 0.12 (100%)	0.56 ± 0.18 (100%)	0.93 ± 0.20 (100%)	1.11 ± 0.26 (100%)	1.07 ± 0.14 (100%)
DOgl–DO	0.71 ± 0.25 (+86%)	0.91 ± 0.23 (+62%)	1.66 ± 0.61* (+78%)	2.06 ± 0.62* (+85%)	2.10 ± 0.67* (+96%)
DPgl–DO	0.49 ± 0.17 (+28%)	0.68 ± 0.14 (+21%)	1.35 ± 0.16* (+45%)	1.55 ± 0.23* (+39%)	1.27 ± 0.34 (+18%)
DOgl–DP	0.77 ± 0.07 (+102%)	1.03 ± 0.10* (+83%)	1.62 ± 0.16* (+74%)	1.92 ± 0.36* (+72%)	1.51 ± 0.37* (+41%)

( ) percentage increase in paw volumes (intensity of inflammatory response) compared to the DPgl-DP group. Values expressed as mean ± SD. ***P<0**,05. The * refers to the difference between the experimental groups (DOgl-DO or DPgl-DO or DOgl-DP) compared to the control group (DPgl-DP).

Influence of OD consumption on the anti-inflammatory activity of nimesulide in Wistar rats

Figure 3 (A and B) shows the evolution of paw oedema in animals fed DP or DO and treated with nimesulide in the carrageenan-induced acute inflammation model. As can be seen, the anti-inflammatory effect of nimesulide (% inhibition of paw oedema) was significantly lower in the DOgl-DO group when compared to the DPgl-DP group. In addition, in the animals in the DPgl-DP group, the anti-inflammatory effect of nimesulide was seen at 120 (38.70%), 180 (38.73%) and 240 min (38.31%), while in the animals in the DOgl-DO group it was only seen at 120 min (42.16%).



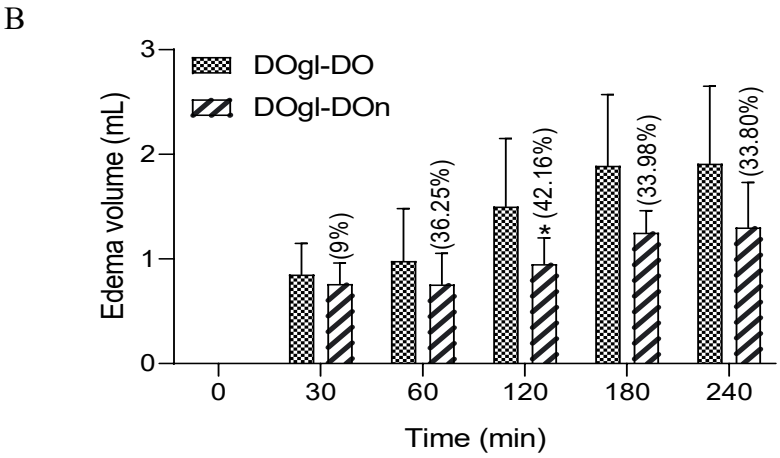


Figure 3 – Effect of treatment with Nimesulide (5 mg/kg, i.p. single application) on paw edema induced by subplantar injection of carrageenan in the DPgl-DPn (A) and DOgl-DOn (B) groups. Values expressed as mean ± SD. Significance obtained from *two-way* ANOVA followed by the *Bonferroni* test. * *P<0*.05 when compared to their respective control groups (DPgl-DP *versus* DPgl- DPn, and DOgl- DO *versus* DOgl- DOn).

IL-6 and TNF-α levels

Figure 4 (A and B) shows the concentrations of the pro-inflammatory cytokines IL- 6 and TNF-α in the homogenate of the subplantar region of the DPgl-DPn, DOgl-DOn, and DPgl-DP and DOgl-DO groups. The results show a significant increase in the levels of these cytokines in the animals in the DOgl-DO groups when compared to the DPgl-DP group. The group treated with nimesulide (DPgl-DPn) showed a significant reduction in IL-6 and TNF-α levels when compared to its control group (DPgl-DP). However, the DOgl-DOn group showed no difference in IL-6 and TNF-α levels when compared to its control group (DOgl-DO).

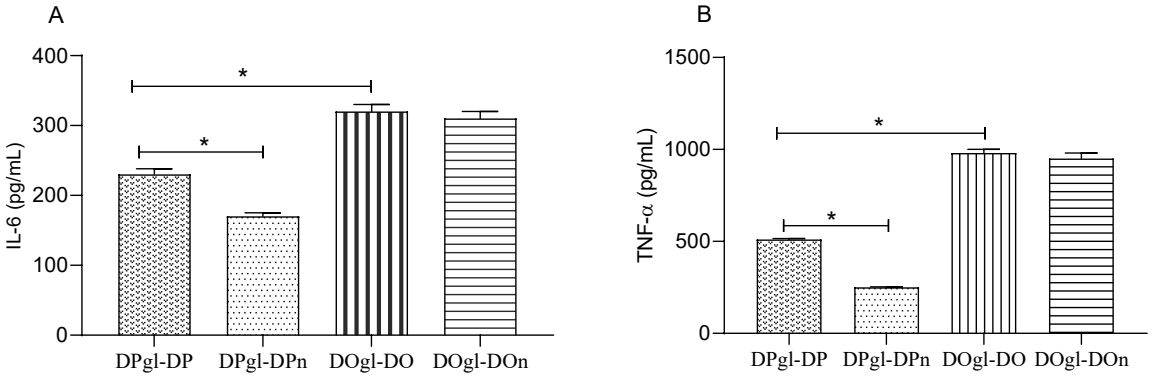


Figure 4 – Levels of IL-6 (A) and TNF-α (B) in the homogenate of the subplantar region of the treated groups (DPgl-DPn, DOgl-DOn) and their respective control groups (DPgl-DP and DOgl- DO) four hours after carrageenan injection. Values expressed as mean ± SD. Significance obtained from *one-way* ANOVA followed by Tukey's test *´ P<0.05

Myeloperoxidase (MPO) enzyme activity

The animals in the DOgl-DO group showed higher MPO activity when compared to the DPgl-DP group (Figure 5). Regarding treatment with nimesulide (5 mg/kg; i.p. single application), the animals in the DPgl-DPn group showed a significant reduction in MPO activity when compared to their respective control group (DPgl-DP), while no change was seen in the DOgl-DOn group in relation to their respective DOgl-DO control.

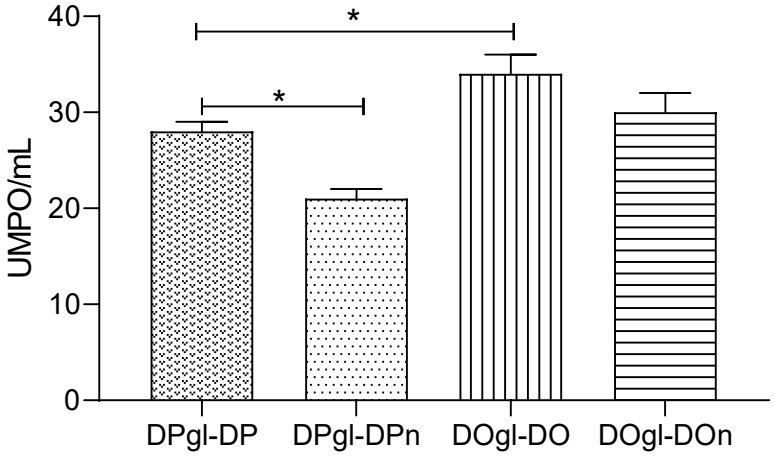


Figure 5 – Activity of the enzyme myeloperoxidase (MPO) in the supernatant of the homogenate of the supplantar region of Wistar rats four hours after the injection of carrageenan in the groups treated with nimesulide (DPgl-DPn, DOgl-DOn) and their respective control groups (DPgl-DP and DOgl-DO). Values expressed as mean ± SD. Significance obtained from one-way ANOVA followed by Tukey's test. P< 0.05*.

DISCUSSION

Studies show that increasing the amount of fat or the energy load mediated by carbohydrates in diets leads to a reduction in food consumption in animal models[19]. In animals without metabolic disorders, this balance probably mediated by an adjustment in the energy balance. Our results align with these authors, showing reduced food consumption during follow-up. This reduction, in turn, was not accompanied by a reduction in body weight; on the contrary, the Western diet caused weight gain in all groups. We therefore suggest that the proportion of energy derived from the different types of fatty acids (saturated and unsaturated) probably contributed to this excessive gain, given that the Western diet has a higher saturated fatty acid content[15]. The metabolism of saturated fats is different from that of unsaturated fats, contributing to greater storage of body fat. In turn, Turchi et al.[20] reported damage to mitochondrial metabolism, with a predisposition to excessive body weight gain in mammals when fed a hypercaloric diet similar to that consumed by the Western population.

Clinical and non-clinical studies suggest that obesity during pregnancy negatively affects the health of the offspring in the short and long term, increases the risk of developing obesity and other comorbidities and can result in premature death[21]. According to Drozdz et al.[22] and Faienza et al.[23], body weight in early life is a key indicator of the risk of metabolic diseases in adulthood. In our study, DO was able to induce an increase in body weight (16%) in the animals that received the diet during gestation, lactation and/or post-weaning.

Desai et al.[24] observed that the different effects caused by the maternal diet on the phenotype of the offspring depend on the period of supply, i.e. during gestation and/or lactation. The same authors found that exposure to a hypercaloric diet during gestation and lactation resulted in an altered phenotype in the offspring, characterized by exaggerated obesity when compared to the group derived from dams fed the same diet only during gestation. In this context, Buzinaro et al.[25] showed that women with gestational diabetes *mellitus* who had high fasting and daily blood glucose levels gave birth to children with higher birth weights and that these children were more likely to be overweight during adolescence. These results suggest that the greater the exposure to diets that lead to obesity and metabolic alterations, the more likely it is that the offspring will have metabolic complications in adulthood. In addition, studies on C57BL/6 mice with diet-induced obesity have found increased adiposity, glucose intolerance, hyperlipidemia and structural changes in the kidneys in their offspring[26].

The results obtained in this study show that all the groups fed DO at different times during the experimental period (DOgl-DO; DOgl-DP and DPgl-DO) had a metabolic profile similar to that of obese animals, showing an exacerbated inflammatory response due to the increase in paw volume and corroborated by an increase in cytokine levels. In this study we demonstrated that the level of TNF-α and IL-6 in animals that consumed a Western diet were higher when compared to animals fed a standard diet. These results suggest an association between obesity and a subclinical state of inflammation in animals exposed to a high-calorie or high-lipid diet.

The cytokine levels of the rats pretreated with nimesulide (5 mg/kg, i.p. single application) in our study showed no statistical difference between the groups (DOgl-DO x DOgl-Don), i.e. nimesulide was unable to decrease the level of TNF-α and IL-6. However, in the work carried out by Azab; Kaplanski[27] on rats pre-treated with nimesulide (30 mg/kg, i.p.), there was a decrease in TNF-α production. We suggest that the dose and vehicle used by Azab; Kaplanski[27] influenced the reduction in the synthesis of this cytokine (5 mg/kg vs. 30 mg/kg; carboxymethylcellulose vs. DMSO). Our results corroborate those found by Liang et al.[28], who showed that nimesulide was unable to reduce the level of TNF-α quantified in the liver and aorta in mice fed a hypercaloric diet.

Similar to the results found in the present study, exacerbated inflammation induced by carrageenan was demonstrated in obese Zucker rats compared to controls[29]. In addition, elevated expression of adhesion molecules and macrophage infiltration was found in response to vascular inflammation in obese Zucker rats[30].

According to Hosogai et al.[31], in obesity, adipocytes are hypertrophied and compromise the vascularization of adipose tissue, causing a restriction in the availability of oxygen, resulting in tissue hypoxia[32]. As the tissue expands, macrophages infiltrate it, activating the production of TNF-α, IL-6, interleukin-1β, leptin, resistin and monocyte chemotactic protein (MCP-1)[33]. Fantuzzi[34] suggests that leptin induces oxidative stress and contributes to the accumulation of macrophages, facilitating the adhesion of macrophages and endothelial cells.

The relationship between increased body weight and neutrophil infiltration in the parenchyma of intra-abdominal adipose tissue was demonstrated after starting to eat a hypercaloric diet in C57BL/6J mice[35]. High fat intake results in increased neutrophil infiltration and expression of MPO activity in epididymal white adipose tissue, along with increased weight and altered insulin signaling[36]. Plasma levels of the MPO enzyme have been shown to be elevated in obese individuals with systemic inflammation, increasing the risk of cardiovascular disease.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as the nimesulide used in this study, are among the categories of drugs most commonly used to mitigate the consequences of an inflammatory stimulus. Their action consists of selectively inhibiting the activity of the enzyme cyclooxygenase-2 (COX2)[37]. Some studies have shown that functional and body composition in obese people can alter

the distribution of the drug, with no effect on the volume of distribution, renal clearance, hepatic clearance and binding to plasma proteins[38-39]. Nimesulide is a lipophilic compound, i.e. it has a greater affinity for fat and the drug's binding to plasma proteins is not altered in obesity. Current studies on the subject are necessary to better understand the pharmacological mechanism in obese individuals, given that obesity is a disease that is growing uncontrollably throughout the world and NSAIDs are the most widely used drugs.

CONCLUSION

It is concluded that the consumption of the Western diet by the dams during the offspring's development period (gestation, lactation and/or post-weaning) causes an increase in the intensity of the inflammatory response in the acute phase, as well as altering the pharmacological efficacy of nimesulide in the offspring of *Wistar* rats.

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