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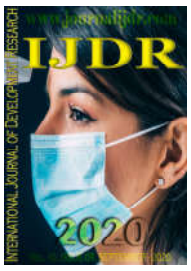

RESEARCH ARTICLE OPEN ACCESS

ARE ANTHROPOMETRIC MEASURES RELATED TO FATTY ACID CONCENTRATIONS?

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ABSTRACT

Objective: To associate the levels of fatty acids in serum with anthropometric markers. **Methods**: A total of 48 subjects were evaluated by quantifying their relative percentages of serum free and esterified fatty acids (EFAs) by gas chromatography. Measures including body mass index (BMI), body fat percentage (BF%), waist circumference (WC) and waist-hip ratio (WHR) were used to characterize anthropometric measurements. We compared the relative percentages of the concentrations of EFAs and FFAs to categories of anthropometric indicators and used the correlation coefficients to evaluate their associations. **Results**: Differences were identified in palmitic and palmitoleic acid concentrations for BMI, WC and WHR; myristic and linoleic acid concentrations for WC and WHR; oleic acid concentrations for WC; stearic acid concentrations for WHR; and palmitoleic acid concentrations for BF%. A moderate and positive association was found between BMI and palmitoleic acid (r = 0.471; r = 0.477), and a moderate and negative association was found between WHR and linoleic acid (r = -0.448). **Conclusion**: The composition of fatty acids was found to be related to anthropometric values, as evidenced by differences in concentrations and measures of association.

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INTRODUCTION

Hypertriglyceridemia exerts atherogenic effects by increasing lipoprotein concentrations and is an independent risk factor for cardiovascular disease and coronary events (CULLEN, 2000; RODULFO; NEGRETTI; CANDIA, 2009). This condition is enhanced when combined with the identification of abdominal obesity, in which a hypertriglyceridemic waist is considered a valid clinical phenotype for screening individuals with risks of developing cardiovascular diseases (LEMIEUX *et al*., 2007). Based on this information, some studies have thoroughly investigated this biochemical marker, seeking to assess the differential effects of fatty acids composing triglycerides and their free forms and to determine the relationships between anthropometric markers (CLEVENGER; STEVENSON; COOPER, 2015; JEYAKUMAR *et al*., 2009; KUNEŠOVÁ *et al*., 2012). Esterified and free fatty acids (EFAs and FFAs, respectively) differ in their forms by the numbers of carbons in their structures, which generate specific characteristics. Their structures influence their metabolism and are indicated as modulators of energy expenditure, contributing to the distribution of total and visceral fat (ROYNETTE *et al*., 2008). Considering the increasing incidence of obesity in many populations (NG *et al*., 2013) and the concomitant changes observed in body weight with metabolic disorders, the goal of this study was to associate the concentrations of fatty acids with anthropometric markers.

MATERIALS AND METHODS

Design, setting, sample and ethical aspects: Rural workers were invited to participate in research involving the assessment of anthropometric, biochemical and cardiorespiratory markers using a cross-sectional design. The convenience sample included the voluntary participation of 48 rural workers from municipalities of Candelaria, Passo do Sobrado, Vale Verde and Santa Cruz do Sul, which are cities located in the state of Rio Grande do Sul, Brazil. Consent to participate was

registered as free and informed consent, and the research project was approved by the Ethics Committee on Human Research at the University of Santa Cruz do Sul (authorization number 726121). The data were collected between 2012 and 2013, starting with a demand identified by the Regional Development Council of the Vale do Rio Pardo and approved by the Department of Science, Innovation and Technological Development. The subjects who participated in the study completed a lifestyle questionnaire conducted by interviewer. Exclusion criteria included a previous history of or treatment for coronary artery disease, stroke or acute myocardial infarction.

Gas chromatography: Blood was collected from the subjects through their median cubital veins with a fasting recommendation of 12 hours. The samples were processed to obtain serum and were frozen until extracting the serum FFAs and EFAs using a method adapted from Yi *et al*. (2006). For this processing procedure, serum aliquots (400 µL) were mixed with 4 mL of potassium hydroxide (KOH), homogenized by vortexing (30 seconds) and then kept at rest for 10 minutes at room temperature. Subsequently, 4 mL of hexane was added, and the sample was reinserted into the vortex; this process was performed twice, generating two phases. The phases were separated, and the supernatant corresponding to the EFAs was evaporated under nitrogen gas to dryness. Traces of water were removed from the remaining samples by adding anhydrous sodium sulfate, and the sample was subsequently mixed with 4 mL of sulfuric acid (H_2SO_4) incubated in a water bath for 30 minutes at 70 ° C. Then, 4 mL of hexane was added, and the mixture was stirred by a vortex; this procedure was repeated, resulting in the extraction of the supernatant to afford FFAs, which were also evaporated under nitrogen. After the extraction procedure, 500 µL of hexane was added to the vials to prepare the samples for injection into the chromatograph. Readings were collected in triplicate and quantified by gas chromatography with a flame ionization detector (GC-FID). The quantified components were oleic, palmitoleic, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, linoleic and lignoceric acids. The equipment used for this process was a Shimadzu GC-2010 (Shimadzu Co., Kyoto, Japan) connected to a GCMS-QP2010. A 75-minute reading time was used for each replicate with a ZB-5ms capillary column (60 m × 0.25 mm × 0.25 µm) and the following temperature program: 100.0 °C (10 minutes) - 3.2 °C/minute - 180.0 °C (0 minute) - 8.0 °C/minute - 260.0 °C (30 minutes). Helium was used as the carrier gas at a constant flow of 30 mL/min. GC data were expressed as the relative percentage area of each esterified analyte in relation to the total sample. The identification of the methyl esters of each fatty acid contained in the samples was used to determine the patterns of the respective acid methyl esters (Sigma-Aldrich).

Anthropometry: Evaluations of total and visceral obesity were performed using anthropometric measurements, similar to the blood collection procedure, and consisted of measuring the body mass index (BMI), body fat percentage (BF%), waist circumference (WC) and waist-hip ratio (WHR). Subjects were barefoot and wore light clothes during these measurements. For BMI measurements, the weights and heights of the participants were determined on an anthropometric scale, the BMIs were calculated using a formula (BMI = weight/height^2), and the data were oriented to maintain the Frankfurt Plan. For categorical evaluation, the subjects were dichotomized into a desirable weight group (BMI <25 kg/m^2) and an overweight group (BMI ≥ 25 kg/m^2) (WORLD HEALTH ORGANIZATION, 2006). Seven skinfolds (i.e., chest, triceps, medium axillary, subscapular, supra iliac, abdominal and thigh) were measured with a Lange compass, and the values were inserted into the body density formula of Jackson and Pollock and later in a Siri equation (SIRI, 1961) to determine the participants' BF%. To compare the groups, we considered using the criteria described in Golding *et al*. (1989), which unified categories in groups that had desirable (excellent, good, above average and average) and undesirable (below average, bad and very bad) BF% values. The visceral obesity measurements consisted of WC, where the waist was defined as the midpoint between the last rib and the upper region of iliac crest, as categorized in Lean, Han and Morrison (1995); these measurements indicated desirable and undesirable cardiovascular risks, with the latter obtained as the sum of increased and high risks. Hip circumference (HC) were measured to calculate WHR (WHR = WC/HC), and these values were adopted as references for the maximum extension of buttocks and to define groups with desirable (low and moderate) and undesirable (high and very high) cardiovascular risks (NORTON; OLDS, 2000). Circumferences were measured in centimeters using anthropometric tape.

Statistical methods

Central tendency and dispersion measurements are used to present the data. The normality of the continuous data was evaluated by a Shapiro-Wilk test. Student's t-tests and Mann-Whitney tests were used to calculate the percentage differences in the relative concentrations of fatty acids according to the groups of each of the anthropometric measurements. The association of the parameters (i.e., fatty acids and anthropometric markers) was assessed using Pearson and Spearman tests and coefficient correlations. The significance level was set at $p < 0.05$.

RESULTS

A sample of 48 subjects (28 women) with an average age of 50 years (SD: 11.6) was studied. Of this sample group, 26 subjects belonged to the B economy class, 21 belonged to the C class, and 35 subjects were married. No differences were observed between the anthropometric groups regarding the subjects' sex; however, those identified with undesirable BF%s had a mean age that was lower that that of subjects with desirable BF% (41.1 years *versus* 53.4 years, $p = 0.001$). Regarding drug consumption, six workers regularly used antihypertensive agents, four used lipid-lowering agents, and two simultaneously used both; however, there was no difference in the frequency between individuals in the desirable and undesirable categories for BMI, BF%, WC and WHR. Subjects identified with excess weight showed higher concentrations of EFA palmitic acid ($p = 0.007$) and FFA palmitoleic acid ($p = 0.025$); similarly, those with undesirable levels of BF% showed higher palmitoleic acid concentrations (EFA: $p = 0.045$; FFA: $p = 0.039$) (Table 1). Higher concentrations of EFA myristic, palmitoleic, palmitic and oleic acids and lower concentrations of linoleic acid were found in subjects with WC values above the desired level ($p < 0.05$). The same trend was observed for the WHR and EFA values for myristic and palmitic acids. For FFAs, differences were identified between WC measurements and palmitic acid concentrations, WHR and stearic acid concentrations, and for both of the anthropometric markers of visceral obesity with

Table 1. Comparison of fatty acids for BMI and BF% groups

Fatty Acids		BMI		p	BF%		p
		Desirable (n=9)	Overweight (n=39) *		Desirable (n=33) *	Undesirable (n=15)	
Myristic	EFAs	0.55 (0.19)	0.77 (0.32)	0.062†	0.69 (0.31)	0.80 (0.31)	0.271 †
	FFAs	0.85 (0.16)	0.95 (0.37)	0.463 †	0.94(0.30)	0.91(0.43)	0.757 †
Palmitoleic	EFAs	3.05(1.04)	3.97(1.46)	0.082 †	3.52 (1.37)	4.41 (1.42)	0.045 †
	FFAs	2.17(0.34)	2.54(0.66)	0.025 †	2.34 (0.56)	2.74 (0.70)	0.039 †
Palmitic	EFAs	17.70(1.75)	20.61(2.94)	0.007 †	19.98 (3.06)	20.25 (2.84)	0.767 †
	FFAs	23.72(0.81)	24.20(2.61)	0.345 †	23.76 (2.59)	24.84 (1.66)	0.146 †
Linoleic	EFAs	18.50(2.78)	17.10(2.68)	0.168 †	17.39 (2.97)	17.29 (2.20)	0.908 †
	FFAs	18.15(2.25)	15.73(3.64)	0.064 †	16.13 (3.86)	16.32 (2.83)	0.869 †
Oleic	EFAs	14.79 (2.14)	18.51 (5.06)	0.270‡	17.78 (5.12)	17.89 (4.42)	0.664‡
	FFAs	19.72 (2.03)	19.82 (2.82)	0.947‡	19.74 (3.02)	19.94 (1.77)	0.973‡
Stearic	EFAs	7.16 (0.79)	8.03 (1.45)	0.081‡	8.07 (1.58)	7.43 (0.66)	0.291‡
	FFAs	9.34(0.45)	8.83(1.06)	0.168 †	9.04 (1.00)	8.70 (0.99)	0.282 †
Others	EFAs	37.82(5.07)	30.59(6.96)	0.005 †	32.12 (7.57)	31.54 (6.50)	0.799 †
	FFAs	25.00 (2.24)	26.95 (6.99)	0.863 †	27.04 (7.30)	25.57 (3.79)	0.909 †

BMI - Body Mass Index; BF% - Body Fat Percentage; EFAs - Esterified Fatty Acids; FFAs - Free Fatty Acids; * - 1 FFA missing; † - Student's t-test; ‡ - Mann-Whitney.

Table 2. Comparison of fatty acids for WC and WHR groups

Fatty Acids		WC		p	WHR		p
		Desirable (n=19)	Undesirable (n=29)*		Desirable (n=23)*	Undesirable (n=25)	
Myristic	EFAs	0.59 (0.27)	0.82 (0.30)	0.0126 †	0.59 (0.25)	0.85 (0.31)	0.003 †
	FFAs	0.87 (0.31)	0.97 (0.36)	0.371 †	0.85 (0.20)	1.00 (0.42)	0.148 †
Palmitoleic	EFAs	3.17 (1.28)	4.20 (1.40)	0.013 †	3.48 (1.30)	4.09 (1.51)	0.142 †
	FFAs	2.08 (0.40)	2.74 (0.62)	<0.001 †	2.21 (0.38)	2.70 (0.72)	0.006 †
Palmitic	EFAs	18.45 (2.30)	21.12 (2.91)	0.002 †	19.14 (2.75)	20.91 (2.96)	0.038 †
	FFAs	22.98 (2.19)	24.86 (2.21)	0.006 †	23.80 (2.12)	24.38 (2.59)	0.408 †
Linoleic	EFAs	19.04 (2.53)	16.26 (2.28)	<0.001 †	17.98 (3.00)	16.79 (2.37)	0.133 †
	FFAs	18.04 (3.50)	14.94 (3.02)	0.002 †	17.32 (3.58)	15.19 (3.24)	0.037 †
Oleic	EFAs	15.52 (3.37)	19.31 (5.15)	0.006‡	16.80 (4.45)	18.74 (5.13)	0.110‡
	FFAs	18.93 (2.43)	20.39 (2.70)	0.074‡	19.95 (2.54)	19.67 (2.82)	0.949‡
Stearic	EFAs	7.74 (1.42)	7.96 (1.39)	0.605‡	7.97 (1.71)	7.77 (1.04)	0.959‡
	FFAs	9.18 (0.74)	8.76 (1.12)	0.155 †	9.33 (0.86)	8.58 (0.99)	0.008 †
Others	EFAs	34.99 (6.50)	29.95 (7.01)	0.016 †	33.58 (7.20)	30.44 (6.98)	0.132 †
	FFAs	26.90 (7.21)	26.35 (5.89)	0.948 †	25.51 (5.14)	27.50 (7.29)	0.306 †

WC - Waist circumference; WHR - Waist-hip ratio; EFAs - Esterified Fatty Acids; FFAs - Free Fatty Acids; * - 1 FFA missing; † - Student's t-test; ‡ - Mann-Whitney.

Table 3. Association between fatty acids and anthropometric measures

Fatty Acids		BMI*		BF%*		WC*		WHR*,†	
		EFAs	FFAs‡	EFAs	FFAs‡	EFAs	FFAs‡	EFAs	FFAs‡
Myristic	r	0.206	0.143	0.122	0.210	0.192	0.076	0.192	0.005
	p	0.160	0.337	0.407	0.157	0.192	0.614	0.191	0.973
Palmitoleic	r	0.471	0.477	0.300	0.285	0.352	0.355	-0.008	0.041
	p	0.001	0.001	0.039	0.052	0.014	0.014	0.957	0.786
Palmitic	r	0.270	0.339	0.050	0.262	0.327	0.152	0.323	-0.174
	p	0.064	0.020	0.736	0.075	0.023	0.307	0.025	0.243
Linoleic	r	-0.320	-0.253	-0.106	-0.013	-0.276	-0.310	-0.309	-0.448
	p	0.027	0.086	0.475	0.933	0.057	0.034	0.033	0.002
Oleic	r	0.207	0.057	-0.085	-0.131	0.308	0.063	0.326*	0.102
	p	0.158	0.702	0.567	0.380	0.033	0.672	0.024	0.495
Stearic	r	0.049	-0.192	0.064	-0.041	0.141	-0.204	0.231*	-0.156
	p	0.740	0.197	0.664	0.787	0.340	0.170	0.114	0.296
Others	r	-0.227	-0.004	0.041	0.026	-0.333	0.063	-0.320	0.210
	p	0.122	0.979	0.784	0.863	0.021	0.673	0.027	0.156

BMI - Body Mass Index; BF% - Body Fat Percentage; WC - Waist circumference; WHR - Waist-hip ratio; EFAs - Esterified Fatty Acids; FFAs - Free Fatty Acids; * - Spearman; † - Pearson; ‡ 1 missing.

palmitoleic and linoleic acid concentrations (Table 2). When the anthropometric measurements were associated with the percentages of the relative areas of the fatty acids, we found a moderate positive correlation between BMI and palmitoleic EFA concentrations and a weak association of the analyte with BF% and WC. Palmitic and oleic EFA were positively associated with central obesity measures but exhibited a weak correlation. Weak and inverse associations were found between linoleic EFA and BMI and WHR (Table 3). Moderate associations were found between BMI and palmitoleic FFA (positive) and between linoleic FFA and WHR (reverse). Weak and positive associations were found between palmitic FFA and BMI and between palmitoleic FFA and WC, indicating that the concentration of linoleic acid was inversely related to WC measurements (Table 3).

DISCUSSION

This study revealed differences in the percentages of the relative areas of FFA and EFA peaks between the desirable and undesirable categories of anthropometric measures, particularly for WC measurements. When the profiles of fatty

acids and body composition values were correlated, moderate to weak associations were observed. We believe that the sample size may have been a limiting factor for the detection of correlations and that additional assessments of the participants' diets could contribute to a better understanding of the relationship between the evaluated parameters. In addition, an assessment of the participants' body composition images could have generated accurate results; however, it should be noted that anthropometric measures are widely used in epidemiological studies; represent low-cost, viable indicators; generate highly reproducible results; and allow for the screening of large populations (PITANGA, 2011). Understanding the influence of fatty acids on the development of obesity and its implications is still being investigated and requires further research to expand our understanding of their associations. Some types of fatty acids are known to act in weight management and are used as predictors in dietary interventions (KUNEŠOVÁ *et al*., 2012). In our study, palmitic acid showed differences in concentration between groups with different BMI, WC and WHR values. The highest concentrations were detected in undesirable groups. Indeed, a high intake of this saturated fatty acid (KIEN; BUNN; UGRASBUL, 2005) has been shown to increase obesity rates and is a major component of animal fat.

Similar to observations of palmitic acid concentrations, other long-chain saturated fatty acids also showed significant results. Myristic EFA, which is present in milk and dairy products, was found in lower concentrations in subjects with low cardiovascular risks for both WC ($p = 0.012$) and WHR ($p = 0.003$). Stearic acid is already in its free form and was found to occur in higher concentrations in workers with undesirable cardiovascular risks, as determined by WHR; this is a common fatty acid found in in fat, cocoa, milk and lard. It has been reported that the oxidation of unsaturated fatty acids and long-chain saturated fatty acids decreases as the number of carbons in the molecular structures increases (DELANY *et al*., 2000). Palmitoleic acid exhibited higher concentrations in all groups with undesirable anthropometric measures. Reports have already demonstrated that a higher initial percentage of this fatty acid is associated with decreased maintenance of weight loss after dietary intervention (KUNEŠOVÁ *et al*., 2012). The concentration of esterified oleic acid (monounsaturated) differed between different WC categories (Desirable: 15.52 versus Undesirable: 19.31), while linoleic (polyunsaturated) acid was found in high concentrations in subjects with central fat-level measurements indicating good health. There is no consensus regarding which compound undergoes increased oxidation with a consequent increase in postprandial energy expenditure because the relevant studies used different evaluation techniques. For example, Jones *et al*. (2008) reported that oleic acid was more efficient in increasing energy expenditure than linoleic acid, contrary to our findings. We also evaluated the correlations between biochemical and anthropometric markers, identifying a moderate association between BMI and palmitoleic acid, positive relationships between EFA ($r = 0.471$) and FFA ($r = 0.477$), and a moderate inverse association between linoleic FFA and WHR ($r = -0.448$). In children, a positive association between arachidonic acid contents in fat tissue and BMI has been reported (SAVVA *et al*., 2004), as has a negative relationship between stearic acid concentrations and BMI (KUNEŠOVÁ *et al*., 2012). However, our results did not indicate that the concentration of this fatty acid was associated with any of the assessed anthropometric parameters. Fatty acids have chemical characteristics that contribute to their diversity, including the length of their carbon chains, number of double bonds in their carbon chains and the placement of these double bonds. These structural differences have been shown to influence the oxidation rate of fatty acids (DELANY *et al*., 2000). An ecological study that evaluated data from 168 countries noted an inverse relationship between the availability of monounsaturated fatty acids (MUFAs) and weight gain, whereas saturated fatty acids (SFAs) and polyunsaturated (PUFAs) have been associated with the prevalence of obesity. Although studies on ecological design could not establish a relationship between the causes and effects of these compounds, some authors have suggested that there may be a higher intake of MUFAs in countries with lower prevalences of obesity prevalences, while the transition from MUFA consumption to PUFA consumption may be closely related to increasing incidences of obesity (MOUSSAVANI, GAVINO, RECEVEUR, 2008).

Other studies support findings of Moussavi, Gavino and Receveur (2008), who reported that concentrations of saturated fats, the most beneficial unsaturated fat for metabolism (especially monounsaturated fats), were associated with increased participation in obesity genesis. These findings were based on measurements of thermogenesis markers induced by diet and the evaluation of fat oxidation after meals (CLEVENGER; STEVENSON; COOPER, 2015; KRISHNAN; COOPER, 2014). Considering that the sample consisted of 31% women over 50 years old who were undergoing hormonal changes, we must consider whether menopause may be related to changes in body fat. Indeed, fat preferentially deposits in the abdomen because of a reduction in estrogen levels (POEHLMAN; TOTH; GARDNER, 1995). Abdominal fat accumulation increases FFA production in the visceral abdominal area, which stimulates triglycerides hepatic synthesis through portal circulation (PITANGA, 2011).

Conclusions

Finally, the data indicated that fatty acid composition is related to anthropometric parameters, as evidenced by differences in the concentrations and measures of association. Thereby, they act on an organism's metabolism and may influence the development of obesity; thus, recommendations have been created as strategies to help treat overweight subjects.

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