

Bioelectrical impedance analysis and anthropometry for the determination of body composition in rats: effects of high-fat and high-sucrose diets

Bioimpedância elétrica e antropometria na determinação da composição corporal de ratos: efeitos das dietas ricas em lipídeos e sacarose

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ABSTRACT

Objective

The aim of the present study was to determine the impedance of Wistar rats treated with high-fat and high-sucrose diets and correlate their biochemical and anthropometric parameters with chemical analysis of the carcass.

Methods

Twenty-four male Wistar rats were fed a standard (AIN-93), high-fat (50% fat) or high-sucrose (59% of sucrose) diet for 4 weeks. Abdominal and thoracic circumference and body length were measured. Bioelectrical impedance analysis was used to determine resistance and reactance. Final body composition was determined by chemical analysis.

Results

Higher fat intake led to a high percentage of liver fat and cholesterol and low total body water in the High-Fat group, but these changes in the biochemical profile were not reflected by the anthropometric measurements

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or bioelectrical impedance analysis variables. Anthropometric and bioelectrical impedance analysis changes were not observed in the High-Sucrose group. However, a positive association was found between body fat and three anthropometric variables: body mass index, Lee index and abdominal circumference.

Conclusion

Bioelectrical impedance analysis did not prove to be sensitive for detecting changes in body composition, but body mass index, Lee index and abdominal circumference can be used for estimating the body composition of rats.

Indexing terms: Anthropometry. Bioelectrical impedance analysis. Diet. Rats.

RESUMO

Objetivo

Determinar a impedância de ratos tratados com dietas ricas em lipídeos e sacarose por meio do método da bioimpedância elétrica e correlacionar com a análise direta da carcaça, os parâmetros bioquímicos e antropométricos.

Métodos

Foram utilizados 24 ratos machos (Wistar), os quais foram alimentados com dieta padrão, rica em lipídeos ou rica em sacarose por 4 semanas. O grupo-controle recebeu dieta padrão AIN-93; o hiperlipídico, dieta contendo 50% de lipídeos, dos quais 70% eram gordura saturada; e o grupo com rico em sacarose foi alimentado com maior proporção de carboidratos simples, sem alteração na quantidade total.

Resultados

A maior ingestão de lipídeos levou a um aumento da porcentagem de gordura hepática e de colesterol e reduziu a quantidade de água corporal total no grupo hiperlipídico, contudo essas alterações no perfil bioquímico não se refletiram em mudanças antropométricas ou alterações nos parâmetros da bioimpedância elétrica. Mudanças na antropometria e bioimpedância elétrica não foram observadas no grupo com rico em sacarose. No entanto, uma associação positiva foi observada entre a gordura da carcaça e três parâmetros antropométricos, ou seja, índice de massa corporal, índice de Lee e circunferência abdominal.

Conclusão

A impedância bioelétrica não se mostrou sensível em detectar mudanças na composição corporal, entretanto observou-se que parâmetros antropométricos - índice de massa corporal, índice de Lee e circunferência do abdômen - podem ser utilizados para estimar a composição corporal em ratos.

Termos de indexação: Antropometria. Bioimpedância elétrica. Dieta. Ratos.

INTRODUCTION

Body composition is usually assessed for determining body component deficiencies or excesses, such as lean mass and fat mass, which allow an understanding of nutritional status¹. The great importance of this assessment is that body weight alone does not reflect if there is too much or too little of these body components, which may be hazardous². In addition, changes in body composition may have an impact on metabolism, since adipose tissue modulates lipid and glucose homeostasis³.

Accurate methods for determining the body composition of animals are extremely

important for understanding how the body responds to nutrient intake, and for nutritional and physiological studies^{4,5} that use animal models to investigate the effects of obesity and nutrient deficiencies^{6,7}.

Direct chemical analysis of the carcass is the reference method for determining rat body composition⁸. However, even though direct chemical analysis provides more precise information, it is invasive, time consuming, expensive and requires technical knowledge. Also, it cannot be used in living animals¹, so its use is limited.

METHODS

In an attempt to overcome the disadvantages of direct chemical analysis of the carcass, indirect techniques have been used for determining the body composition of animals⁸. Interest in Bioelectrical Impedance (BIA) has increased recently since it is a noninvasive, inexpensive, fast and reproducible method⁹ that provides a good estimate of body composition¹⁰. Moreover, it can be used repeatedly on the same animal causing minimal disturbance^{1,11}. However, estimation accuracy will depend on the equation used and standardization of the test conditions¹².

Since Bioelectrical Impedance is based on the principle that tissues have different impedances, that is, opposition to the flow of an electric current, which in turn is dependent on water and electrolyte content,¹³ and assuming that the fat-free mass has a constant water content and resistivity, Total Body Water (TBW) and Fat-Free Mass (FFM) can be estimated by measuring the electrical impedance of the body¹⁴.

Studies considering this information for the use of BIA in animals have shown that BIA can be used for predicting the total body water of animals, but there are no specific formulas that distinguish lean from fat mass^{1,11,15}. Furthermore, few studies have used this method in rats and no study has investigated whether diet affects body composition and whether BIA would be capable of detecting subtle changes.

More studies using BIA on live animals are necessary to determine if this technique can determine rats' body composition accurately and if diet affects the body composition of experimental animals.

The objective of the present study was to determine the impedance of Wistar rats fed high-fat and high-sucrose diets by BIA and to verify if the results correlate with those obtained by direct chemical analysis of the carcass and biochemical and anthropometric measurements.

Animals and treatment

The study included 24 male Wistar rats with an initial mean weight of 65g, obtained from the Central Animal Facility of *Ribeirão Preto* School of Medicine, *Universidade de São Paulo* (FMRP/USP). The project was approved by the FMRP/USP Ethics Committee on Animal Research, protocol number 147/2008. The animals were housed in individual cages under an alternating 12-hour light/dark cycle, mean temperature of 22°C, and free access to food and water.

The animals were allowed to adapt to the experimental conditions for one week, all of them receiving the standard AIN-93 diet. They were then divided randomly into 3 groups: Control group (C): group fed the standard diet AIN-93 (n=8); High-Fat group (HF): group fed a high-fat diet (n=8); and High-Sucrose group (HS): group fed a diet with a different type of carbohydrate (n=8).

The control group was given the standard AIN-93 diet¹⁶, and the high-fat group was given a HF diet containing 15% fat, adapted from Reeves *et al.*¹⁶, as described in Table 1¹⁷. Fats in the control diet originated exclusively from soybean oil and in the HF diet, from rendered lard (70.00%) and soybean oil (30.00%). The HS diet had a different carbohydrate composition, that is, more simple carbohydrates: while the control diet contained mainly cornstarch, the HS diet contained 3.50% cornstarch and 59.85% sucrose¹⁸ (Table 1).

The animals were weighed once a week by a Filizola® electronic scale with a capacity of 1,500 grams and accuracy of 1 gram. Food intake was monitored by weighing the feeders three times a week over the study period.

At the end of the 4-week intervention, the animals' body impedance was measured by BIA and the animals were sacrificed by decapitation. The liver and blood were collected, the serum separated and all items were immediately frozen until use. The remainder of the animals was also frozen until direct chemical analysis of the carcass.

Table 1. Composition of the experimental diets. *Ribeirão Preto* (SP, Brazil), 2010.

Ingredients/group (%)		Control Diet (AIN-93)	High-fat diet	High-sucrose diet
Carbohydrates	Cornstarch	53.00	17.2*	3.50*
	Sucrose	10.00	3.30*	59.8
Lipids	Saturated soy oil	7.00	15.00	7.00
	Industrialized lard	-	35.00	-
Casein		20.00	20.00	20.00
Fibers		5.00	5.00	5.00
Vitamin mix		1.00	1.00	1.00
Mineral mix		3.50	3.50	3.50
Choline		0.25	0.25	0.25
L-Cystine		0.30	0.30	0.30*
Tert butylhydroquinone		0.01	0.01	0.01

* Values corrected according to lipid plus sucrose values.

Anthropometric measurements

Body length was measured from the nostril to the base of the tail (pelvic-caudal junction); abdominal circumference at the point immediately anterior to the forefoot; and chest circumference at the site immediately behind the foreleg¹⁹. The rats were anaesthetized with 2% tribromoethanol for the measurements.

A non-elastic tape measure was used for all measurements.

BMI was determined by dividing the animal's weight (g) by the square of its length (cm). The Lee index was determined by dividing the cube root of the body weight (g) by the nose-to-anus length (cm)²⁰.

Bioelectrical impedance

Whole Body Resistance (WBR) and Reactance (WBXc) were measured by a phase-sensitive tetrapolar bioelectrical impedance analyzer (Byodynamics BIA 310E). Standard hypodermic needles were used as electrodes. The rats were anesthetized and put on their stomachs on a non-conductive surface to eliminate interference from electrical induction. The dorsal surfaces of the head and body were shaved for placement of the needle electrodes. Source electrode 1 was placed on the midline on the anterior margin of the orbit and source electrode

2 was placed 4cm from the base of the tail. Detector electrode 1 was placed on the anterior opening of the ear and detector electrode 2 was placed in the median region of the pelvis¹. All impedance measurements were done in well fed and hydrated animals.

Total body water (W_{TBW} , g) was estimated by the empirical formula given by Hall *et al.*¹: $W_{TBW} = 15.47 + 97.44 L^2 / WBR$, where L is the length of the body (cm) and WBR is the total body resistance (Ω) according to BIA.

Laboratory methods

Biochemical analyses

Hepatic fat was determined as suggested by Bligh & Dyer²¹. Total serum cholesterol and protein, and blood glucose were determined by colorimetric reactions, using the LABTEST® enzyme kit.

Direct chemical analysis of the carcass

At the end of the four-week intervention, the animals were sacrificed by decapitation and frozen until chemical analysis of the carcass. Skin, viscera, head and feet were discarded, using only bones and muscles for quantitative water, fat and protein analysis²². Water content was determined by placing the empty carcasses individually in

aluminum sheets in an oven at 105°C for 24 hours. The amount of water present in the carcass was calculated by subtracting the dry carcass weight from the baseline carcass weight. The dry carcasses were then macerated and the fat extracted by intermittent extraction using petroleum ether and a Soxhlet extractor. Carcass fat was calculated by the difference in weight. The protein content was calculated by the micro-Kjeldahl method²³, an indirect nitrogen determination method, using the 6.25 factor for conversion to protein. All analyses were repeated three times at the FMRP/USP Nutrition and Metabolism Laboratory.

Statistical analysis

Data are expressed as Means (M) and Standard Deviation (SD). Analysis of Variance (ANOVA) followed by the Tukey post-hoc test

were used for investigating possible differences in the study parameters, and linear regression for investigating possible correlations between the study variables. The significance level was set at 5% ($p < 0.05$) for all analyses.

RESULTS

Table 2 shows the weight and food intake of the groups. The HF group consumed significantly less food (g/week) than groups C and HS, but the groups did not differ with respect to energy intake (kcal/week). All animals had similar baseline and final weights, and weight gain during the intervention.

The HF group had significantly higher hepatic fat content and serum cholesterol level than group C. Meanwhile, the HS group had significantly higher blood glucose level than the

Table 2. Comparison of final weight, weight gain and food and energy intake among the study groups. *Ribeirão Preto* (SP, Brazil), 2010.

	C		HF		HS	
	M	SD	M	SD	M	SD
Final weight (g)	290.1	18.3 ^a	269.1	55.8 ^a	302.6	4.7 ^a
Weight gain (g)	229.3	16.7 ^a	204.5	52.7 ^a	240.1	31.9 ^a
Food intake (g/week)	40.8	12.7 ^a	27.5	6.5 ^b	42.0	8.8 ^a
Energy intake (kcal/week)	161.1	50.3 ^a	168.6	39.9 ^a	166.4	35.0 ^a
Fat intake (g/week)	2.8	1.2 ^a	13.7	3.4 ^b	2.9	1.2 ^a
Sucrose intake (g/week)	4.1	1.4 ^a	0.9	0.1 ^b	25.1	5.3 ^c

C: Control group; HF: High-Fat group/ HS: High-Sucrose group; Mean values; M: Media; SD: Standard Deviation; followed by different letters indicate a significant difference for $p < 0,05$.

Table 3. Body composition of the rats studied evaluated by direct chemical analysis of the carcass after 4 weeks of the experiment. *Ribeirão Preto* (SP, Brazil), 2010.

	C	HF	HS
Total body water (g)	M=80.1; SD=12.3 ^a	M=69.7; SD=12.1 ^b	M=82.7; SD=11.7 ^a
%	50.8 ^a	43.9 ^a	51.2 ^a
Fat-free mass (g)	M=147.7; SD=1.2 ^a	M=147.4; SD=0.9 ^a	M=146.4; SD=1.4 ^a
%	90.6 ^a	90.4 ^a	89.8 ^a
Protein (g)	M=52.8; SD=9.7 ^a	M=41.7; SD=5.9 ^b	M=51.2; SD=8.5 ^a
%	33.6 ^a	26.6 ^a	31.7 ^a
Body fat (g)	M=14.9; SD=1.9 ^a	M=15.4; SD=3.0 ^a	M=16.4; SD=1.8 ^a
%	9.4 ^a	9.6 ^a	10.2 ^a

C: Control group; HF: High-Fat group; HS: High-Sucrose group. Values are reported as means; M: Media; SD: Standard Deviation mean values followed by different letters indicate a significant difference ($p < 0.05$, ANOVA followed by the Tukey *post-hoc* test).

Table 4. Anthropometric and bioelectrical impedance analysis indices. *Ribeirão Preto (SP, Brazil), 2010.*

Anthropometric Indices	C		HF		HS	
	M	SD	M	SD	M	SD
Length (cm)	22.25	0.5 ^a	22.14	1.6 ^a	22.78	0.9 ^a
Chest circumference (cm)	12.10	0.7 ^a	11.60	0.9 ^a	12.10	0.6 ^a
Abdominal circumference (cm)	13.60	1.0 ^a	13.30	0.7 ^a	12.90	0.3 ^a
Abdomen: chest ratio	1.13	0.07 ^a	1.15	0.07 ^a	1.07	0.04 ^a
BMI (g/cm ²)	0.59	0.04 ^a	0.59	0.06 ^a	0.58	0.06 ^a
Lee index	0.77	0.02 ^a	0.77	0.04 ^a	0.76	0.04 ^a
Bioelectrical impedance Indices						
Resistance (ohms)	251.10	26.4 ^a	279.10	28.4 ^a	260.10	11.7 ^a
Reactance (ohms)	19.60	6.1 ^a	18.90	6.4 ^a	18.20	5.3 ^a
Total body water	210.06	24.3 ^a	195.02	17.1 ^a	210.99	17.9 ^a

C: Control group; HF: High-Fat group; HS: High-Sucrose group. Values are reported as M: Means and SD: Standard Deviation.

HF group. Group HF had significantly less total protein than groups C and HS.

Table 3 shows the circumferences, BMI and Lee index of the animals. These variables did not differ significantly among the groups, nor did resistance or reactance Table 4.

Table 5 shows the composition of the carcasses determined by direct chemical analysis. Only TBW and protein differed significantly among the groups ($p < 0.05$): they were lower in the HF group than in the C and HS groups.

Figure 1 shows the positive correlations found between carcass fat and BMI, Lee index and abdominal circumference. A negative correlation ($p < 0.05$) was found between carcass fat and reactance ($r = -0.51$). However, no correlation was found between carcass fat and resistance determined by BIA.

DISCUSSION

The effects of different macronutrient intakes have been extensively studied in laboratory animals. However, little information is available about the effect of different macronutrient intakes on the body composition and anthropometric variables of rodents in general. Also, little is known about the validity of these methods for the anthropometric assessment of these animals.

There are several experimental studies investigating the effects of high-fat and high-sucrose diets on rats since these diets promote metabolic changes, but they usually only assess their effect on body weight²⁴⁻²⁶. BIA can accurately measure lean and fat mass and this distinction is important because excess body fat compromises health, and may promote the development of glucose intolerance and dyslipidemia. Importantly, BIA can be used repeatedly for determining the body composition of live animals, while direct chemical analysis requires sacrificing.

In the present experiment, abdominal circumference, Lee index and BMI (Figure 1) correlated significantly with body composition. The positive correlation found between carcass fat and BMI is in agreement with Novelli *et al.*¹⁹, who suggested that BMI can reliably estimate body fat in rats even though it is not sensitive enough to detect body changes stemming from diets with different macronutrient compositions. Contrary to the present experiment, the cited study did not show the data regarding the correlation between carcass fat and the Lee index and abdominal circumference. Thus, future studies should investigate how accurately these variables can reflect body composition changes.

Bioelectrical Impedance is used in humans as a fast, noninvasive and reproducible method for determining body composition and water content^{27,28}. However, few studies have used this

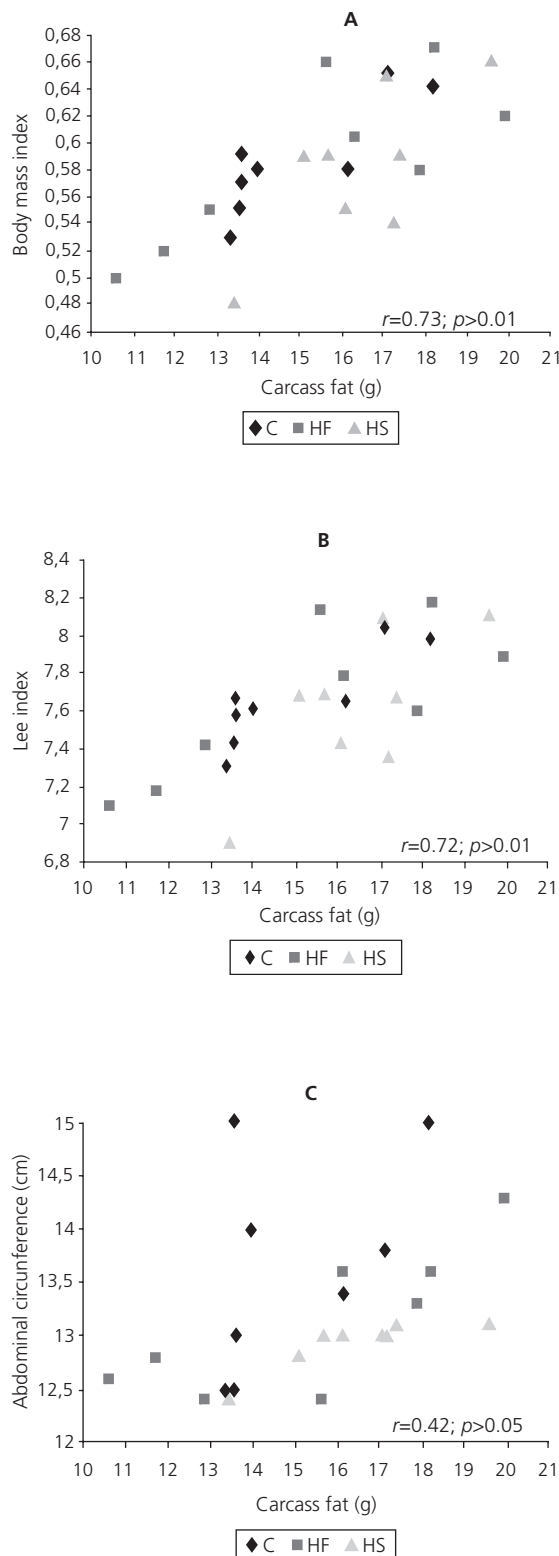


Figure 1. Correlations between carcass fat and BMI (a), Lee index (b) and abdominal circumference (c) 4 weeks after the dietary intervention. Ribeirão Preto (SP) Brazil, 2010.

Note: C: Control group; HF: High-Fat group; HS: High-Sucrose.

technique in laboratory animals^{11,29,30}, so this method needs to be further investigated.

Surprisingly, the present results demonstrated that resistance data do not correlate with carcass fat determined chemically, suggesting that BIA is not sufficiently sensitive to measure the body composition of rats or detect the differences in groups receiving different diets. Another disagreement concerned reactance, which was negatively correlated with carcass fat, in contrast with Hall *et al.*¹, in which reactance, when compared with resistance, was not considered a strong predictor of any body component. This study also found wide intragroup variation, showing the heterogeneity of these animals and their different responses to the same diet.

In contrast to the present results, other studies have shown that BIA is sufficiently sensitive to determine rat body composition. In a pioneering study, Hall *et al.*¹ developed an appropriate method for using BIA in rats and found a strong negative correlation between Whole Body Resistance (WBR) measured at 50kHz and total body water and protein. Yoki *et al.*³¹ used the empirical formula proposed by Hall *et al.*¹ to estimate the total body water of rats fed a control diet and a diet supplemented with methionine or homocysteine, and demonstrated that this formula was capable of detecting differences between the groups. In contrast to the present study, there were no correlations between this formula and body composition variables. Also, there were no differences among groups fed different diets. Rutter *et al.*¹¹ noted that BIA could be used to estimate the total body water of control rats, although the method was less accurate when the procedure was used in rats fed a high-fat diet.

The present results show that the fat intake of the HF group and the sucrose intake of the HS group were considerably higher (HF:389% and HS:512%) than those of the control group during the intervention period, despite lower food

intake and same energy intake. Rat tendency to consume less high-fat food is confirmed by other studies^{32,33}. This change in food intake may be due to a mechanism in rats that regulates food intake, reducing food intake when the diet is energy-dense³⁴.

However, higher fat intake increases hepatic fat and cholesterol and reduces total body water, demonstrating that high-fat diets change the lipid profile of the animals. Nevertheless, these changes in blood biochemistry were not reflected by anthropometric or BIA variables, which is in disagreement with most literature reports^{11,29}. Thus, it is possible that the duration of the study intervention was not enough to change body composition but enough to change the biochemical profile, since it changes more readily, or that a diet with different fat proportions would be necessary. Also, some studies have shown that high fat accumulation in rats does not depend on the age or sex of the animals, although genetics may influence fat retention³⁵. BIA standardization in rats with the development of smaller devices and specific equations could provide more accurate results.

CONCLUSION

In conclusion, BIA was not capable of detecting body composition changes in rats fed high-fat and high-sucrose diets. However, carcass fat was significantly associated with BMI, Lee index and abdominal circumference, suggesting that these parameters may be used for estimating rat body composition. More research is needed using BIA to assess the body fat of animals fed different diets. The associations between diet and body composition, and how body composition changes over time, could be investigated without having to kill the animals.

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CONTRIBUTIONS

LRN ANGÉLOCO and R DEMINICE planned and carried out the experiment, analyzed data and wrote the manuscript. IA LEME helped carry out the experiment, standardized BIA in rats, analyzed data and wrote the manuscript. RC LATARO helped carry out the experiment, collected laboratory data and analyzed the data. AA JORDÃO helped to plan the experiment, analyzed data and wrote the manuscript.

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