The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

Echography analysis altered in musculoskeletal, heart and liver associated with endothelial dysfunction of obese rat

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	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	Title, page 1 **Echography analysis altered in musculoskeletal, heart and liver associated with endothelial dysfunction of obese rat**
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	Abstract, Page 2 Background: Modern imaging plays a central role in the care of obese patients, with an integral focus on its use and accessibility in individuals into this condition with alterations of various organs. Objective. To perform an echographical analysis of musculoskeletal system disorders, endothelial dysfunction and the left ventricle in obese rats. Methods. Sprague Dawley rats (250±5 g) were used and divided in two groups: control group (C) fed with a standard diet, and the obese group (Ob) fed with a hyper caloric diet of high fructose-fat for 4 months. Body weight, cholesterol, triglycerides, glucose, inflammatory cytokines and adhesion molecules (ICAM-1, VCAM-1) were measured. Additionally, two-dimensional echocardiography, abdominal ultrasound and musculoskeletal system studies were performed in the lower extremities. Results. Body weight in the Ob group was increased compared to the control group, ($p < 0.001$); in addition, increased glucose, cholesterol and triglycerides were found in the Ob group vs the C group, ($p < 0.05$), and as well as increased adhesion molecules ICAM-1 and, VCAM-1 ($p < 0.01$). On ultrasound, 75% of the Ob group presented, showed 75% fatty liver and distal joint abnormalities. Conclusion. Endothelial dysfunction and changes at the level of the musculoskeletal system with the presence of joint cysts in the posterior region of the distal joint of the lower extremities and fat liver were observed in obese rodents.
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	Paragraphs 3, page 3 and Paragraphs 1 page 4 In obese rat have been demonstrated excess lipid accumulates in other tissues including the liver, the skeletal muscle and the heart, also, these are associated with an increase in adipose mass and free fatty acids. Likewise, in liver together with other intrahepatic signals leading to derangement in glucostatic and lipidostatic functions, are generating to greater vulnerability to hepatic steatosis [8, 9]. While echography is usually used in the clinical setting for the diagnosis and follow-up of patients with nonalcoholic fatty liver disease (NAFLD); also, this analysis is a good method which allows to examine arteries, cardiopathies and fatty tissue [10]. However, in obese murine models no study has assessed sonographic findings of several organs in obesity, this could be identified to integration and relationship of dysfunctions of mechanism different in other organs or comorbidities in same organism.
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	Paragraphs 3, page 4 Therefore, we report here in this study the echographic analysis of the left ventricle, liver, musculoskeletal disorders and endothelial dysfunction in obesity.

METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	Paragraphs 1, page 6 Ethical statement All experiments were performed in accordance with relevant guidelines and regulations of bioterium of the Specialty Hospital of National Center Medical, of the Mexican Social Security Institute (CMN SXXI-IMSS), in accordance with the Official Mexican Standard (NOM-062-ZOO-1999, revised 2001) for the care and use of laboratory animals. Also, we obtained written informed consent to use the animals in our study. This study was approved by the Ethical Committee and the Local Research and Health Committee of the Mexican Social Security Institute, with number registered 3601-2015-95. The animals were treated according to the Official Mexican Standard for the care, use and sacrificing laboratory animals. (NOM-062-ZOO-1999, revised 2001). In this work the obese group had a hypercaloric diet during 4 months, and both groups; C and Ob were studied at 6 months of age. However, their feeding continued of both groups until 12 months of age for others of tissues and organs analysis.
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	Methods: Paragraphs 1-3, page 5 Animals and Model of obesity This was an experimental, cross-sectional and analytical study. The population was a murine model of rats of the Sprague Dawley strain obtained and used from an inbred colony of bioterium of the Specialty Hospital of the National Medical Center, Mexican Social Security Institute (Mexico City, Mexico), a total of 20 males weighing 200-250 g, were used. Animals were randomly allocated and were divided in two groups: the control group C (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA) and the Ob group (n=8) was fed with a high fat-fructose diet (standard diet supplemented with 10% lard and 30% fructose mixed and dissolved in drinking water) by 16 weeks ad libitum, both groups had this diet until six months of age. Food and water intake, as well as, the animals weight, were recorded daily during these weeks. We have worked with this experimental model in other projects [5]. This animal model employed in this work with the ingestion of a high fat-fructose diet is clearly associated with insulin resistance development, disturbed glucose homeostasis and endothelial dysfunction in rodents [4-6]. The hypercaloric diet was used to induce obesity in (Ob group) experimental group. All cages contained wood shavings, bedding and a cardboard tube for environmental enrichment. All rats of each group had ad libitum access to their pellet diet and water; they were housed in groups of 4 rats, in conventional cages at room temperature (22-25°C), under a light cycle of 12 hours' light/12 hours' dark since at the start of the trial and hygienically controlled room.
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	Methods: Paragraphs 2, page 5 and page 6-7 Animals were randomly allocated and were divided in two groups: the control group C (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA) and the Ob group (n=8) was fed with a high fat-fructose diet (standard diet supplemented with 10% lard and 30% fructose mixed and dissolved in drinking water) by 16 weeks ad libitum, both groups had this diet until six months of age. both groups had this diet until six months of age. All experiments were performed in accordance with relevant guidelines and regulations of bioterium of the Specialty Hospital of National Center Medical, of the Mexican Social Security Institute (CMN SXXI-IMSS). We have worked with this experimental model in other projects [5]. The hypercaloric diet was used to induce obesity and insulin resistance in (Ob group) experimental rats.

Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	Methods: Paragraphs 1, page 5 This was an experimental, cross-sectional and analytical study. The population was a murine model of rats of the Sprague Dawley strain obtained and used from an inbred colony of bioterium of the Specialty Hospital of the National Medical Center, Mexican Social Security Institute (Mexico City, Mexico), a total of 20 males weighing 200-250 g, were used. N/A
Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	Methods: Paragraphs 1-3, page 5 Animals and Model of obesity This was an experimental, cross-sectional and analytical study. The population was a murine model of rats of the Sprague Dawley strain obtained and used from an inbred colony of bioterium of the Specialty Hospital of the National Medical Center, Mexican Social Security Institute (Mexico City, Mexico), a total of 20 males weighing 200-250 g, were used. Animals were randomly allocated and were divided in two groups: the control group C (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA) and the Ob group (n=8) was fed with a high fat-fructose diet (standard diet supplemented with 10% lard and 30% fructose mixed and dissolved in drinking water) by 16 weeks ad libitum, both groups had this diet until six months of age. Food and water intake, as well as, the animals weight, were recorded daily during these weeks. We have worked with this experimental model in other projects [5]. This animal model employed in this work with the ingestion of a high fat-fructose diet is clearly associated with insulin resistance development, disturbed glucose homeostasis and endothelial dysfunction in rodents [4-6]. The hypercaloric diet was used to induce obesity in (Ob group) experimental group. All cages contained wood shavings, bedding and a cardboard tube for environmental enrichment. All rats of each group had ad libitum access to their pellet diet and water; they were housed in groups of 4 rats, in conventional cages at room temperature (22-25°C), under a light cycle of 12 hours' light/12 hours' dark since at the start of the trial and hygienically controlled room.
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	Methods: Paragraphs 1-2, page 5 a total of 20 males weighing 200-250 g, were used. Animals were randomly allocated and were divided in two groups: the control group C (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA) and the Ob group (n=8) was fed with a high fat-fructose diet (standard diet supplemented with 10% lard and 30% fructose mixed and dissolved in drinking water) by 16 weeks ad libitum, both groups had this diet until six months of age.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	Methods: Paragraphs 1-2, page 5 a total of 20 males weighing 200-250 g, were used. Animals were randomly allocated and were divided in two groups: the control group C (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA) and the Ob group (n=8) was fed with a high fat-fructose diet (standard diet supplemented with 10% lard and 30% fructose mixed and dissolved in drinking water) by 16 weeks ad libitum, both groups had this diet until six months of age.

Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Abstract: Paragraphs 3, page 2 Body weight in the Ob group was increased compared to the control group, (p <0.001); in addition, increased glucose, cholesterol and triglycerides were found in the Ob group vs the C group, (p<0.05), and as well as increased adhesion molecules ICAM-1 and, VCAM-1 (p<0.01). On ultrasound, 75% of the Ob group presented, showed 75% fatty liver and distal joint abnormalities.
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	**Statistical Analysis** Paragraphs 3, page 7 The study groups were statistically analyzed the difference in means between group C *vs* Ob with a Student t test, the level of statistical significance was considered with a value of p <0.05. The data are presented as means ± standard deviation (SD) of each group
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	**Statistical Analysis** Paragraphs 3, page 7 The data are presented as means ± standard deviation (SD) of each group. The study groups were statistically analyzed the difference in means between group C vs Ob with a Student t test, the level of statistical significance was considered with a value of p <0.05. For each study variable: glucose, insulin, lipid profile, HOMA-IR index, proinflammatory cytokines, TNF-α, IL-6, adhesion molecules ICAM-1 and VCAM-1 statistical analysis was carried out using Graph Pad Prism. (GraphPad Prism 8 for Windows, San Diego, CA); p <0.05 was considered significant.
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.	Methods: Paragraphs 1-2, page 5 a total of 20 males weighing 200-250 g, were used. Animals were randomly allocated and were divided in two groups: the control group C (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA) and the Ob group (n=8) was fed with a high fat-fructose diet (standard diet supplemented with 10% lard and 30% fructose mixed and dissolved in drinking water) by 16 weeks ad libitum, both groups had this diet until six months of age. N/A
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	Table 1, Table 2, and Table 3.

	C (n=12)	Ob (n=8)	% ± SD
Body weight (g)	385±20	472±25**	22.5
Fasting Glucose (mg/dL)	69±2	165±45*	139.1
HOMA-IR	4±1	16±3***	300
Triglycerides (mg/dL)	109±7	225±19***	106.4
Cholesterol (mg/dL)	70±5	98±3***	40
HDL (mg/dL)	50±5	32±3***	-64
LDL (mg/dL)	25±2	58±4***	132
VLDL (mg/dL)	33±2	48±4***	45.4

Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	N/A
DISCUSSION			
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	Discussion: Paragraphs 2, page 10 In this study, we showed relation among obesity, endothelial dysfunction and alterations in several organs as musculoskeletal system, kidney, liver and heart through a noninvasive method as is echography. Thus, our result showed that high fat-fructose diet increased intake food, with marked weight gain due the caloric contribution provided by the fat and carbohydrate in obese group. We also found metabolic alterations, such as rise in glucose, triglycerides, cholesterol and HOMA-IR index, similar as other studies where were used hypercaloric diets [14,15]. Hence, with the obesity is triggered dysmetabolism, in our results observed hyperglycemia and hypertriglyceridemia, also, an increase of the levels of inflammatory cytokines (TNF-a, IL-6). Likewise, as a consequence of obesity can be manifested alterations in other organs that could be analyzed in ultrasound. In this work was relevant the results in muscle-skeletal and articular levels to show the presence of cysts at the articular level, in the posterior region of the distal joint, where it was possible to view the damage due accumulated of fat. Other alterations were presented in heart, kidney, musculoskeletal and liver analyzed by echography.
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	Conclusions: Paragraphs 1, page 13 This study we observed a relationship between endothelial dysfunction and the changes observed at the level of the musculoskeletal system, liver and heart with the presence of joint cysts in the posterior region of the distal joint of the lower extremities in obese rodents. Thus, we suggest that ultrasound is an excellent diagnostic tool that is accessible and easy to use in the chronic diseases that are increasing in our population, and it allows the integration of alterations in different organs, joints and tissues, besides, better monitoring and support to reducing complications and improving the quality of life of patients.
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	Funding: Paragraphs 7, page 13 This study was supported by Mexican Social Security Institute (IMSS) (Grant No. FIS/IMSS/PROT/G14/1666). The funder had no role on the design of study and collection, analysis, and interpretation of data and in writing the manuscript in this study

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.