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Use of murinometrics indices and bioelectrical impedance (BIA) in the determination of experimental obesity in oophorectomized rats

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ABSTRACT. In this study, we tested the use of murinometric indices and bioimpedance (BIA) to determine obesity in rats. Female Wistar rats (8 weeks/130-160 g) were divided into control and oophorectomy group. The Body Mass Index (BMI) and Lee index (LI) were used as anthropometric techniques to determine obesity, and the determination of body composition by BIA, as a way to partition body weight into fat mass and lean mass components. The dissection of muscle tissues and adipose deposits was used as a direct determination of body fat content. The groups had body weight gain ($p < 0.05$) after the trial period, with a differential gain in body fat ($p < 0.05$) observed by the dissection of tissue in the oophorectomy group. This gain in body fat was detected more accurately by BIA, due to the greater ability of this method to distinguish lean from fat mass. BIA was able to measure the differential gain of body fat in a BMI considered as eutrophic by murinometric indices.

Keywords: body composition, bioimpedance, anthropometry, menopause.

Uso de índices murinométricos e impedância bioelétrica (BIA) na determinação de obesidade experimental em ratas ooforectomizadas

RESUMO. Neste estudo, foi testado o uso de índices murinométricos e da bioimpedância (BIA) na determinação da obesidade em ratos. Ratas Wistar (8 semanas/130-160g) foram divididas em dois grupos: controle e ooforectomia. O Índice de Massa Corporal (IMC) e o índice de Lee (IL) foram utilizados como técnicas antropométricas para a determinação da obesidade e da composição corporal por BIA, como um meio de fracionamento do peso corporal em sua massa de gordura e componentes de massa magra. A dissecação dos tecidos musculares e depósitos adiposos foi utilizada como uma forma direta de determinação do teor de gordura corporal. Os grupos tiveram ganho de peso corporal ($p < 0,05$) após o período experimental, com o grupo ooforectomia com ganho diferencial na gordura corporal ($p < 0,05$), observada na dissecação do tecido adiposo. Esse ganho de gordura corporal foi percebido com maior precisão pela BIA devido à maior capacidade de diferenciação da massa corporal magra e da massa de gordura no peso corporal por meio do método. A BIA foi capaz de perceber o ganho diferencial da fração de gordura corporal em um IMC proposto como eutrófico pelos índices murinométricos.

Palavras-chave: composição corporal, bioimpedância, antropometria, menopausa.

Introduction

Obesity is a disease and a risk factor for various cardiovascular, metabolic, orthopedic and psychosocial disorders (Brandalize & Leite, 2010; Grundy, 2004; Vaidya, 2006), with high and still increasing prevalence and incidence in the world (Kelly, Yang, Chen, Reynolds, & He, 2008). One of the criteria generally used for identification and monitoring is to determine the increase in body weight by body mass index (BMI) (Collaboration, 2009). However, obesity is in fact characterized by the differential increase in body fat, which is not only an energy reserve, but an endocrine organ (Ahima & Flier, 2000; Wellen & Hotamisligil, 2003). In this way, it has aroused discussion, about not only its absolute amount but also its relative participation in body composition and topographic distribution (Goodpaster, Thaete, & Kelley, 2000).

It is known that, in menopause, the female body undergoes metabolic changes that can activate subsequent changes in health condition. The change

in gynecoid to android fat distribution (Raskin, Pinto-Neto, Paiva, Raskin, & Martinez, 2000; Trémollieres, Pouilles, & Ribot, 1996) can cause excessive accumulation of visceral adipose tissue and trigger chronic inflammation (Abu-Taha et al., 2009; Pfeilschifter, Köditz, Pfohl, & Schatz, 2002).

In this context, it is important to use animal models in order to simulate and evaluate specific experimental conditions of postmenopausal obesity. Experimental oophorectomy triggers changes in metabolism that are compatible with the development of postmenopausal excess body fat by deprivation of estrogen in female rats (Ignacio et al., 2009; Torrezan et al., 2008).

Most articles describing animal models monitor body weight as a standard of development evaluation. In the early the last century, Donaldson (1915) showed the importance of direct methods for analyzing the partition of body weight and indirect methods with wider applicability *in vivo*. Among the indirect methods of estimating body composition, measures and metric indices are widely used, because they are non-invasive, having greater clinical applicability and therefore convenient tools in monitoring body composition (Bernardis & Patterson, 1968; Dahms & Glass, 1982; Novelli et al., 2007; Simson & Gold, 1982).

Bioelectric techniques have been proposed as low cost and less invasive indirect methods for assessing more precisely body composition in experimental animals (Angéloco et al., 2012; Hall, Lukaski, & Marchello, 1989; Rutter, Hennoste, Ward, Cornish, & Thomas, 1998), as well as several indices, which, in this case, are called murinometric (from Latin *murinae* - rodent and Greek *metri* - measure). These indices are methodologically similar to anthropometric measures in the human body and are very discussed in relation to their efficiency in defining the framework of obesity in rats (Dahms & Glass, 1982; Simson & Gold, 1982). Regarding the use of these indices, Novelli et al. (2007) showed that body mass index (BMI) has a greater sensitivity than the Lee Index as obesity determination index for experimental animals. However, no studies were found comparing murinometric indices with bioelectric measures, or evaluating the effectiveness of the analysis in experimental models of postmenopausal obesity. Thus, the objective was to evaluate and discuss the use of bioimpedance (BIA) to identify the obesity framework in oophorectomized female rats.

Material and methods

Animals

Ten young adult Wistar rats (8 weeks) weighing between 130-160 g, from the Animal House of the *Universidade Federal do Piauí* (UFPI) were kept at 22-25°C, 12 hours light/dark cycle, and received water and food *ad libitum* (LABCIL COBAIAS 6522). All procedures related to the use of animals were performed according to standards of the Brazilian Society of Science in Laboratory Animals (SBCAL) and according to the Normative Resolutions of the National Council for Animal Experimentation Control (CONCEA). The research project was approved by the Ethics Committee on Animal Experimentation of *Universidade Federal do Piauí* (017-2009).

Experimental groups

The animals were randomly assigned to the control group (N = 5), in which the animals were subjected to false oophorectomy surgery, and the oophorectomy group (N = 5) underwent a bilateral oophorectomy.

Surgical procedures

Surgical procedures were performed under intraperitoneal anesthesia (ketamine 60 mg kg^{-1} and xylazine 8 mg kg^{-1}), confirmed by testing sensory responses to pressure stimulus. Midline incision of 1.5 cm in length was made in the skin and subcutaneous tissue on the animal's back below the last rib, bilaterally, the ovaries were exposed and isolated with suture thread #5, followed by excision and replacement of the fallopian tubes and suture. After disinfection with iodine solution (Friezol, São Paulo, Brazil), analgesic administration and anti-inflammatory treatment with Pencivet Plus® (Intervet, Cotia, São Paulo, Brazil) intramuscularly at a dose 1 ml kg^{-1} was provided. The control group underwent the entire surgical procedure except excision of the ovaries.

Murinometric and bioelectrical evaluation

After 4 weeks, the animals were weighed on an analytical balance (Marte/AD500) under anesthesia and the naso-anal length (NAL), the length from nose to anus, was measured with a stadiometer, with the animal in the prone position. The Body Mass Index (BMI) and Lee index were calculated according to the following equations:

$$BMI = \frac{Weight}{NAL^2} \qquad \text{Lee Index} = \frac{\sqrt[3]{Weight}}{NAL} \times 1000$$

For bioelectrical impedance analysis, dual electrodes were used, subdermal needle type (26 x 10, stainless steel), with 1 cm between needles. The animals were kept in left lateral decubitus and the electrodes were introduced in the upper intramuscular region (thigh) in the foreleg and back of the right hemisphere. After set, the distance between the electrodes (DBE) was determined using inextensible millimeter measuring tape. The resistance at 50 KHz (R_{50}) and reactance (Xc) were determined using a tetrapolar bioimpedance analyzer at 50 kHz Model BIA-101Q (RJL Systems Clinton Township, MI, USA), which emits an alternating current of 800 μA at 50 KHz.

The formulas for the determination of body composition were used in accordance to Cornish, Ward, and Thomas (1992) and Ilagan, Bhutani, Archer, Lin, and Jen (1993). The following equations enable the determination of total body water (TBW) and fat-free mass (FFM):

$$TBW = 309.9 \times \frac{L^2}{Zc} + 30.0$$

$$FFM = 0.38 \times BW + 13.8 \times \frac{L^2}{R_{50}} + 70.9$$

in which L corresponds to DBE, R50 is the resistance in 50 KHz, BW is Body Weight and Zc is Impedance ($Zc = \sqrt{R^2 + X^2}$). Body fat was calculated as the difference between body weight and FFM and then the fat percentage was determined by proximate relationship between body fat and body weight of the animal.

Murinometric evaluation of body tissues

After dissection and removal of gastrocnemius and soleus muscles, adipose pads concerning periovarian and retroperitoneal fat were weighed on an analytical balance (Marte/AD500). The left tibia was dissected and measured with a caliper Digimess #100.150 (São Paulo, São Paulo, Brazil). The ratios for tissue weights were determined dividing tissue weight by the length of the animal tibia.

Statistical analysis

The results were presented as descriptive statistics (mean and standard error). The comparison between groups was performed by Student's t test for independent samples and Pearson correlation using GraphPad prism 6.0® program. Statistical significance was considered when the results presented likelihood of null hypothesis lower than 5% ($p < 0.05$). The post-hoc results relative to power of analysis (1 - β error probability) and effect size of t-test in Lee Index, Body Mass Index and Bioimpedance was calculated using the software G*Power 3.1.9.2 (Universität Düsseldorf, Germany).

Results

Table 1 shows the murinometric and bioimpedance data demonstrating that, during the experimental period, both groups gained body weight, the oophorectomized group presented a mean weight 46.3% higher than the control group at the end of the experiment. This weight gain as a result of hormone deprivation was reflected in increased muscle weight, 39.7%, and body fat weight, with an increase of 108.9%, in the oophorectomized group.

Based on these results, with respect to relative tissue weight normalized to tibia length, only the fat weight ratio was significantly different between the groups (Table 1). This fact, added to a total dissected fat mass of about 100% higher in oophorectomized animals compared to the control group, indicates that the average weight gain of this group was mainly due to a differential increase in body fat gain.

Table 1. Murinometric and Bioelectrical data (mean ± standard error) of animals and body tissue in oophorectomy and control groups. (*) = significant difference to control ($p < 0.05$). (TBW = total body water; FFM = fat-free mass).

	Control	Oophorectomy	P value
Murinometric data			
Initial body weight (g)	138.1 ± 4.90	148.2 ± 1.10	0.0800
Final body weight (g)	175.6 ± 4.30	256.6 ± 7.67*	<0.0001
Muscle weight (g)	1.16 ± 0.03	1.62 ± 0.05*	<0.0001
Fat weight (g)	5.87 ± 0.98	12.26 ± 0.64*	0.0013
Muscle/tibia length ratio ($x10^{-3}$)	23.55 ± 2.57	30.16 ± 3.94	0.1643
Fat/tibia length ratio ($x10^{-3}$)	157.4 ± 26.66	306.3 ± 18.10*	0.0033
Bioelectrical data			
Resistance (ohms)	360.0 ± 28.64	367.3 ± 14.56	0.8414
Reactance (ohms)	86.60 ± 2.50	83.50 ± 3.43	0.4781
Impedance (ohms)	370.5 ± 27.96	376.6 ± 14.86	0.8634
TBW (g)	140.4 ± 9.96	152.4 ± 14.25	0.4997
FFM (g)	142.7 ± 1.94	173.9 ± 3.50*	<0.0001
Fat weight (g)	32.89 ± 2.43	82.40 ± 4.20*	<0.0001

Bioelectrical data were able to sensitively predict the gain of FFM and proportional gain of body fat in oophorectomy group compared to the control (Figure 1). In relative terms, the BIA technique has evidenced that fat percentage of this group was 72.6% higher than the control, while the changes in obesity assessment using the murinometric indices, LEE status and BMI, although significant, were relatively low, 8.4% and 32.6% respectively (Figure 1).

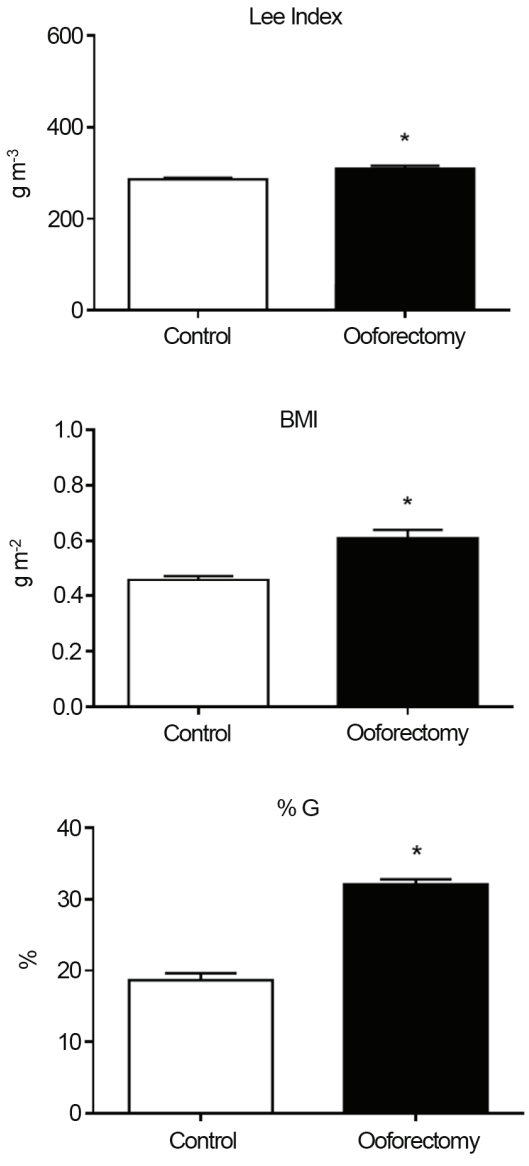

Figure 1. Body composition index (mean ± standard error) in oophorectomy and control groups. (*) = significant difference to control ($p < 0.05$). (BMI = body mass index; %G = body fat percentage).

When analyzing the statistical power of the results, the percent variation observed in results in Figure 1 was confirmed by the post-hoc test of effect size and power (Table 2). Thereby, the accuracy of the BIA was demonstrated, as effect size and power of analysis were similar or even higher than the direct methods.

Table 2. Post-hoc results relative to the power of analysis (1 - β error probability) and effect size of t-test in LEE Index, Body Mass Index and Fat calculated by Bioimpedance, dissecation and fat/tibia length ratio.

Analysis	Effect Size	Power
Lee Index	2.322834	0.8938870
Body Mass Index	3.078142	0.9883145
Bioimpedance % G	7.330463	1.0000000
Dissected Fat	7.720680	1.0000000
Fat/Tibia length ratio	6.534833	1.0000000

Discussion

The detection of variations in the "body complexion" due to ovarian castration in young Wistar rats was possible by the different strategies proposed here: dissection of the adipose tissue, murinometric and BIA indices. However, the approach of a bioelectrical method revealed a greater variation in fat percentage between groups than variations found using murinometric indices.

Despite the general anabolic state and tissue weight gain due to hormonal deprivation (Gavin, Cooper, Raymer, & Hickner, 2013), bioimpedance was further able to identify that the body weight gain of oophorectomized rats mainly derived from adipose tissue gain, as discussed below.

Although all indirect methods for obesity determination were able to identify differences in body weight, the sharp increase of adipose tissue observed in the oophorectomized group, was best determined using BIA, while murinometric indices apparently do not distinguish well between body weight and composition. Murinometric indices exhibited similar responses for the body weight gain in animals, but with different sensitivity, as shown by Novelli et al. (2007). The Lee index showed only a small difference (8.4%) between control and oophorectomized rats, although metabolic disorder was already settled in the oophorectomized group accompanied by a substantial increase in body fat, as also commonly described for this hormone deprivation model (Ignacio et al., 2009; Torrezan et al., 2008). Again, the BMI presented a greater distinction between the groups, thereby representing a higher detection capability of metabolic disorders related to obesity (Novelli et al., 2007) than the Lee index.

In comparison with the *post-mortem* tissue dissection, we observed that the BIA was able to predict the increase in absolute body lean and fat mass of the oophorectomized animals, reflecting the data obtained from the dissection of muscle and

adipose tissue (Table 1). These results were also confirmed by the analysis of power of the measurements (Table 2). These analyses show that BIA obtained a greater effect size and a smaller possibility of error in hypothesis testing in relation to BMI and Lee index. Angéloco et al. (2012) failed to validate BIA as a co-equal method for chemical fractionation as a direct method, since they disregarded the distance between the electrodes in the prediction of FFM and Fat Mass contemplated by Cornish et al. (1992), Ilagan et al. (1993) and Rutter et al. (1998), and successfully applied in our study. Our results also corroborated the capability in detecting changes in body composition by BIA, as already described by Skalicky, Narath, and Viidik (2001) and Narath, Skalicky, and Viidik (2001), who also considered the distance between the electrodes.

Nevertheless, murinometric measures in animal models are still widely used because of their simplicity, such as division of specific tissue weight by body weight, body length and/or long bone length (Dantas et al., 2010). We used murinometric indices in order to determine the differential gain of fat content relative to body lean mass in body weight composition, but these indices are also utilized in more sophisticated applications such as for obesity prediction indices (Simson & Gold, 1982; White et al., 2013). In this context, the Lee Index (Bernardis & Patterson, 1968) was considered inadvisable by Novelli et al. (2007) to estimate the fat mass and obesity in rats. These authors also claimed that BMI applied to rats reliably estimates the body fat content, and normal weight, "eutrophism", would be determined as the range of 0.45 to 0.68 g cm^{-2}. Applying this range to our data, both control and oophorectomized animals would be considered as eutrophic with BMIs of 0.46 g cm^{-2} and 0.61 g cm^{-2} (Figure 1). But dissection of body fat and BIA revealed a fat percentage approximately 2 times higher in oophorectomized animals. These findings demonstrate that the BMI range proposed by Novelli et al. (2007) is not suitable to predict changes in body composition and is also a disadvantage the use of BMI in rats, thus requiring further discussion. We conclude that bioimpedance has advantages over murinometric techniques in terms of evaluating body composition and obesity rates.

Conclusion

Murinometric indices as techniques for determining body fat and obesity in oophorectomized rats, as well as other models already studied, should be used with caution as they may underestimate the differential gain of fat tissue in these animals. In this sense, the BIA, as an indirect method to determine body composition, was able to detect the increase of adipose tissue, in this post-menopausal obesity model. Additionally, it revealed that this increase in body fat occurred within a BMI range proposed as eutrophic.

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