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CAFETERIA DIET AND OBESITY: MUTAGENICITY IN WISTAR RATS AND CONSEQUENCES ON FEMALE OFFSPRING

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ABSTRACT

Besides genetic factors, diet components may contribute to the development of obesity, and it is known that cafeteria diet is characterized by a high caloric intake. Thus, the present study evaluated the mutagenic effects that cafeteria diet exerted on *Wistar* rats and its consequences on female offspring. Twelve females (generation 1) were separated in control group (CTL) and Cafeteria Diet group (CAF). At 70 days of age, females were mated with non-obese control males. Weaning at 21 days of age, female offspring (2nd generation) were separated into daughters of CTL or CAF mothers and subdivided according to previous diet. Ratio of polychromatic erythrocytes (PCE) / normochromatic erythrocytes (NCE) in a total of 1000 cells and Micronuclei (MN) 1000 PCE were evaluated. Were observed high MN frequencies, body weight, retroperitoneal and perigonadal fat; and low PCE/NCE ratio in generation 1. In generation 2, cafeteria diet caused high body weight, retroperitoneal and perigonadal fat and perpetuation of cytotoxicity in offspring, regardless of the cafeteria diet. Changing the diet of offspring, no statistically differences were observed in MN frequencies, PCE/NCE ratio, body weight and retroperitoneal fat, showing the importance of diet even with a disadvantageous mother background.

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INTRODUCTION

In recent decades, global prevalence of obesity has substantially increased (Ogden *et al.* 2007). It is estimated to 2025 that the number of children under five years old overweight or obese increases to 70 million (WHO 2013a). Furthermore, in 2048, all American adult should present obesity or overweight (WHO 2013b). These are disturbing data given the association between obesity and various metabolic disorders, such as insulin resistance and hyperglycemia, increased risk of cardiovascular diseases, type 2 diabetes and some types of cancer (Calle *et al.* 2003; Reilly and Rader 2003; Feng *et al.* 2010; Ohtani *et al.* 2014). In addition, an estimative of $ 147 billion is annually spent for the treatment of obesity only in America (Zamosky 2013), which makes this dysfunction a public health problem and a major target for study. Obesity is a multifactorial disease, resulting from complex interactions between genetic and environmental factors (Martinez *et al.* 2008; Schwenk *et al.* 2013). It is known that the consumption habits of industrialized, fast and practical foods (characterized as western diet) have increased, and it is composed of high fat and sugars (Popkin 2006). As similar, the cafeteria diet is characterized by a high caloric intake. Associated with the standard rodent feed, it consists of biscuits, salty snacks, salami, bread, gas-free refrigerant, among others (Sampey *et al.* 2011; Goularte *et al.* 2012).

Moreover, cafeteria diet components as preservatives and dyes may contribute to the development of diseases and exert a mutagenic and / or carcinogenic effect. Two epidemiological studies created the concept of "developmental programming", referring to the fact that stimuli can promote changes in fetus that could be reflected in a late development risk for diseases (Barker *et al.* 1989; Barker *et al.* 1990). Fetal response to environment stress is related to molecular and physiological changes that may remain during adulthood (Roseboom *et al.* 2002; Gonzalez-Barranco *et al*. 2003; Godfrey *et al.* 2011). In this context, maternal obesity during pregnancy is considered one of the stressors that can affect fetal development, and numerous animal studies showed this relationship with development of insulin resistance, obesity and metabolic syndrome in offspring (Bayol *et al.* 2007; Glavas *et al.* 2010).
The micronucleus test is a simple and easily applied method used to detect chromosomal changes caused by mutagenic and / or carcinogenic agents. It is possible to analyze the frequency of micronuclei in blood and epithelial cells, bone marrow and other tissues (Ribeiro 2006). The ratio between polychromatic and normochromatic erythrocytes is considered a parameter of cytotoxicity or cell depression (Ribeiro 2006). Thus, the present study evaluated the mutagenic effect that a cafeteria diet exerted on *Wistar* rats and its consequences for female offspring, considering mother background and diet.

METHODS

Groups and crossings

All procedures were approved by the Animal Use Ethics Committee of the State University of the West Paraná (UNIOESTE), Campus of Cascavel / PR under 0021/2013 – CE certificate number. *Wistar* rats delivered from State University of West Parana were maintained under day-night conditions (light from 07:00 a.m. to 7:00 p.m.), with free access to food and water. A total of twelve females (generation 1) aged 21 days were randomly separated in two groups: control group (CTL) were fed with rodents standard ration and water at will (standard diet); Cafeteria Diet group (CAF) were fed with cafeteria diet adapted from previous studies (Sampey *et al*. 2011; Goularte *et al.* 2012) and water at will. At 70 days of age, females were mated with non-obese control males (two females to one male) for five days. The pregnant females were housed individually. After birth and weaning at 21 days of age, female offspring (2nd generation) were separated into daughters of CTL mothers and subdivided according to diet: CTL - CTL (n = 4), which received a standard diet and CTL - CAF (n = 5), which received a cafeteria diet; and daughters of CAF mothers were divided into CAF - CTL (n = 6), which received a standard diet and CAF - CAF (n = 6) which received a cafeteria diet.

Euthanasia was performed by guillotine decapitation at 100º day of age, both of mothers and daughters, and 6µL of blood was used for making smears on clean glass slides (two per animal). After 24 hours, the slides were immersed in absolute methanol for 10 minutes followed for 24 hours of rest. Staining procedure were performed with 5% Giemsa diluted in phosphate buffer, pH 6.8. The slides were stored in refrigerator until cytological analyses. In blind test, the ratio of polychromatic erythrocytes (PCE) / normochromatic erythrocytes (NCE) in a total of 1000 cells were verified in a 100x light microscope. Micronuclei (MN) frequency was evaluated in 1000 PCE, as described by Hayashi *et al.* (1994).

Statistical Analysis

Data were analyzed using Statistica 7.0 Software (SoftStat). The frequencies of MN / 1000 PCE and PCE / NCE were compared between groups in generation 1 by ANOVA one way, followed by the Tukey test. To compare the data of the generation 2 groups: CTL - CTL, CTL - CAF, CAF - CTL and CAF - CAF, the multivariate ANOVA test was adopted, taking account, the maternal diet followed by the Tukey 's test. Body weight, retroperitoneal fat and perigonadal fat were compared between the generation 1 and 2 groups, separately by generation. For this purpose, ANOVA was used, followed by Dunnett's test. In generation 2, all groups were compared with CTL-CTL. For all tests, $p < 0.05$ was considered. The effect of the mother's diet on the fat and weight of the daughters were also compared by multivariate ANOVA, followed by Tukey´s test when significant.

RESULTS

Tables 1 and 2 shows, respectively, data of generation 1 (late gestation) and generation 2. Table 3 shows the frequencies of MN / 1000 PCEs and PCE / NCEs. Were observed statistically ($p<0.05$) increasing in body weight, retroperitoneal and perigonadal fat in CAF, indicating obesity (Generation 1 - Table 1).

Table 1. Demographic data of generation 1 (mean ± standard deviation)

Groups	Body weight (g)	Retroperitoneal Fat (g)	Perigonadal Fat (g)
CTL	265,4±5,8	1,4±0,1	3,1±0,1
CAF	320,3±9,2*	2,6±0,1*	5,3±0,3*

g: Grams; CTL: control group; CAF: cafeteria group.
*Statistically different of CTL ($p<0.05$)

Table 2 – Demographic data of generation 2 (mean ± standard deviation)

Groups	Body weight (g)	Retroperitoneal Fat (g)	Perigonadal Fat (g)
CTL-CTL	203±9,46	2,01±0,55	2,45±0,55
CTL-CAF	256,4±4,98*	4,82±0,32*	13,33±0,79*
CAF-CTL	217,5±4,37	2,03±0,22	5,48±0,36*
CAF-CAF	230±2,25*	4,13±0,26*	10,1±0,65*

g: Grams; CTL-CTL: Control group daughter with standard diet; CTL-CAF: Control group daughter with cafeteria diet; CAF-CTL: Cafeteria group with standard diet; CAF-CAF: Cafeteria group with cafeteria diet.
*Statistically different of CTL-CTL ($p<0.05$)

Moreover, statistically significant decrease in PCE/NCE frequencies and a higher in MN frequencies were observed in the CAF group (Table 3). In table 2 (generation 2) were observed that CTL-CAF and CAF-CAF showed higher body weight, retroperitoneal and perigonadal fat ($p<0.05$) compared to CTL-CTL group. Furthermore, all parameters of CTL-CAF are even higher than CAF-CAF, showing the effect of cafeteria diet, despite mother background. CAF-CTL showed higher perigonadal fat compared to CTL-CTL, but no differences in body weight or retroperitoneal fat, suggesting some protection caused by the standard diet, even with the CAF genetic background. No statistically significant difference in PCE / NCE and MN frequencies were observed in Generation 2 (Table 3). However, tendencies to increase were observed in MN frequencies of CTL-CAF and CAF-CAF groups, compared to CTL-CTL. Regarding the PCE / NCE ratio, it showed a tendency to decrease in the CAF-CTL and CAF-CAF groups, but with no statistically differences.

Table 3. Means and standard error of MN/1000 PCEs and PCE/NCE ratio on Generations 1 and 2

	Generation 1		Generation 2			
	CTL	CAF	CTL-CTL	CTL-CAF	CAF-CTL	CAF-CAF
MN	0,0005±0,0002	0,0023±0,0009*	0,0005±0,0003	0,0037±0,0024	0,0005±0,0003	0,0012±0,0002
PCE/NCE	0,0125±0,0027	0,0055±0,0014*	0,0109±0,0021	0,0120±0,0016	0,0085±0,0005	0,00849±0,0008

*Statistically significant compared to CTL-CTL ($p<0.05$).

These data demonstrated the impact of the cafeteria diet on results in both generations. Using the diet of the mothers as variable in analysis, were observed p=0.057 in the comparison of MN frequencies of CAF and CTL groups, and p=0.7 in the comparison of PCE/NCE frequencies of the same groups (Data not showed). No statistically differences were observed, but a relation with mothers diet and genetic background can be observed.

DISCUSSION

An association between maternal obesity and high energy consumption with offspring metabolic dysfunctions had been previously described (Khan *et al*. 2005; Oben *et al*. 2010). A high-fat diet (eg, cafeteria diet) is the most common method to induce obesity in rodents (Xiao *et al*. 2007; Vogt *et al*. 2014). The progression of polychromatic erythrocytes to normochromatic erythrocytes in the bone marrow is an important cytotoxicity indicator that can be evaluated in the MN test (Venkatesh *et al*. 2007), and is an indicator of acceleration or inhibition of erythropoiesis. However, the mechanisms that link the metabolic dysfunction of the offspring, and the changes due to the obesogenic diet (genetic background), are not yet well established. First, we observed that the cafeteria diet induced obesity, DNA damage and cytotoxicity in rats (Table 1), with high body weight, retroperitoneal and perigonadal fat, high MN frequencies and decreased PCE/NCE ration, in the cafeteria group. In addition, our work showed some cytogenetic consequences to the offspring, that continued or not the mother´s diet. Thus, in generation 2, were observed the consequences of the cafeteria diet in offspring and the ability to reverse changes in body weight and retroperitoneal fat only by changing diet, regardless of the mother genetic background.

Furthermore, even when we did not observe a statistical difference, were observed a tendency to decrease in the frequency of PCE/NCE, not related to diet (only to mother´s genetic background); and increase in the frequency of MN independent of the mothers genetic background (only by diet), when compared to CTL-CTL. Cafeteria diet showed to cause genetic instability in the experimental model, and capacity of damage perpetuation to the offspring. Leffa *et al*. (2014) observed high levels of DNA damage in obesity. It is proved that maternal obesity alone is an aggravating factor to the development of diseases at offspring later life. In addition, the cafeteria diet, which is rich in dyes, preservatives and other mutagenic substances (Hassanane 2014), showed to maximize this poor-prognosis. In fact, food preservatives such as benzoic acid salts (Zerzin *et al*. 2011) nitrite and nitrate and dyes such as amaranth, red allura, new coccine, tartrazine, erythrosine, floxin, rose bengal and caramel are considered cytotoxic and mutagenic (Sampey *et al*. 2011; Goularte *et al*. 2012). As a basic research, firstly were demonstrated that cafeteria diet caused obesity, DNA damage and cytotoxicity in generation 1; secondly, cafeteria diet caused high body weight, retroperitoneal and perigonadal fat, and possible genetic instability with increased DNA damage independently of maternal obesity background; and perpetuation of cytotoxicity in offspring due to maternal obesity, regardless of the cafeteria diet. Changing the diet of offspring, no statistically differences were observed in MN frequencies, PCE/NCE ratio, body weight and retroperitoneal fat, showing the importance of diet even with a disadvantageous mother background.

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