Hypercaloric Cafeteria Diet-Induces Obesity, Dyslipidemia, Insulin Resistance, Inflammation and Oxidative Stress in Wistar Rats

Palma-Jacinto José Antonio[1], Santiago-Roque Isela[2], Arroyo-Helguera Omar Elind[3*]

[1]Centro de Investigaciones Biomédicas, Universidad Veracruzana, Av. Luis Castelazo Ayala S/N, Col. Industrial Ánimas, Xalapa, Veracruz, México. CP. 91190
[2]Laboratory of Biochemistry and Neurotoxicology, Universidad Veracruzana. Odontólogos W/N, U.H del Bosque, Xalapa, Veracruz, México.
[3]Laboratorio de Biomedicina y Salud, Instituto de Salud Pública, Universidad Veracruzana. Av. Luís Castelazo Ayala S/N, Col. Industrial Animas, Xalapa, Veracruz CP. 91190, México

Abstract

Obesity is a public health problem associated with oxidative stress, chronic inflammation, and insulin resistance. The present study analyzed the cafeteria diet's effects on obesity, oxidative stress, chronic inflammation, and insulin resistance in Wistar rats. Thirty-five male rats were divided into four groups: 1, control (n=10); 2, cafeteria diet (n=9) during 26 weeks (age relative to human adolescence); 3, control 38 weeks (n=9); 4, cafeteria diet during 38 weeks (n=7) (age relative to human adults). The percentage of total adipose tissue (TAT), body mass index, Lee's index, and insulin sensitivity (QUICKI-HOMA) were calculated, as well as metabolic parameters such as lipid profile, glucose levels, glycosylated hemoglobin, antioxidant and oxidative status, TNF-α, and IL-6. Both rat groups with cafeteria diet increased their weight 45.13±15.73 g and TAT 7.75 ± 0.64 g significantly at 26-week, and 46.7±9.05 g weight and TAT 9.97 ± 0.46 at 38 weeks compared to the control group ($p<0.05$ and $p<0.01$, respectively). Total cholesterol, HDL, LDL, triglycerides, glucose, Insulin, TBARS, GSH, and catalase activity levels increased in both 26 and 38-week groups vs. control ($p<0.01$), and TNF-α only at 26 weeks ($p<0.01$). In conclusion, the cafeteria diet induces obesity in rats, accompanied by hyperglycemia, dyslipidemia, oxidative stress, inflammation, insulin resistance, and TAT. Induction of obesity with a cafeteria diet could be used to study the mechanisms involved in the genesis of overweight, obesity, and comorbidities to establish intervention strategies to prevent these pathologies during adolescence and adulthood.

Keywords: Inflammation, Oxidative Stress, Total Adipose Tissue, QUICKI-HOMA.

INTRODUCTION

Obesity is considered a risk factor for developing comorbidities such as type 2 diabetes mellitus (T2DM), metabolic syndrome, dyslipidemia, coronary heart disease, hepatic steatosis, and cancer [1-5]. Obesity has a multifactorial etiology involving environmental factors and unhealthy lifestyles. A sedentary lifestyle with hypercaloric foods rich in fats or carbohydrates is related to overweight and obesity [6-11]. Other causal factors contributing to obesity are hormonal changes [12,13] and hereditary genetic factors [14,15].

The obesity is a risk factor to metabolic changes involved with dyslipidemia, lipotoxicity [16], oxidative stress, inflammation, and insulin resistance [17,18]. To study obesity and its comorbidities, animal models were used to understand this pathophysiology and find new therapies. These models include gene alterations by depleting a specific gene or polygenic models [19]. These models show the association between genes and obesity development [20]. In contrast, the most frequent models for studying obesity involved feeding rodents with a hypercaloric diet high in fat [21-23]. The cafeteria diet uses highly appetizing foods such as cookies, fries potatoes, cakes, salami, and pate [24]. These foods are characterized by their high fat, carbohydrates, sodium content, and additional additives. It is similar to the human diet and more effective in producing obesity and metabolic alterations than high-fat or high-calorie meals diets only [25,26], such as dyslipidemia, increased adipose tissue, inflammation, oxidative stress, hyperglycemia and even insulin resistance [27-32]. These models have the disadvantage that the first diet needs to be standardized, and the calorie content varies depending on the type of food given to rodents. Furthermore, additives such as preservatives, colorants, vitamins, and minerals may alter the amount-consumed depending on the hedonic neural signals received [33-35]. Another disadvantage is the five weeks of general diet administration; this age of onset is six weeks old, correlating with adolescence in humans [27-32].

In this study, a cafeteria diet was used, which consisted of high-calorie, industrialized foods that were labeled junk food, such as cookies, cupcakes, fries potatoes, and pate. In México, these foods are

*Correspondence Address:
Omar Elind Arroyo Helguera
E-mail : oarroyo@uv.mx / elindirene@hotmail.com
Address : Instituto de Salud Pública, Universidad Veracruzana, Veracruz CP. 91190, México

the most popular among children aged 1-11 years old and adults [36]. Therefore, the aim of the present work was to study the cafeteria diet effects at 26 and 38 weeks on physiological and metabolic status in Wistar rats.

MATERIAL AND METHODS

Experimental Design and Animals

Thirty-five male rats Wistar strain, 28-day-old were used (Laboratorio de Bioquímica y Neurotoxicología, Facultad de Bioanálisis, Universidad Veracruzana, Xalapa, Veracruz, Mexico). The animals were housed in 58 x 36 x 20 cm acrylic boxes in an inverted cycle of 12 h light/dark, temperature 19.08 ± 0.33°C, and humidity 58.87 ± 0.57%. The rats were randomly divided into four groups: 1, the control group fed with Purina® rodent Chow kibbles 26 weeks; 2, cafeteria diet 26 weeks (W26); 3, the control group fed with Purina® rodent Chow kibbles 26 weeks 38 weeks; 4, cafeteria diet 38 weeks (W38) fed with a human-like designed cafeteria diet with highly palatable foods commonly consumed in Mexico (Table 1). All animals received filtered water and food ad libitum, which was weighed and measured daily. At the beginning of the experiment, with a 4-hour fast, a blood sample was obtained from the rats, and the serum levels of glucose, cholesterol, and triglycerides were quantified. Weight and height were measured weekly, and the criteria for obese rats were according to the growth curve [37], BMI, and Lee's index [38]. Two cuts were made: one at week 26 of the experiment with two groups 1, control (n = 10) and 2, group W26 (n = 9), and another cut at week 38; 1, control (n = 9) and 2, group W36 (n = 7), at the end of each experiment with eight h of fasting the rats were euthanized with and an overdose of pentobarbital 120 $mg.kg^{-1}$ intraperitoneal injection, in a volume of 1 $mL.100g^{-1}$ of body-weight, respectively.

Blood samples were obtained by cardiac puncture. The serum and plasma were stored at -80°C for biochemical determinations. Finally, the visceral, epididymal, and subcutaneous adipose tissue was extracted and weighed, and the BMI and Lee's index were obtained. The Internal Committee for the Care and Use of Laboratory Animals approved the experiment (CICUAL) from Unidad de Ciencias de la Salud, Universidad Veracruzana, Xalapa region, Veracruz, Mexico, with the number of approval CICUAL-UCS-004).

Metabolic Markers

The serum levels of glucose, total cholesterol, triglycerides (TG), HDL-cholesterol, and LDL-cholesterol were quantified by enzymatic assays with commercial kits (BioSystems SA, Barcelona, Spain), and the VLDL-cholesterol values were calculated (VLDL-cholesterol = TG/5). Commercial kit cartridges (Abbot, Green Oaks, Ill, USA) determined the percentage of glycosylated Hemoglobin (HbA1C). Serum insulin levels were quantified by ELISA using a commercial kit (RAB0904, Sigma Aldrich, St. Louis, MO, USA). Insulin sensitivity was determined using the Quantitative Insulin Sensitivity Check Index (QUICKI) (1 / log glucose ($mg.dL^{-1}$) + insulin ($\mu IU.mL^{-1}$) and the Homeostasis Model Assessment using the formula (HOMA-IR) (glucose ($mg.dL^{-1}$) x insulin ($\mu IU.mL^{-1}$) / 2430) according to previous studies [39,40].

Inflammation Markers

Tumor necrosis factor alpha (TNF-α) (RAB0479, Sigma Aldrich, St. Louis, MO) and interleukin 6 (IL-6) (RAB0312, Sigma Aldrich, St. Louis, MO) levels were quantified by ELISA following the manufacturer's instructions.

Markers of Antioxidant Status and Oxidative Stress

Antioxidant status was determined by catalase activity (CAT), superoxide dismutase, and glutathione (GSH) content that were quantified by colorimetric methods using a standard curve with known concentrations. Oxidative stress through the determination of plasma levels of malondialdehyde (MDA) following the manufacturer's instructions (STA-330, Cell Bio labs, INC, San Diego, CA, E.U.).

Table 1. Control diet and cafeteria diet (100 g of diet) composition

Composition	Purina Rodent Chow		Cafeteria diet	
	g	Kcal %	g %	Kcal %
Carbohydrates	48.70	194.80	51.62	206.48
Fat	5.00	45.00	16.39	147.51
Protein	23.90	95.60	12.76	51.04
Total $Kcal.g^{-1}$	**3.35**		**4.05**	

Notes: Cafeteria diet ingredients= pastries, cream snow cookies, chocolate cookies and shortbread orange cookies; fries´ potatoes, pork liver pate and Rodent Chow® croquettes (Purina, México). Each one administered on demand every 24 hours, except for the pâté, which was available 10 g daily.

Statistical analysis

Results are presented with mean ± standard error of the mean. The significant differences between groups were determined by a t-test or two-way ANOVA of repeated measures with Tukey's post hoc test. The data were analyzed with the SIGMAPLOT program (v. 12.0, Systat Software, Inc., San Jose, California, USA).

RESULTS AND DISCUSSION

The Cafeteria Diet Induces Obesity, Dyslipidemia and Hyperglycemia at Weeks 26 and 38

Table 2 reveals that rats fed with a cafeteria diet increased their weight by 45.13 ± 15.73 g at week 26 compared to rats with a control diet (P <0.05, Table 2). Rats fed with the cafeteria diet at week 26 had an increase in BMI> 0.7, Lee's index of > 0.3, and fat tissue deposits (Table 2). Meanwhile, the group fed with the cafeteria diet until 38 weeks increased weight by 46.71 ± 9.05 g (P <0.001), BMI 0.07 ± 0.0112 $g.cm^{-2}$ (P <0.001), the Lee index 0.01 ± 0.000935 $g.cm^{-1}$ (P <0.001) and, the accumulation of adipose tissue compared with a cafeteria control group 26 week. Table 3 shows that rats fed with a cafeteria diet for 26 weeks had a significant increase in glucose levels (P <0.001), HbA1C (P <0.001), and insulin (P <0.001).

Compared to control rats —observed insulin resistance (increase in HOMA-IR and decrease in QUICKI). At week 26 of the experiment, the lipid profile indicates an increase in total cholesterol (P <0.001), VLDL-cholesterol (P <0.001), triglycerides (P <0.001), and total lipids (P <0.001) compared to control rats (Table 3). This significance was maintained in the rats that continued until week 38 of the experiment, when the levels of glucose (P= 0.014), total cholesterol (P <0.001), and triglycerides (P <0.001) increased significantly when the group of cafeteria diet at week 26 was compared to the group of cafeteria diet during 38 weeks (Table 3). At week 38 of the experiment, the levels of VLDL-cholesterol (P <0.001) and total lipids (P <0.001) increased significantly in the group fed with cafeteria diet compared with control rats (Table 3).

Effect of Cafeteria Diet

Table 4 shows an increase in the TNF-α levels (P <0.001) with a tendency to increase IL-6 levels in the group fed with cafeteria diet at 26 and 38 weeks compared with the control diet group. In both groups fed with cafeteria diet was observed a significant increase of TBARS and the activity of the enzymes catalase (P <0.001) and glutathione (P <0.001, Table 4).

Table 2. Hypercaloric cafeteria diet effects on physical variables at weeks 26 and 38

Variable	Control	W26	Control	W38
Final weight (g)	425.08 ± 7.51	470.21 ± 15.73*	424.96 ± 14.63	502.00 ± 14.89*
BMI ($g.cm^{-2}$)	0.66 ± 0.01	0.73 ± 0.02*	0.70 ± 0.01	0.82 ± 0.03*
Lee's index ($g.cm^{-1}$)	0.30 ± 0.001	0.31 ± 0.003*	0.30 ± 0.003	0.32 ± 0.005*
Total TA (%)	4.06 ± 0.32	11.81 ± 0.96***	5.51 ± 0.41	15.48 ± 0.87***
Visceral TA (%)	2.24 ± 0.12	7.34 ± 0.70***	2.66 ± 0.22	8.06 ± 0.47***
Subcutaneous TA (%)	0.58 ± 0.19	2.47 ± 0.38***	1.43 ± 0.16	4.92 ± 0.55***
Epididymal TA (%)	1.23 ± 0.11	1.97 ± 0.26*	1.41 ± 0.08	2.49 ± 0.020*

Notes: W26=cafeteria diet 26 weeks; W38= cafeteria diet 38 weeks; TA=adipose tissue; Body mass index= BMI. Data are presented as media ± standard error. Level of significance *P <0.05, **P<0.01, ***p<0.001 vs control group. Control W26 n = 10; group W26 n = 9; Control group W38 n = 9 and Group W38 n = 7.

Table 3. Biochemical determinations after of cafeteria diet at week 26 and 38

Variable	Control W26	Group W26	Control W38	Group W38
Glucose ($mg.dL^{-1}$)	98.86 ± 11.17	319.43 ± 19.80***	125.14 ± 27.19	310.97 ± 44.03*
HbA1C (%)	1.28 ± 0.06	3.77 ± 0.16***	1.01 ± 0.22	2.40 ± 0.43
Insuline ($uIU.mL^{-1}$)	26.31 ± 6.27	58.04 ± 9.51***	29.71 ± 6.66	48.70 ± 20.12
HDL-cholesterol ($mg.dL^{-1}$)	48.03 ± 1.77	50.38 ± 2.36	53.47 ± 0.25	54.73 ± 0.95
LDL-cholesterol ($mg.dL^{-1}$)	54.77 ± 0.82	55.80 ± 2.06	58.85 ± 3.13	59.07 ± 7.22
VLDL-cholesterol ($mg.dL^{-1}$)	25.15 ± 1.24	38.70 ± 1.06***	28.30 ± 1.17	53.65 ± 4.59***
Triglyceride ($mg.mL^{-1}$)	125.70 ± 26.21	193.51 ± 5.34***	141.51 ± 25.89	268.26 ± 22.95**
Total Cholesterol ($mg.mL^{-1}$)	58.95 ± 1.50	127.64 ± 12.31***	65.63 ± 3.12	187.47 ± 18.12***
Total Lipids ($mg.dL^{-1}$)	43.60 ± 7.25	148.79 ± 8.80***	142.77 ± 8.83	343.20 ± 34.27***
HOMA-IR	0.42 ± 0.10	4.68 ± 0.73***	5.58 ± 1.26	11.85 ± 4.90
QUICKI	0.38 ± 0.02	0.25 ± 0.01***	0.25 ± 0.01	0.24 ± 0.01

Notes: W26=cafeteria diet 26 weeks; W38= cafeteria diet 38 weeks. Data are presented as media ± standard error. Level of significance *P <0.05, **P<0.01, ***p<0.001 vs control group. Control W26 n=10; group W26 n = 9; Control group W38 n = 9 and Group W38 n=7.

Table 4. Effect of cafeteria diet in oxidative stress, antioxidant status and inflammation parameters

Variable	Control W26	Group W26	Control W38	Group W38
TNF-α (pg.mL^{-1})	342.87 ± 98.28	4779.78 ± 996.32***	1032.02 ± 341.82	2271.17 ± 529.81
IL-6 (pg.mL^{-1})	21.78 ± 8.01	41.06 ± 9.34	21.12 ± 4.53	38.85 ± 17.42
TBARS (μM MDA)	6.62 ± 0.30	10.87 ± 0.36***	6.45 ± 0.42	17.37 ± 3.01***
GSH (nmoles.mL^{-1})	6.79 ± 0.45	13.67 ± 1.00***	20.92 ± 2.57	36.22 ± 2.83*
Catalase (% de cat.mg de prot^{-1})	18.48 ± 0.28	34.97 ± 0.84***	22.91 ± 3.23	52.18 ± 5.47***
SOD	ND	ND	79.72 ± 1.21	70.01 ± 2.94*

Notes: TBARS = Thiobarbituric Acid Reactive Species; GSH = Glutathione; SOD = Superoxide dismutase; IL-6 = Interleukin; TNF-α = Tumor necrosis factor alpha; ND= not determined. Level of significance *P <0.05, **P<0.01, ***p<0.001 vs control group. Control W26 n = 10; group W26 n = 9; Control group W38 n = 9 and Group W38 n = 7.

In our study, the cookies, cupcakes, fries' potatoes, and pate were featured on a cafeteria diet that contained high-calorie, processed meals that were labeled junk in our research. This form of eating is more widespread in the age groups between 1-11 years old in México [36], and it promotes the development of obesity in adulthood. In our study, cafeteria diet causes weight gain and the creation of excess adipose tissue, mainly abdominal adipose tissue, supporting what has previously been observed in young rats given a cafeteria diet [41,42].

The physical variables of BMI, Lee's index, and the growth curve are used to diagnose obesity in rodents [37,38]. In this study, rats fed with a cafeteria diet had a higher Lee index and BMI and a significant weight increase in accord with the intake of hypercaloric foods from a cafeteria-type diet as described in other studies [27,29-32,39,42].

Although various obesity models have aided the understanding of pathophysiological mechanisms and their comorbidities [43], they have limitations. For example, the model with mono and polygenic alterations to induce obesity by depleting the leptin gene or polygenic models that include the interaction of more genes with the environment and development of obesity [19]. These models only allow us to investigate a gene linked to obesity or to induce a mutation in a single gene to produce an obese phenotype [20]. Hypercaloric diets are used more frequently to induce obesity [21-23]. In addition, the BMI increase has been associated with leptin and adiponectin, both satiety and hunger hormones. Future studies are being conducted to examine the association between consuming a cafeteria diet with leptin and adiponectin hormones [44].

The increase in caloric intake resulted in increased serum glucose levels in the rat fed with cafeteria diet, in contrast with other studies that report lower levels [25,26,29,41]. It is possible that this increase could be a longer cafeteria diet administration time, up to 26 weeks. In addition, glucose levels increased when insulin resistance was established [16]; in this study, the cafeteria diet is capable of inducing alterations in the HOMA-IR and QUICKI compared with the control group after 26 weeks. These observations are reported in other models [25,26,33,41].

The inflammatory process mediated by cytokines such as TNF-α and IL-6, oxidative stress, and dyslipidemias is the mechanisms linked to insulin resistance [17,18]. In accordance with these, serum levels of total cholesterol, VLDL-cholesterol, LDL-cholesterol, and triglycerides are increased in rats fed with the cafeteria diet. In addition, an increase in TNF-α was found after 26 and 38 weeks with cafeteria diet, in accord with previous reports that show that high consumption of foods rich in lipids causes a more significant accumulation of adipose tissue and induces an inflammatory state [31].

In addition, pro-inflammatory cytokines such as TNF-α and IL-6 are used as biomarkers to determine an individual's inflammatory status. The increase in TNF-α has been reported in rodents with a diet rich in fat [33, 45]. However, the levels reported are lower compared to our results. These differences could be by the more prolonged cafeteria diet of 26 and 38 weeks that increases TNF- α, but the levels of IL-6 are not significantly different with the control group. Bortolin *et al.* [33] show similar results, and IL-6 seric level was not significantly different from the control group. It could indicate that the inflammatory state is mediated mainly by TNF-α and not by IL-6. An increase in the production of free radicals and the activity of antioxidant enzymes accompanies this homeostatic alteration. Our results show a significant increase in lipoperoxidation, catalase activity, and total glutathione levels in the group fed with cafeteria diet. However, similar studies do not report rodents' antioxidant status to demonstrate the effect of cafeteria diet [41,42].

CONCLUSION

Cafeteria diet induces obesity and insulin resistance with alterations in the inflammatory state, oxidative stress, antioxidant status, and lipid profile in both rat groups at 26 and 38 weeks. These parameters are similar to those found in obese humans during adolescence and adults. The induction of obesity models with cafeteria diet could be used to carry out studies to understand the mechanisms involved in the genesis of overweight, obesity, and their comorbidities and establish intervention strategies to combat these pathologies during adolescence and adult.

Acknowledgement

We are grateful for the valuable help of Sandra Anell Hernández Landa for her support in the dissection and collection of samples from rodents. We also thank Irene Xochihua Rosas for proofreading. Thanks to CONACyT for granting the 931891 doctoral scholarship.

REFERENCES

[1] Aronne, L.J. 2002. Classification of obesity and assessment of obesity-related health risks. *Obesity Research.* 10(2). 105-115.

[2] Atawia, R.T., K.L. Bunch, H.A. Toque, R.B. Caldwell, R.W. Caldwell. 2019. Mechanisms of obesity-induced metabolic and vascular dysfunctions. *Front. Biosci*. 24. 890-934.

[3] Caballero, B. 2019. Humans against obesity: Who will win? *Adv. Nutr*. 10(1). S4-S9.

[4] Engin, A. 2017. The definition and prevalence of obesity and metabolic syndrome. *Adv. Exp. Med. Biol*. 960. 1-17.

[5] Guh, D.P., W. Zhang, N. Bansback, Z. Amarsi, C.L. Birmingham, A.H. Anis. 2009. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health*. 88(9). 1-22.

[6] Tremblay, M.S., R.C. Colley, T.J. Saunders, G.N. Healy, N. Owen. 2010. Physiological and health implications of a sedentary lifestyle. *Appl. Physiol. Nutr. Metab.* 35(6). 725-740.

[7] Shrestha, N., Z. Pedisic, S. Neil-Sztramko, K.T. Kukkonen-Harjula, V. Hermans. 2016. The impact of obesity in the workplace: A review of contributing factors, consequences and potential solutions. *Curr. Obes. Rep*. 5(3). 344-360.

[8] Allom, V., B. Mullan, E. Smith, P. Hay, J. Raman. 2018. Breaking bad habits by improving executive function in individuals with obesity. *BMC Public Health*. 18(505). 1-8.

[9] Nicolaidis, S. 2019. Environment and obesity. *Metabolism*. 88(2009). 153942.

[10] Ishida, Y., D. Yoshida, T. Honda, Y. Hirakawa, M. Shibata, S. Sakata, *et al.* 2020. Influence of the accumulation of unhealthy eating habits on obesity in a general Japanese population: The Hisayama study. *Nutrients.* 12(10). 1-10.

[11] Healt, W.O. 2020. Physical inactivity: A global public health problem. Available at: https://www.who.int/dietphysicalactivity/factsheet_inactivity/en/.

[12] Martinez-Escude, A., G. Pera, I. Arteaga, C. Exposito, L. Rodriguez, P. Toran, *et al.* 2020. Relationship between hypothyroidism and non-alcoholic fatty liver disease in the Spanish population. *Med. Clin. (Barc)*. 154(1). 1-6.

[13] Nieman, L.K. 2015. Cushing's syndrome: update on signs, symptoms and biochemical screening. *Eur. J. Endocrinol*. 173(4). M33-38.

[14] Duicu, C., C.O. Marginean, S. Voidazan, F. Tripon, C. Banescu. 2016. FTO rs 9939609 SNP is associated with adiponectin and leptin levels and the risk of obesity in a cohort of Romanian children population. *Medicine (Baltimore)*. 95(20). e3709.

[15] Singh, R.K., P. Kumar, K. Mahalingam. 2017. Molecular genetics of human obesity: A comprehensive review. *C. R. Biol*. 340(2). 87-108.

[16] Barazzoni, R., G. Gortan-Cappellari, M. Ragni, E. Nisoli. 2018. Insulin resistance in obesity: an overview of fundamental alterations. *Eat Weight Disord*. 23(2). 149-157.

[17] Hotamisligil, G.S. 2006. Inflammation and metabolic disorders. *Nature*. 444(7121). 860-7.

[18] Kahn, S.E., R.L. Hull, K.M. Utzschneider 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 444(7121). 840-846.

[19] Barsh, G.S., I.S. Farooqi, S. O'Rahilly. 2000. Genetics of body-weight regulation. *Nature*. 404. 644-651.

[20] Speakman, J., C. Hambly, S. Mitchell, E. Krol. 2007. Contribución de los modelos animales al estudio de la obesidad. *Lab. Anim*. 42. 413-432.

[21] Buettner, R., J. Schölmerich, C. Bollheimer. 2007. High-fat diets: modeling the

metabolic disorders of human obesity in rodents. *Obesity*. 15(4). 798-808.

[22] Fernandes, M.R., N.V. Lima, K.S. Rezende, I.C. Santos, I.S. Silva, R.C. Guimaraes. 2016. Animal models of obesity in rodents. An integrative review. *Acta Cir. Bras*. 31(12). 840-844.

[23] Kimura, Y., A. Yamada, Y. Takabayashi, T. Tsubota, H. Kasuga. 2018. Development of a new diet-induced obesity (DIO) model using Wistar lean rats. *Exp. Anim*. 67(2). 155-161.

[24] Leigh, S.J., M.D. Kendig, M.J. Morris. 2019. Palatable western-style cafeteria diet as a reliable method for modeling diet-induced obesity in rodents. *J. Vis. Exp*. (153).

[25] Higa, T.S., A.V. Spinola, M.H. Fonseca-Alaniz, F. Sant'Anna Evangelista. 2014. Comparison between cafeteria and high-fat diets in the induction of metabolic dysfunction in mice. *Int. J. Physiol. Pathophysiol. Pharmacol*. 6(1). 47–54

[26] Sampey, B.P., A.M. Vanhoose, H.M. Winfield, A.J. Freemerman, M.J. Muehlbauer, P.T. Fueger, *et al.* 2011. Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. *Obesity (Silver Spring)*. 19(6). 1109-1117.

[27] Castell-Auvi, A., L. Cedo, V. Pallares, M. Blay, A. Ardevol, M. Pinent. 2012. The effects of a cafeteria diet on insulin production and clearance in rats. *Br. J. Nutr*. 108(7). 1155-1162.

[28] de Castro Ghizoni, C.V., F.R. Gasparin, A.S. Junior, F.O. Carreno, R.P. Constantin, A. Bracht, *et al.* 2013. Catabolism of amino acids in livers from cafeteria-fed rats. *Mol. Cell Biochem*. 373(1-2). 265-277.

[29] Gil-Cardoso, K., I. Gines, M. Pinent, A. Ardevol, X. Terra, M. Blay. 2017. A cafeteria diet triggers intestinal inflammation and oxidative stress in obese rats. *Br. J. Nutr*. 117(2). 218-229.

[30] Johnson, A.R., M.D. Wilkerson, B.P. Sampey, M.A. Troester, D.N. Hayes, L. Makowski. 2016. Cafeteria diet-induced obesity causes oxidative damage in white adipose. *Biochem. Biophys. Res. Commun.* 473(2). 545-550.

[31] Liu, J., L. Han, L. Zhu, Y. Yu. 2016. Free fatty acids, not triglycerides, are associated with non-alcoholic liver injury progression in high fat diet induced obese rats. *Lipids Health Dis*. 15(27). 1-9.

[32] Namekawa, J., Y. Takagi, K. Wakabayashi, Y. Nakamura, A. Watanabe, D. Nagakubo, *et al.* 2017. Effects of high-fat diet and fructose-rich diet on obesity, dyslipidemia and hyperglycemia in the WBN/Kob-Lepr(fa) rat, a new model of type 2 diabetes mellitus. *J. Vet. Med. Sci*. 79(6). 988-991.

[33] Bortolin, R.C., A.R. Vargas, J. Gasparotto, P.R. Chaves, C.E. Schnorr, K.B. Martinello, *et al.* 2018. A new animal diet based on human Western diet is a robust diet-induced obesity model: comparison to high-fat and cafeteria diets in term of metabolic and gut microbiota disruption. *Int. J. Obes. (Lond)*. 42(3). 525-534.

[34] Johnson, P.M., P.J. Kenny 2010. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat. Neurosci*. 13(5). 635-641.

[35] Steele, C.C., J.R.A. Pirkle, I.R. Davis, K. Kirkpatrick. 2019. Dietary effects on the determinants of food choice: Impulsive choice, discrimination, incentive motivation, preference, and liking in male rats. *Appetite*. 136. 160-172.

[36] González-Block, M.A., A. Figueroa-Lara, L. Ávila-Burgos, D.A. Balandrán-Duarte, B. Aracena-Genao, L. Cahuana-Hurtado, *et al.* 2017. Retos a la Encuesta Nacional de Salud y Nutrición 2017. *Salud Publica de Mexico*. 59(2). 126-127.

[37] Cossio-Bolaños, M., R. Gómez-Campos, R. Vargas-Vitoria, R.T. Hochmuller, M. de Arruda. 2013. Curvas de referencia para valorar el crecimiento físico de ratas machos Wistar. *Nutrición hospitalaria*. 28(6). 2151-2156.

[38] Novelli, E., Y. Diniz, C. Galhardi, G. Ebaid, H. Rodrigues, F. Mani, *et al.* 2007. Anthropometrical parameters and markers of obesity in rats. *Lab. Anim*. 41. 111-119.

[39] Cacho, J., J. Sevillano, J. de Castro, E. Herrera, M.P. Ramos 2008. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am. J. Physiol. Endocrinol. Metab*. 295(5). E1269-1276.

[40] Katz, A., S. Nambi, K. Mather, A. Baron, D.D. Follman, G. Sullivan, *et al.* 2000. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J. Clin. Endocrinol. Metab*. 86(7). 2402-2410.

[41] Buyukdere, Y., A. Gulec, A. Akyol. 2019. Cafeteria diet increased adiposity in

comparison to high fat diet in young male rats. *PeerJ*. 7(e6656). 1-15.

[42] Maeda Junior, A.S., J. Constantin, K.S. Utsunomiya, E.H. Gilglioni, F.R.S. Gasparin, F.O. Carreno, *et al.* 2018. Cafeteria diet feeding in young rats leads to hepatic steatosis and increased gluconeogenesis under fatty acids and glucagon influence. *Nutrients*. 10(11). 1-25.

[43] Kleinert, M., C. Clemmensen, S.M. Hofmann, M.C. Moore, S. Renner, S.C. Woods, *et al.* 2018. Animal models of obesity and diabetes mellitus. *Nat. Rev. Endocrinol.* 14(3). 140-162.

[44] Cruz-Mejia, S., H.H. Duran-Lopez, M. Navarro-Meza, I. Xochihua-Rosas, S. de la Pena, O.E. Arroyo-Helguera. 2018. Body mass index is associated with interleukin-1, adiponectin, oxidative stress and ioduria levels in healthy adults. *Nutr. Hosp*. 35(4). 841-846.

[45] Xu, Z.J., J.G. Fan, X.D. Ding, L. Qiao, G.L. Wang 2010. Characterization of high-fat, diet-induced, non-alcoholic steatohepatitis with fibrosis in rats. *Dig. Dis Sci*. 55(4). 931-940.