The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

Echography analysis of musculoskeletal, heart and liver alterations associated with endothelial dysfunction in obese rats

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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 of musculoskeletal, heart and liver alterations associated with endothelial dysfunction in obese rats**
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, and there is an integral focus on its use and accessibility in individuals who have alterations of various in various organs. The objective in this study was to perform an echographic analysis of musculoskeletal system disorders, endothelial dysfunction and the left ventricle (LV) in obese rats. Methods: Sprague Dawley rats (250±5g) were obtained and divided into two groups: the control (C) group was fed with a standard diet, and the obese (Ob) group was fed hyper caloric diet with a high fructose-fat content 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: The body weight in the Ob group was increased compared to that in the control group, ($p<0.001$); in addition, increased glucose, cholesterol and triglyceride concentrations ($p<0.05$) as well as increased levels of the adhesion molecules ICAM-1 and, VCAM-1 ($p<0.01$) were found in the Ob group vs the C group. On ultrasound, 75% of the Ob group presented fatty liver and distal joint abnormalities. Conclusion: Obese rats exhibit endothelial dysfunction and musculoskeletal changes, also, fatty liver and articular cysts in the posterior region of the distal lower- extremity joints.
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 rats, excess lipid accumulates in other tissues, including the liver, skeletal muscle and heart, and this condition is associated with an increase in adipose mass and free fatty acids. Likewise, in the liver, this accumulation along with other intrahepatic signals leads to a derangement in glucostatic and lipidostatic functions, and generate a greater vulnerability to hepatic steatosis [8, 9]. Echography is commonly used in the clinical setting for the diagnosis and follow-up of patients with nonalcoholic fatty liver disease (NAFLD); in addition, this analysis is a good method that allows the examination of arteries, cardiopathies and fatty tissue [10]. However, no study has used murine models to assess the sonographic findings of several organs in obesity, which could be identified as the integration and relationship of dysfunctions in various mechanisms in other organs or comorbidities with in the 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 herein report the echographic analysis of the left ventricle, hepatic, 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 the relevant guidelines and regulations of the 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. Additionally, 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 (registration number 3601-2015-95). The animals were treated according to the Official Mexican Standard for the care, use and sacrifice of laboratory animals. (NOM-062-ZOO-1999, revised 2001). In this work, the obese group had a hypercaloric diet for 4 months, and both groups, C and Ob, were studied at 6 months of age. However, to facilitate other tissue and organ analyses, the feeding continued in both groups until the animals reached 12 months of age.
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 murine model that was used consisted of a population of Sprague Dawley rats strain that were obtained from an inbred colony in the 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 divided into two groups: the control (C) group (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA), and the obese (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) during 16 weeks ad libitum; both groups received this diet until reaching six months of age. Food and water intake, as well as, animal weight, were recorded daily during this period. We have worked with this experimental model in other projects [5]. In this murine model the ingestion of a high fat-fructose diet is clearly associated with the development of insulin resistance, disturbed glucose homeostasis and endothelial dysfunction [4-6]. The hypercaloric diet was used to induce obesity in the Ob 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 drinking water and were housed in a hygienically controlled room 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.
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 divided into two groups: the control (C) group (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA), and the obese (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) during 16 weeks ad libitum; both groups received this diet until reaching six months of age. both groups received this diet until reaching six months of age. All experiments were performed in accordance with the relevant guidelines and regulations of the 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. We have worked with this experimental model in other projects [5]. In this murine model the ingestion of a high fat-fructose diet is clearly associated with the development of insulin resistance, disturbed glucose homeostasis and endothelial dysfunction [4-6].

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 murine model that was used consisted of a population of Sprague Dawley rats strain that were obtained from an inbred colony in the 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 murine model that was used consisted of a population of Sprague Dawley rats strain that were obtained from an inbred colony in the 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 divided into two groups: the control (C) group (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA), and the obese (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) during 16 weeks ad libitum; both groups received this diet until reaching six months of age. Food and water intake, as well as, animal weight, were recorded daily during this period. We have worked with this experimental model in other projects [5]. In this murine model the ingestion of a high fat-fructose diet is clearly associated with the development of insulin resistance, disturbed glucose homeostasis and endothelial dysfunction [4-6]. The hypercaloric diet was used to induce obesity in the Ob 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 drinking water and were housed in a hygienically controlled room 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.
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 divided into two groups: the control (C) group (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA), and the obese (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) during 16 weeks ad libitum; both groups received this diet until reaching 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 divided into two groups: the control (C) group (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA), and the obese (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) during 16 weeks ad libitum; both groups received this diet until reaching 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 The body weight in the Ob group was increased compared to that in the control group, ($p<0.001$); in addition, increased glucose, cholesterol and triglyceride concentrations ($p<0.05$) as well as

			increased levels of the adhesion molecules ICAM-1 and, VCAM-1 (p<0.01) were found in the Ob group vs the C group. On ultrasound, 75% of the Ob group presented 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 2, page 8 The data are presented as the means ± standard deviation (SD) of each group. The study groups were statistically analyzed for the difference in means between group C and group Ob with Student´s t test, and a value of p<0.05 was considered to indicate statistical significance. For each study variable (glucose, insulin, lipid profile, HOMA-IR index, proinflammatory cytokines, TNF-α, IL-6, adhesion molecules ICAM-1 and VCAM-1), a statistical analysis was carried out using GraphPad 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 divided into two groups: the control (C) group (n=12) was fed with the standard diet (Formulab 5008 Diet; PMI Nutrition International, Brentwood, MO, USA), and the obese (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) during 16 weeks ad libitum; both groups received this diet until reaching 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. **Table 1.** Metabolic parameters of the control (C) and hypercaloric diet (Obese, Ob) rats C n=12 / Ob n=8 / % difference between Ob and C 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±5 / 58±4*** / 132 VLDL (mg/dL): 33±2 / 48±4*** / 45.5 Determination of body weight (g), fasting glucose, cholesterol and triglyceride levels in control (C) and obese (Ob) rats. Values are represented as the mean ± SD of 8-12 animals per group. Values of *p<0.05, **p<0.001 and ***p<0.0001 indicate a significant difference from the C group. The % difference is based on a control value of 100%.
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 show the relationships among obesity, endothelial dysfunction and alterations in several organs, such as the musculoskeletal system, kidney, liver and heart, through noninvasive methods such as echography. Thus, our results showed that a high fat-fructose diet increased food intake, with marked weight gain due to the caloric contribution provided by fat and carbohydrates in obese group. We also found metabolic alterations, such as increases in glucose, triglycerides, cholesterol and the HOMA-IR index, similar to other studies that have used hypercaloric diets [14,15]. Hence, our results showed that with obesity triggered dysmetabolism, hyperglycemia and hypertriglyceridemia, as well as an increase of the levels of inflammatory cytokines (TNF-a, IL-6), are present. Likewise, as a consequence of obesity, alterations in other organs can manifest and these changes can be analyzed by ultrasound. In this work the results at the muscle-skeletal and articular levels showed the presence of articular cysts, in the posterior region of the distal joints, where it was possible to view the damage due to fat accumulation. Other alterations were present in the heart, kidney, musculoskeletal system 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 In 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 articular cysts in the posterior region of the distal lower-extremity joint in obese rodents. Thus, we suggest that ultrasound is an excellent diagnostic tool that is accessible and easy to use to access the chronic diseases that are increasing in our population. Moreover, ultrasound allows an integral assessment of the alterations in different organs, joints and tissues, as well as, better monitoring and support to reduce complications and improve 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 the Mexican Social Security Institute (IMSS) (Grant No. FIS/IMSS/PROT/G14/1666). The funder had no role in the study design; the collection, analysis, and interpretation of the data; or in the writing of the manuscript.

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.