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Morphometric and Anatomical Evaluation of the Heart of Wistar Rats

Evaluación Morfométrica y Anatómica del Corazón de Ratas Wistar

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SUMMARY: The use of animals in scientific research is not recent. The rat has been used in experiments since before Christ and, in the XIX century, with the intensification of the research reports, the use of this species increased considerably. Although this fact happens, the literature regarding the heart of these animals is not very detailed and descriptive. The objective of this research is to study the macroscopic morphometric of several parameters that have not been described yet regarding the hearth of Wistar rats in different phases of their lives. Thirty-six adult male and female rats (*Rattus norvegicus*) weighing 150-770 g were randomly divided into 6 experimental groups: 6 animals of the group I male-weighting 150-249g (I-M); 6 animals of the group I female-weighting 150-249g (I-F); 6 animals of the group II male-weighting 250-350g (II-M); 6 animals of the group II-female–weighting (II-F); 6 animals of the group III-male weighting more than 35 g (III-M); 6 animals of the group III-female weighting more than 351g (III-F). The heart was then carefully dissected and removed from the chest. After this process, the heart had their weight measured on a precision scale HELMAC HM100, and for each front section of the heart were taken related measures through a semi-automatic image analysis of Kontron Build Analyse (Minimop). The percentage ratio between the relative heart weight to body weight was between 0.5 and 0.7% in the animals with body weight up to 650g, moreover, the wall thickness of the non-septal left ventricle was always lower for the females in all groups, while the interatrial septum have similar action in males and females and the thickness of the interventricular septum demonstrated to be similar to the wall thickness of the non-septal left ventricle.

KEYWORDS: Heart; Ventricle; Atrium; Rats.

INTRODUCCIÓN

Rats, among other animals, have been used in scientific experiments since before Christ. Since the nineteenth century with the intensification of research, there has been an increase in the number of animals used. In 1909, the first albino strain was obtained from successive pairings for the production of a blood line to recessive genes that have this hair color. The albino rat has many phenotypic changes compared to wild brown rats *(Rattus norvegicus)* probably originating from trade caravans of the northern regions of Europe (Norway) or another possibility is that it would be from the north of the Himalayas coming to Europe in the eighteenth century (Harkness & Wagner, 1993).

Two major genetic variants of albino rats have been developed: the Wistar and Sprague-Dawley rats. Both have been developed from colonies in the United States of America. Wistar animals have wider head and smaller tail when compared to other strains since there are currently over 200 of them defined (Tirapegui *et al*., 1995).

Approximately four million rats are used annually for research only in the U.S. The works in which they are most used are on aging, cancer, effects and toxicity of drugs, dental cavities, lipid metabolism, effects of vitamins, behavior, alcoholism, cirrhosis, phenylketonuria, jaundice, intolerance, hypertension, embryology, teratology, infectious diseases and diabetes insipidus (Harkness, 1993).

The "Atlas of Microscopic Anatomy of the Rat" has a chapter on the Circulatory System in which the heart is described histologically in three layers: endocardium, consisting of flat polygonal endothelial cells, the myocardium which consists of cardiac muscle fibers and a little loose connective tissue, the pericardium that has two leaflets. The first layer is formed by a visceral serous membrane covering the heart muscle in its outer surface and the second layer, unlike the previous parietal that has little fat in its outer surface (Junqueira, 1947).

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The book "Anatomy and Embryology of the Laboratory Rat" features a chapter on the Circulatory System in which it describes the starting rat heart by the pericardium and its limits, discusses the variation of weight on the dynamics of entry and exit of the arteries and veins in relation to the cardiac chambers and on some peculiarities in relation to macroscopic and microscopic differences between these chambers. Moreover, the conduction system of nerve impulses, in the heart of these animals, and also the behavior of the cardiac coronary arteries are described. The book does not make any reference to the external morphological and morphometric parameters and neither to the morphometric parameters of the cardiac chambers (Hebel, 1986).

Although there is a relative literature on the heart of rats, practically few is described about the macroscopic morphometry of the heart in these animals. Therefore, this study aimed to study the macroscopic morphometry of several parameters, not described yet, of the hearth of male and female Wistar rats in different phases of their lives, these findings may be useful and provide anatomical support for the research using animal model experiments that require more detailed knowledge in cardiac anatomy.

MATERIAL AND METHOD

The experiments were carried out according to the Ethical Principles for Experimental Animals (COBAO) and the study was approved by the Animal Experimentation Committee (CETEA) of the Faculty of Medicine of Ribeirão Preto, University of São Paulo (Protocol nº 0347/2005). Thirty-six adult male and female rats (*Rattus norvegicus*) weighting 150-770g were used. The animals were randomly divided into 6 experimental groups: 6 animals of the group I male-weighting 150-249 g (I-M); 6 animals of the group I female-weighting 150-249g (I-F); 6 animals of the group II male-weighting 250-350g (II-M); 6 animals of the group II-female–weighting (II-F); 6 animals of the group III-male weighting more than 351g (III-M); 6 animals of the group III-female weighting more than 351g (III-F).

Experimental Procedures. The heart was then carefully dissected and removed from the chest by the section of the great vessels distally as possible of their mouth or emergency cardiac chambers for subsequent detailed dissection. After 48 hours, the hearts were kept immersed in formaldehyde, preceded a thorough dissection of the vessels. For standardization of parts, vena cava was immediately cut before connecting to the right atrium, the aorta was severed immediately before the emergence of the innominate artery and the pulmonary trunk, immediately before the bifurcation into right and left pulmonary arteries. After this process, the hearts had their weight measured using a HELMAC HM100 precision scale, and two measurements were also performed with whole hearts: the measurements of the circumference of the coronary (atrioventricular), and measurement of the distance of the coronary, and reference to its intersection with the main pulmonary artery, to the apex of the heart. To do this, we used a nylon ribbon supported along the surface of the heart. Once rectified, the tape was measured with a caliper MAUb analog.

Morphometric Analysis. For each front section of the heart, we made related measurements through a semi-automatic image analysis of Kontron Build Analyse (Minimop) coupled to a Carl-Zeiss binocular microscope equipped with camera and a compatible IBM-PC.

The measurement of atrial and ventricular wall thickness was performed in three distinct regions non-septum of each chamber to obtain the average thickness. The measurement of the thickness of the interatrial and interventricular septum was performed according to the same protocol.

Statistical Analysis. The data concerning the mean of the morphometric and anatomical analysis in the various groups were statistically analyzed by ANOVA using the PROC GLM SAS, version 9 for Windows. The level of significance was set at $p < 0.05$ for two-tailed tests. For determining the linear correlation coefficient, it was suggested the Pearson correlation coefficient, denoted by r. (Pagano & Gauvreau, 2004).

RESULTS

Intragroup comparisons of the weight of the hearts showed significant difference between males and females in groups II and III. ($p<0.05$). In comparison among the groups of females were observed statistically significant differences between groups I and III, and between group II and group III. As for males, it was observed statistical difference between groups I and III and between group II and group III (Table I).

The variable non-septal wall thickness of the left ventricle in the intragroup comparisons showed statistically significant results for groups I and II. In intergroup comparisons, females showed statistically significant results for comparisons between groups I and III and between groups II and III. In males only the comparison was statistically different between groups I versus III (Table II).

Table I. Comparison Intragroup and intergroup comparision, in grams, weight of the hearts.

Comparisons	Average (a)	Average (a)	Estimate (b)	p-value
Groups				
Group I: F X M	1,50	1,77	0,28	0,08
Group II: F X M	1,66	2,04	0,38	0,02*
Group III: F X M	2,21	2,86	0,66	<0,01*
Sex F				
Group I *vs* Group II	1,50	1,66	0,16	0,30
Group I *vs* Group III	1,50	2,21	0,71	<0,01*
Group II *vs* Group III	1,66	2,21	0,55	<0,01*
Sex M				
Group I *vs* Group II	1,77	2,04	0,26	0,09
Group I *vs* Group III	1,77	2,86	1,09	<0,01*
Group II *vs* Group III	2,04	2,86	0,83	<0,01*

Table II. Results in millimeters, thickness obtained for the non-septal wall of the left ventricle.

Comparisons	Average (a)	Average (a)	Estimate (b)	p-value
Groups				
Group I: F X M	2,08	2,66	0,58	0,02*
Group II: F X M	2,22	2,91	0,69	<0,01*
Group III: F X M	2,78	2,97	0,19	0,06
Sex F				
Group I vs Group II	2,08	2,22	0,14	0,75
Group I vs Group III	2,08	2,78	0,70	<0,01*
Group II vs Group III	2,22	2,78	0,56	0,02*
Sex M				
Group I vs Group II	2,66	2,91	0,25	0,07
Group I vs Group III	2,66	2,97	0,31	0,02*
Group II vs Group III	2,91	2,97	0,07	0,87

Regarding intergroup relations in the thickness of the interatrial septum (TSIA), there were no statistically significant results. In relations among groups, females had statistically significant results in comparisons between groups I and III. In the relations between groups of males, there were no statistically significant results (Fig. 1).

The variable wall thickness of non-septal left ventricular (TVL) in intragroup comparisons showed statistically significant results for groups I and II. In intergroup comparisons, females showed statistically significant results for comparisons between groups I and III and between groups II and III. In males only the comparison was statistically different between group I x III (Fig. 2). The variable thickness of the interventricular septum (TSIV) in their relations within groups was not statistically significant. In intergroup relations, the females did not show statistically significant results and males showed significant data both in the comparisons between groups I and II and in the comparisons between groups I and III (Fig. 3).

DISCUSSION

The data obtained in relation to the weights of dissected hearts of males and females were not statistically different for the animals in group I. These values have become different only in groups II and III, from the moment when the animals reached approximately 250 g of body weight.

The weight of the hearts of males and females when compared among groups showed a similar behavior, group I was compared statistically with group III, where there was a large difference in body weight of these animals, and between groups II and III due to the variability in the weight range of group III, as described above.

Krames (1966) described the methodology for measurement of hearts. They were dissected to remove the pericardium, fat and great vessels were cut on the surface of the heart. The hearts were then dried, and their weight was determined. For males, the ratio of heart weight to body weight averaged 0.23 and females were 0.29.

Medeiros *et al*. (2000) in a similar work established the weight of the whole heart of rats, corrected for body weight, a value of 2.98 ± 0.15 mg/g of body weight, with $p < 0.05$ in 16 animals with body weight of 180 to 220 grams. Although the authors did not described in details the methodology used for obtaining these hearts. These same authors in a paper in 2004 described the ratio of heart weight/body weight in 12 male Wistar rats, weighing 200-220 g and 2.82 ± 0.16 mg/g, where the hearts were removed and the dissection of the thoracic cavity was described only as the acquisition of hearts after the removal of non-cardiac tissues.

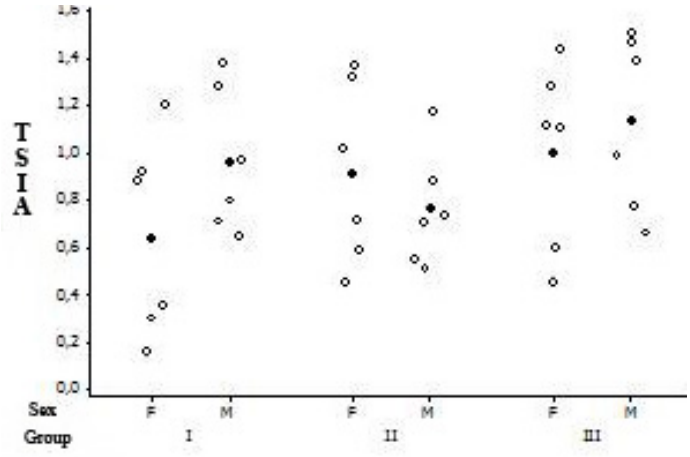

Fig. 1. Dispersion of the variable of thickness of the interatrial septum (TSIA) in relation to different groups and genders. The filled points represent the mean values.

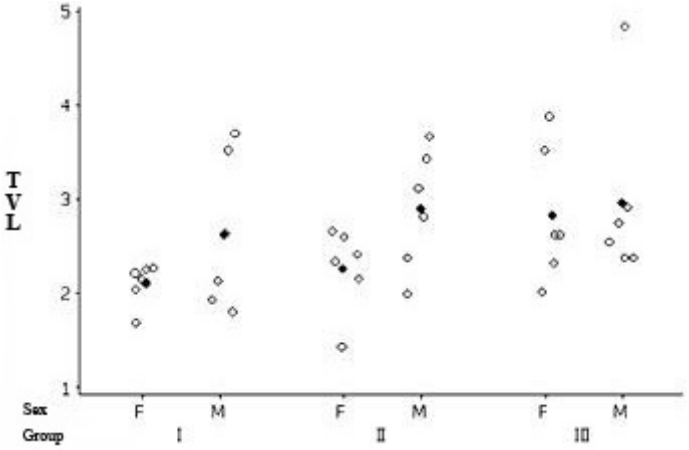

Fig. 2. Dispersion of the variable wall thickness non-septal left ventricular (TVL). The filled points represent the mean values.

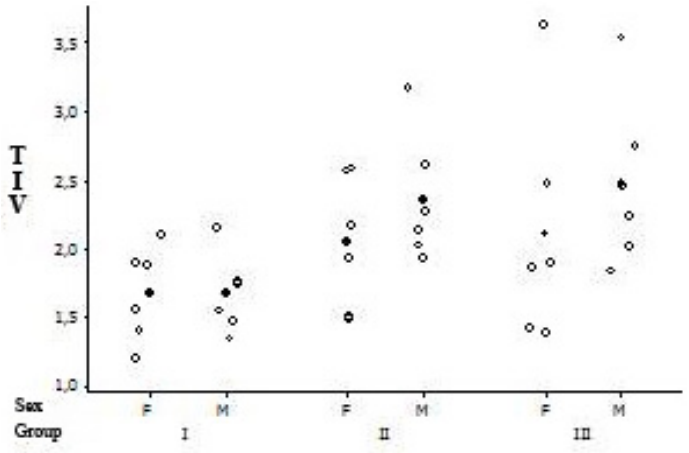

Fig. 3. Dispersion of the variable of thickness of the interventricular septum (TSIV) in relation to different groups and genders. The filled points represent the mean values.

According to Caster *et al*. (1956), the values obtained for these parameters of the left ventricle are directly related to the ejection of the ventricle (stroke volume), because higher values ??indicate greater ejection and this fits the metabolic needs, forthe musculoskeletal system of animals, representing nearly half (45.5%) of their body weight. The results of the groups, regarding the wall thickness of the left ventricle were different for groups I and II and the absolute value of the averages of these parameters was always lower for the females in all groups, thus characterizing left ventricular thinner walls in relation to male animals, which must be understood as an expected result, since the females had lighter hearts and theoretically have less skeletal muscle mass. The measurement of these septa is justified by the fact that each part of both structures of each ventricle, the lobby of each respectively. (Medeiros *et al*., 2000, Medeiros *et al.*, 2004). Our results are in accordance with the data observed for the non-septal wall thickness of the left ventricle and do not match the thickness of the non-septal wall of the right ventricle, an indication that the myocardium of the septum should contribute more effectively to the debt of left ventricular stroke.

In summary, it was observed by morphological and anatomical analysis. The percentage ratio between the relative heart weight to body weight is between 0.5 and 0.7% in the animals with body weight up to 650g, moreover, the wall thickness of the non-septal left ventricle was always lower for the females in all groups, while the interatrial septum has a similar action in males and females and the thickness of the interventricular septum demonstrated to be similar to the wall thickness of the non-septal left ventricle.

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RESUMEN: El uso de animales en la investigación científica no es reciente. La rata se ha utilizado en experimentos desde antes de Cristo y, en el siglo XIX, con la intensificación de los trabajos de investigación, el uso de esta especie ha aumentado considerablemente. Aunque se produce lo anterior, literatura sobre el corazón de estos animales no es muy detallada y descriptiva. El objetivo de esta investigación fue estudiar la morfometría macroscópica de varios parámetros no descritos en relación con el corazón de ratas Wistar en diferentes etapas de la vida. Treinta y seis ratas adultas machos y hembras (*Rattus norvegicus*) con peso entre 150-770 g se dividieron al azar en 6 grupos experimentales: 6 animales del grupo I machos de ponderación 150-249g (IM), 6 animales del grupo I hembra de ponderación 150-

249g (SI); 6 animales del grupo II macho de ponderación de 250-350g (II-M); 6 animales de la II-hembra-ponderación grupo (II-F); 6 animales de la ponderación III-grupo macho más de 35 g (III-M); 6 animales del grupo de ponderación III-hembra más de 351G (III-C). Posteriormente, el corazón se diseccionó cuidadosamente y se retiró del tórax. Después de este proceso, el corazón fue pesado en una balanza de precisión Helmac HM100. Para cada sección frontal del corazón se tomaron medidas relacionadas a través de un análisis de imágenes semi-automática de Kontron Build Analizar (Minimop). La relación porcentual entre el peso del corazón respecto al peso corporal fue entre 0,5 y 0,7% en los animales con peso corporal de hasta 650 g, por otra parte, el espesor de la pared del ventrículo izquierdo no fue siempre menor para las hembras de todos los grupos, mientras el tabique interatrial tienen acción similar en machos y hembras y el espesor del septo interventricular demostró ser similar a la del espesor de la pared del ventrículo izquierdo no septal.

PALABRAS CLAVE: Corazón; Ventrículo; Atrio; Ratas.

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