Research Square

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

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Research article

Keywords: Obesity, Endothelial dysfunction, ICAM-1, Echographic analysis.

Posted Date: June 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17948/v2

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Version of Record: A version of this preprint was published on August 14th, 2020. See the published version at https://doi.org/10.1186/s12902-020-00603-7.

Abstract

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.

Background

Overweight and obesity are defined as an abnormal or excessive accumulation of fat that is harmful to health and it is an important risk factor for non-communicable diseases, such as cardiovascular diseases (mainly heart disease and strokes), associated to metabolic disorders (glucose intolerance, insulin resistance, hyperlipidemia, diabetes and hypertension), musculoskeletal disorders (especially osteoarthritis) and some cancers (endometrial, breast, ovarian, prostate, liver, gallbladder, kidney and colon) [1].

Some mechanisms that are triggered by obesity involve the inflammatory process, with the production of proinflammatory cytokines such as interleukine-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α). Thus, transendothelial migration of inflammatory cells (leucocytes) and production of cytokines are an early step in endothelial dysfunction, which continues with the activation of adhesion molecules (ICAM-1, VCAM-1); this contributed, from early endothelial dysfunction to the atherosclerotic plaques, causing vascular complications [2-6]. To understand these pathophysiological mechanisms, experimental models are used, as murine models (rats) which are a valuable tool to understand these mechanisms linked to the comorbidities of the metabolic disease [7].

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.

Thus, ultrasound is an economical, accessible, fast, precise, simple, comfortable, and noninvasive procedure. It does not cause pain, radiation is not used, it has high sensitivity and accuracy to obtain images and it is essential in the study of a variety of organs such as the liver and the musculoskeletal system. Two-dimensional echocardiography is the study of the heart in two dimensions; it allows us to analyze the organ as a whole and the relationships that the cardiac structures maintain with each other. Two-dimensional echocardiography is very useful in the study of congenital anomalies, in the differentiation between thrombi and intracardiac masses and in the analysis of regions of difficult access with the one-dimensional echocardiogram [11].

Although there are ultrasound studies in rats where the various morphological and functional aspects are both analyzed at the cardiac level, mainly in the left ventricle, none of them has been considered important for a joint analysis of both, the left ventricle and the hepatic, renal and musculoskeletal systems represented in humans [12]. Therefore, we report here in this study the echographic analysis of the left ventricle, liver, musculoskeletal disorders and endothelial dysfunction in obesity.

Methods

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.

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.

Metabolic parameters and Body weight

Body weight. The weight of both groups: group C (n=12) and Ob (n=8) was recorded from the begging to the end of the study. Food and water intake were recorded per week.

Blood samples were collected at 8 am (during a 7 hours fasting at 6 month of age) from the tail vein in order to measure the biochemical blood parameters and inflammatory cytokines. The blood samples were transferred into tubes containing anticoagulant for measurement of biochemical blood parameters and inflammatory cytokines, respectively. The samples were centrifuged at 5200 g for 15 minutes. Plasma was separated and stored at -70 °C until use for bioassay analyses.

Blood glucose level was determined by One drop of blood was placed on the blood glucose test strips of the FreeStyle Optium Xceed glucometer (Abbot Diabetes Care Ltd, OYL, UK). In each animal was twice recorded.

Determinations of levels of total cholesterol, triglycerides (TG), high density lipoproteins (HDL) and low-density lipoproteins (LDL) were made on a Cardiocheck apparatus after placing one drop of blood (see previous section on blood samples) on a reactive strip, according to the manufacturer's instructions.

Quantification of insulin and index HOMA-IR, insulin was measured by chemioluminiscence, using Insulin IMMULITE kit (LKIN1 insulin IMMULITE);

Insulin resistance was calculated the index HOMA-IR through the formula of homeostasis model assessment-insulin resistance (HOMA-IR) index: insulin (μU/mL) × glucose (mg/dL)/405.

TNF-α, IL-6, adhesion molecules ICAM-1 and VCAM-1 were analyzed using enzyme immunoassay kits by ELISA R&D kit (R&D Systems, Inc., Minneapolis, MN, USA).

Ultrasound analysis

To carry out the study, the rat was placed in the left lateral decubitus position, with a slight inclination of the head and placing the transducer in the different acoustic windows. The equipment used was a Philips Affiniti 70 with a linear transducer of 9 mHz, where the results were obtained in millimeters. During the echocardiographic examination, two cuts were used for the evaluation of the heart: the left longitudinal parasternal section and the right longitudinal parasternal section. In the left longitudinal sternal section, it is possible to visualize the right ventricle, aorta with aortic valve, left atrium, mitral valve and left ventricle [11].

The right longitudinal sternal section that appears on the left and the base (atria) on the right, with the rotation of the transducer in the right direction, shows the left ventricular outlet, aortic valve, aortic root and proximal ascending aorta [11,12].

The measurement of the left ventricle in diastole was performed from the left septal endocardium to the posterior wall endocardium, measuring below the level of the mitral valve. The interventricular septum is between the mitral ring in its posterior part and the endocardial surface of the high septum in its anterior part. In the free wall of the VI, the diastolic thickness is measured; its value is approximately equal to the diameter of the IVT, and its birth is parallel to it.

Statistical Analysis

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.

Results

Figure 1 shows the differences in food consumption in the study groups, in Obese group with hypercaloric diet had a rise compared with control ($p<0.001$). Also, an increase in body weight was observed in the Ob group *vs* the C group (22.5%; 472 ± 25 *vs* 385 ± 20, $p<0.001$). In addition, the Ob group showed an increase in fasting glucose ($p<0.05$), lipid profile and HOMA-IR index (300%; 4±1 *vs* 16±3, $p<0.0001$); therefore, also because of lipids analyzed, these rats were considered obese rats with metabolic alterations (Table 1).

Table 1. Metabolic parameters of control (C) and hypercaloric diet (Ob) rats

	C n=12	Ob n=8	% difference of Ob vs 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 triglycerides levels in control (C) and obese (Ob) rats. Values represent the mean ± SD of 8-12 animals per group. Values *p<0.05, **p<0.001, ***p<0.0001 significantly different from control group. Value of % difference was considered value of control as 100%.

This experimental model demonstrated endothelial dysfunction, manifested by inflammatory cytokines TNF-α, also adhesion molecules that are indicators of endothelial damage, such as ICAM-1 (87.6%, 122± 9 *vs* 65±5, p <0.0001) and VCAM-1 (29.4% 110±10 *vs* 85±2, p <0.05) (Table 2). ICAM-1 and VCAM-1 are relevant in chronic inflammatory process, with highlights to an increased risk for type 2 diabetes (T2DM) and cardiovascular diseases (CVD) [6,7,13]

Table 2. Effects on cytokines and CAMs in groups of study.

	Control n=12	Obese n=8	% difference of Ob vs C
TNF-α (pg/mL)	5±0.5	16±2**	220
IL-6 (pg/mL)	9±1	23±5**	155.5
IL-10 (pg/mL)	3±1	4±2	33.3
ICAM-1 (ng/mL)	65±5	122±9***	87.6
VCAM-1 (ng/mL)	85±2	110±10*	29.4

Results are presented as values of the mean ± SD, n=8-12 animals per group. Values * p<0.05, ** p<0.001, *** p<0.0001 significantly different from control and obese group. Value of % difference was considered value of control as 100%.

In the Ob group, an increase in these adhesion molecules and in proinflammatory cytokines such as TNF-α was demonstrated, with a significant difference compared with the C group (220%, 16±2 *vs* 5±0.05, p<0.001), and similar was for IL-6 (155%, 23±5 vs 9±1, p<0.001). According to the measurements of the metabolic parameters and cytokines, the Ob group, with the consumption of the hypercaloric diet, presented endothelial damage. In addition, modern imaging allowed us to evaluate an integral approach of the control group, where the aorta and left ventricle (VI) were observed. For VI, an increase in the septum and wall was found in the Ob group (Table 3, Figure 2).

However, in Figure 3A, the analysis of the two-dimensional ultrasound being more complete, in the obese group was observed fat filtration, with the presence of fatty liver (Table 3); on the other hand, a homogeneous hepatic parenchyma was found being isoechoic in the control group (Figure 3B).

Another analysis of the ultrasound dimensions, that was obtained in the Ob group showed alterations in pelvic dilatation in the kidney compared to the control group where the kidney was echogenic, with no evidence of dilation (Figure 3C, D).

In the ultrasound images at the musculoskeletal level, we can observe alterations in the Ob group, where these changes were found due to fat deposits, demonstrating the presence of cysts at the articular level, in the posterior region of the distal joint, where it is possible to view the damage due to accumulated fat (Table 3, Figure 3E).

Table 3. Descriptions of two-dimensional echocardiography of kidney and skeletal muscle (lower extremities) and left ventricles (LV), partition IV y wall of LV express in mm

C			Ob		
Left ventricle	10 mm	9 mm	6 mm	8 mm	13 mm
Partition IV	2 mm	2 mm	1 mm	1 mm	1 mm
Wall LV	2 mm	2 mm	2 mm	2 mm	1 mm
Liver	normal	normal	non-alcoholic fatty liver	non-alcoholic fatty liver	non-alcoholic fatty liver
Skeletal muscle system	normal	normal	without changes	change in fat	cystic lesions
Other		normal			Pielica dilation at the bilateral renal level

Discussion

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.

In this study we used a noninvasive and fast method, in the evaluation of the ventricular anatomy, although the cut-off point for normal relative parietal thickness in humans is 0.42 mm, this criterion was highly specific in our animals because the rats presented a relative parietal thickness below that limit [11, 12]. Also, in this work was found the presence of Baker's cyst (QB), or popliteal cyst, which was first described in 1840 by Adams [16] and later by Baker [17]. In 1877, Baker published his experience on this entity, which gave him a rise name going to designate this type of cyst. Baker’s cyst is defined as an abnormal cluster of synovial fluid in the gastrocnemius-semimembranosus bursa or, failing that, a herniation of the posterior joint capsule with synovial fluid tension [17-19]. Changes in both static and dynamic alignment of the lower extremities could alter balance and gait, and trigger pain throughout the lower limbs [20]. Besides, ultrasound changes in the liver were found as fatty liver in 75% of the rats (6 exemplary) in obese rats. It is known that one of the main organs affected by obesity is the liver, where long-term the increase in lipogenesis and the decrease in mitochondrial β-oxidation of nonesterified fatty acids, as well as hepatic triglyceride secretion, can contribute to the accumulation of fat in the liver, leading to the appearance of liver steatosis. As well as, in a prospective longitudinal study, 86% of patients with NAFLD and progressive fibrosis were obese [13, 15, 21].

At the renal level, changes such as pelvic dilation were found in 50% of the obese group. On the other hand, changes were observed 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 of the rodents. Thus, with an excessive body weight creates a greater load stress, which in the musculoskeletal system affects muscle-skeletal and articular levels, causing joint misalignment (deformities) in the lower extremities and inflammatory and degenerative processes. Which could decrease physical functioning due associations with mobility and pain [22].

In several studies, it is known that the mechanical stress caused by overload or repetitive use can trigger tendon pathology. There are also extrinsic factors (posture and activity) and intrinsic factors (genetics

and metabolic characteristics) that can interfere with their development [23]. This condition and metabolic factors (hyperglycemia, dyslipidemia and endothelial dysfunction) affect quality life of subjects.

Regarding to the inflammatory process and endothelial dysfunction, we also found an increase in proinflammatory cytokines, such as TNF-α and IL-6, these molecules augment monocytes adhesion to endothelial cell. It is known that in both, humans and mice, an imbalance between TNF-α, and adiponectin (adipokine of adipose tissue) participate in the progression to steatohepatitis [24-26]. So, the adipose tissue releases inflammatory mediators, proinflammatory molecules as TNF-α and IL-6, and some other mediators such leptin, adiponectin, and resistin. IL-6, TNF-α and leptin act on immune cells and cause local and systemic inflammation. In several studies, in endothelial damage is demonstrated augment molecules such as monocyte attractant chemoprotein 1 (MCP-1), ICAM-1, VCAM-1 and plasminogen activator inhibitor (PAI-1), that contributing in the vascular complications [6,7,27,28].

Thus, our results of adhesion molecules showed an increase in the Ob group, which confirms endothelial damage, which has an impact on the joints. In addition, ICAM-1 and VCAM-1 are known being activating molecules of endothelial dysfunction because they play a crucial role in the adhesion of cells to the endothelial surfaces and in the integrity of the vascular wall, generating an accumulation of cells and sparking oxidative stress, that can be modulated by body composition and eating pattern [4,6,29,30].

Conclusion

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.

Abbreviations

C: control group; ELISA: Enzyme immunoassay; HDL: High Density Lipoprotein; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance Index; ICAM-1: Intercellular Adhesion Molecule-1; IL-6: Interleukine-6; IMSS: Mexican Social Security Institute; LDL: Low Density Lipoprotein; MCP-1: Monocyte Attractant Chemoprotein 1; NAFLD: Non-Alcoholic Fatty Liver Disease; Ob: hypercaloric diet group; PAI-1: Plasminogen Activator Inhibitor; QB: Baker's cyst; SD: standard deviation; TG: Triglycerides; TNF-a: Tumor Necrosis Factor-a; VCAM-1: Vascular Adhesion Molecule-1; VI: left ventricle; VLDL: Very Low Density Lipoprotein.

Declarations

Acknowledgements

We acknowledge the support Mexican Social Security Institute (IMSS) (FIS/IMSS/PROT/G14/1666) and SNI/CONACYT. NAEC is scholarship for CONACYT whit number 401729 and of CIS-IMSS whit number 99096806. We thank to MVZ César Hernández Chicharo, Bioterium of UMAE Cardiology, Hospital CMN SXXI, IMSS.

Authors' contributions

All the authors engaged in the surveys. AMC and LMA designed this article. NAEC, MIPO and DMC acquired and collected the data. AMC, DMC and LMA organized and analyzed all the information. AMC, NAEC and LMA drafted the manuscript. All the authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this article.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by the Ethical Committee and the Local Research in 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. In this work the obese group had a hypercaloric diet by 4-months, and both groups; C and Ob were studied at 6 months of age. However, after, of this work all rats followed their feeding continued until 12 months of age. In this moment, rats were sacrificed with deeply anesthetized and was administered pentobarbital (25 mg/kg, i.p.) [14] (NOM-062-ZOO-1999, revised 2001). For collection of blood samples was by cardiac puncture, with the samples was followed analysis of other cytokines and others of tissues analysis.

Funding

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.

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Figures

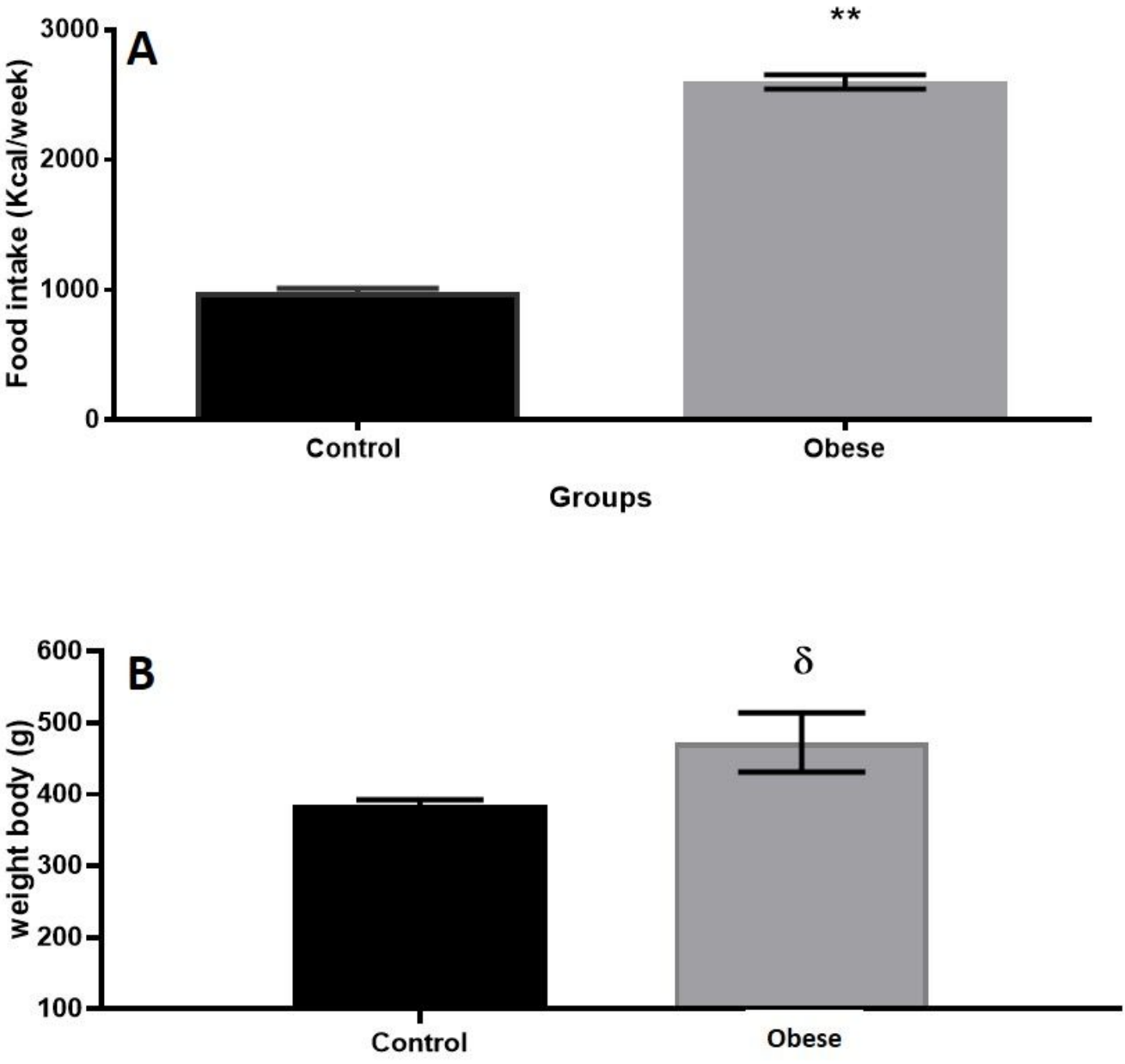

Figure 1

A) Effect on Food intake of groups of study. B) Represent weight body in different groups. Results are presented as values of the mean ± SD, n=8-12 animals per group. Values *p<0.05, **p<0.001, t Student test, significantly different from control group

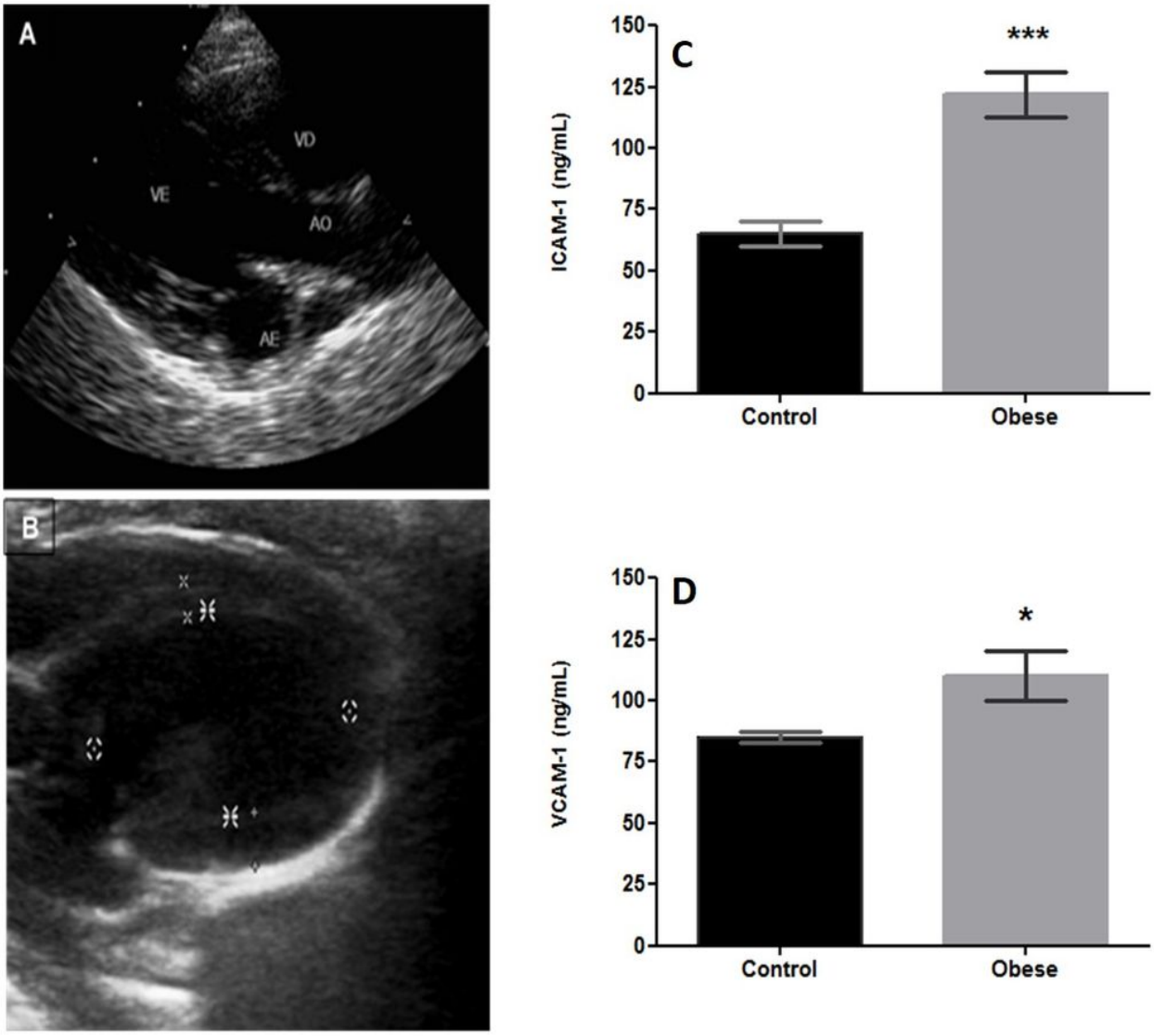

Figure 2

Two-dimensional echocardiography. A: The left longitudinal parasternal section, where it is possible to evaluate the aorta (AO), the left atrium (AE), and the right (RV) and left (Vi) ventricles in the control group. B: The approach for the left ventricle. The markers of endothelial dysfunction as CAMs in C; ICAM-1 and D, VCAM-1 are presented results as values of the mean ± SD.

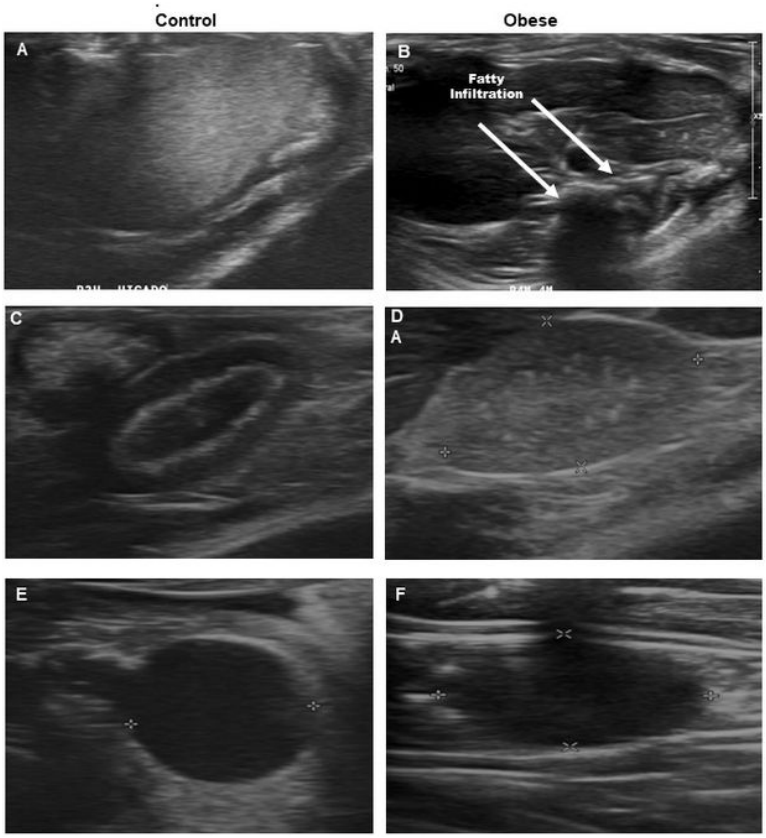

Figure 3

Two-dimensional echocardiography. A: The right hepatic lobe with fatty infiltration and an increase in echogenicity in a homogenous manner in the male Ob group. B: The hepatic parenchyma is homogeneous and isoechoic in the control group. C. The right kidney in a longitudinal section where we can visualize the pelvic dilation presented by the Ob group. D: The right kidney of another specimen can be seen where the renal sinus is echogenic, with no evidence of dilation in the Control group. E. in both images, there is a level approach to the distal joints of the hind legs of the rodents where cystic lesions were found. Two-dimensional echocardiography of: In both images, the approach is at the level of the distal joints of the hind legs of the rodents where cystic lesions were found that predominated in the Ob female group. F, Damage from these lesions can be observed.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ResultsratsBMCEndocrineDisorders2020.xlsx
- NC3RsARRIVEGuidelinesChecklistjune.pdf