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Increased resting metabolism in neurofibromatosis type 1

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SUMMARY

Background & aims: Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disease that is characterized by neurocutaneous changes with multisystem involvement. A previous study with adults with NF1 revealed that changes in total energy expenditure were related to food consumption and body composition. Resting energy expenditure (REE), a measure of energy that the body expends to maintain vital functions, has not been assessed in NF1 populations. This study aimed to assess REE in individuals with NF1 using indirect calorimetry (IC) and evaluate its correlation with body composition and muscle strength.

Methods: Twenty-six adults with NF1 (14 men) aged 18–45 years underwent IC for assessing REE, respiratory quotient (RQ), and substrate utilization. Body composition was assessed by dual energy X-ray absorptiometry. Weight, height, and waist circumference (WC) were also measured. Maximum muscular strength (Smax) was measured by handgrip test using a dynamometer. Patients in the NF1 group were compared to 26 healthy controls in the control group, who were matched by sex, age, body mass index (BMI), and physical activity level.

Results: There were no differences in weight, WC, fat mass, and body fat percentage (BFP). Appendicular lean mass (ALM) adjusted by BMI (ALM_{BMI}) (0.828 ± 0.161 versus 0.743 ± 0.190; $P = 0.048$) and Smax (37.5 ± 10.6 versus 31.1 ± 12.2; $P = 0.035$) was lower in the NF1 group than in the control group. No differences in body composition, strength, and anthropometric parameters were observed in men, but women with NF1 presented lower body surface area (BSA), lean body mass (LBM), ALM, ALM_{BMI}, and Smax. REE adjusted by weight, LBM, or ALM was higher in the NF1 group than in the control group (medians, 21.9 versus 26.3, $P = 0.046$; 36.5 versus 41.1, $P = 0.012$; and 82.3 versus 92.4, $P = 0.006$, respectively), and these differences were observed only among women. RQ was lower in the NF1 group than in the control group (0.9 ± 0.1 versus 0.8 ± 0.1; $P = 0.008$), revealing that individuals with NF1 oxidized more lipids and fewer carbohydrates than controls. REE correlated negatively with BFP and positively with weight, height, BMI, WC, BSA, LBM, ALM, ALM_{BMI}, bone mineral content, and Smax.

Conclusions: Individuals with NF1, particularly women, presented with increased REE (adjusted by weight, LBM, or ALM) and lower RQ compared to healthy controls. These findings were associated with lower ALM_{BMI} and Smax, possibly indicating premature sarcopenia in this population. Further investigation concerning energy metabolism in NF1 and gender differences may be helpful in explaining underlying mechanisms of these changes.

1. Introduction

Neurofibromatoses are a group of genetic diseases characterized by cutaneous symptoms and multiple neural tumors [1]. Neurofibromatosis type 1 (NF1) is the most prevalent disease form and is

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Abbreviations

NF1	Neurofibromatosis type 1
NIH	National Institute of Health
REE	Resting energy expenditure
RMR	Resting metabolic rate
FFM	Fat free mass
BMI	Body mass index
VO_2	Oxygen consumption
VCO_2	Carbon dioxide production
DXA	Dual energy X-ray absorptiometry
LBM	Lean body mass
FM	Fat mass
BMC	Bone mineral content
BFP	Body fat percentage
BFI	Body fat index
ALM	Appendicular lean body mass
WC	Waist circumference
BSA	Body surface area
Smax	Maximum muscle strength

caused by inherited or *de novo* mutations of chromosome 17, resulting in reduced neurofibromin synthesis, which subsequently reduces tumor suppression [1]. The diagnostic criteria for NF1 are almost exclusively clinical and were established by National Institute of Health (NIH) consensus [2]. Although the clinical manifestations of NF1 are well established in the literature, the nutritional aspects associated with this disease have only recently been studied.

Souza et al. [3] published a cross-sectional study of 60 individuals with NF1 (29 men and 31 women) whose ages ranged from 18 to 64 years and evaluated the nutritional status and nutrient intake of this population. This research used a predictive equation to investigate total energy expenditure and showed that 71.7% of patients in the study consumed less calories than their required needs [3]. One of the possible explanations was that predictive equations commonly used in scientific studies may over- or underestimate the energy requirement in individuals with NF1 because these equations were developed for a different study population. In addition, considering that individuals with NF1 are smaller in size [4–6] and may present reduced muscle mass [5,7] and muscle strength [8,9], our initial hypothesis was that resting energy expenditure (REE) in these individuals may be lower.

A recent search of the MEDLINE, SCOPUS, Lilacs, and SciELO databases did not identify any studies regarding REE in NF1 patients. REE or resting metabolic rate (RMR) represents the energy expended by the body to maintain vital functions, including the cardiovascular and respiratory systems along with thermoregulatory mechanisms used to maintain body temperature. REE corresponds to approximately 60%–75% of total energy expenditure in sedentary individuals [10–12].

REE has a large intra- or interpersonal variation depending on body size, body composition, energy balance, age, sex, and genetics [13]. Fat free mass (FFM) is reported as the strongest determinant of REE variability [14]. Part of this variation in REE is not explained by differences in body composition, gender, or age. Other factors, such as circulating leptin levels or pathological conditions, including hypo- or hyperthyroidism, are also associated with changes in REE [13–15].

Indirect calorimetry is the gold standard for REE evaluation and is a non-invasive method that evaluates REE by analyzing oxygen (O_2) and carbon dioxide (CO_2) gases from nutrient metabolism. To calculate the total amount of energy required, this methods assumes that all the O_2 consumed is used to oxidize energy substrates and that all the CO_2 produced is eliminated by respiration [11,12,14]. Thus, using gold standard methodologies for REE and body composition evaluation, the present study aimed to investigate REE in NF1 individuals and verify its associations with body composition and muscle strength.

2. Materials and methods

2.1. Ethical statement

The study was approved by the Ethics Committee of the Federal University of Minas Gerais (#776.524) and all patients provided written informed consent.

2.2. Patient population

This case-control study included NF1 individuals ≥18 years old who were evaluated in a Brazilian Neurofibromatosis outpatient reference center. The NF1 group was compared to unaffected controls (1:1), matched by sex, age, body mass index (BMI), and physical activity level. Patients were excluded based on musculoskeletal limitations, use of medications that might compromise nutritional assessment, the presence of diseases that required a specific diet or food consumption, malignant lesions, hypothyroidism or weight loss ≥10% in the last six months. Also excluded were men over 50 years old and postmenopausal women that presented with the possibility of osteoporosis diagnosis. The sample values were calculated using reference values of REE and standard deviations found in a pre-test performed with ten individuals from each study group. The pre-test had a combined standard deviation between groups of 488 kcal for REE. For this study, we decided to consider a minimum significant difference of 400 kcal and a power of 80%, requiring a minimum of 24 individuals in each group.

2.3. Data collection

Indirect calorimetry was used to evaluate REE. A Quark RMR® open circuit calorimeter (Cosmed, Rome, Italy) was used for this analysis using the canopy system. All individuals had an alimentary fast of at least 5 h [12,16,17], including food, water, and other liquids. Water fasts were necessary because of the evaluation of body water using electrical bioimpedance. As part of the protocol, individuals were asked to not perform physical activities for 24 h prior to the test and not smoke or consume caffeine or stimulants 4 h before the test [12,17]. The calorimeter was switched on for at least 15 min prior to calibration for heating and stabilization tests. All quality parameters recommended by the manufacturer were evaluated and guaranteed before each examination. The tests were performed in the same room in a quiet environment with uniform temperature (23–25 °C) to prevent changes due to cold, heat, or anxiety. Individuals were placed in the supine position for at least 15 min before starting the test [12,17,18]. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were continuously evaluated for approximately 20 min, with data recorded every 5 s. The first five minutes were disregarded to ensure adequate acclimatization, and the mean of the last 15 min was considered in the analysis. The individuals were instructed not to talk or sleep during the evaluation, as well as to avoid yawning, coughing, or agitation [12,17].

The VO_2 and VCO_2 values provided by the equipment were used to calculate REE according to the Weir Equation [19], without using urinary nitrogen levels, usually taken from the equation, because they correspond to <4% of the actual energy expenditure and

contribute to a small error of 1%–2% in the calculation of energy expenditure [17]:

Weir Equation[19]: $REE = (3.9 \times VO_2) + (1.1 \times VCO_2) \times 1.44$

In addition, VO_2 and VCO_2 were also used to calculate the respiratory quotient (RQ) and carbohydrate and fat oxidation per minute (g/min) [12,17]:

$RQ = VCO_2/VO_2$

$\text{Glucose oxidation (g/min)} = (4.585 \times VCO_2) - (3.226 \times VO_2)$

$\text{Lipid oxidation (g/min)} = (1.695 \times VO_2) - (1.701 \times VCO_2)$

Body composition was assessed by dual energy X-ray absorptiometry (DXA) using the Discovery W Hologic® device (Bedford, MA, USA), software version 3.3.0. The results were interpreted by a qualified professional. Total body measurements lasted 6 min, and were performed while individuals were laying in the supine position and with removal of all metal fittings, as recommended by the manufacturer. The room was equipped with air conditioning, and the room temperature was constant during all examinations [20,21]. Lean body mass (LBM), fat mass (FM), bone mineral content (BMC), and body fat percentage (BFP) were used in this study. Body fat index (BFI) was calculated by dividing the fat mass by the squared height [21]. Appendicular lean mass (ALM) was calculated by adding the values of lean mass of arms and legs provided by the DXA, not including the BMC of these regions. ALM was also adjusted by BMI as proposed in previous sarcopenia studies [22].

Anthropometric measures were also evaluated to characterize our study population, including weight, height, waist circumference (WC), and body surface area (BSA). The anthropometric measurements followed the protocol proposed by the WHO [23,24]. The BMI categories used in this study were normal weight (BMI 18.5–25 kg/m^2), underweight (BMI <18.5 kg/m^2), and overweight (BMI ≥25.0 kg/m^2) [23]. BSA were evaluated by DuBois & DuBois predictive equation [25]. Physical activity level were evaluated using the validated International Physical Activity Questionnaire (IPAQ) short version.

Maximum muscle strength (Smax) was measured by handgrip test using the JAMAR® Plus$^+$ digital dynamometer (Asimow Engineering Co., Los Angeles, CA, USA). Three standard measures of Smax (right and left hand alternately) were evaluated, and the mean Smax values were used [8].

2.4. Statistical analyses

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS®) version 19.0 for Windows (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to evaluate normality and determine the appropriate statistical test. Quantitative variables with normal distribution were expressed as means with standard deviation, and compared using the paired Student's *t*-test. Quantitative variables that were not normally distributed were presented as median with minimum and maximum, and compared using the nonparametric Wilcoxon test. Correlations were evaluated by Pearson test for normal distribution or Spearman test for not normal distribution. *P*-values <0.05 were considered statistically significant.

3. Results

Twenty-nine individuals with NF1 were recruited for this study and three were excluded based on the exclusion criteria (one male aged 51 years with hypothyroidism, and two postmenopausal women aged 55 and 57 years). Twenty-six individuals were included in NF1 group, 14 (53.8%) men and 12 (46.2%) women. The NF1 group was compared to 26 unaffected controls, matched by sex, age, BMI, and physical activity level.

Using the BMI categories, there were no differences between the two groups ($P = 0.354$). In the NF1 group, 3 (11.5%) of the 26 patients were classified as underweight, 16 (61.6%) were normal weight, and 7 (26.9%) were overweight. In control group, 2 (7.7%) were classified as underweight, 14 (53.8%) were normal weight, and 10 (38.5%) were overweight.

Demographic, anthropometric, body composition, and muscle strength data are listed in Table 1. There were no differences for age, weight, BMI, WC, BSA, FM, and BFP. The NF1 group showed lower height ($P = 0.003$), BMC ($P = 0.046$), and Smax ($P = 0.035$). ALM_{BMI} values were also lower in NF1 group (0.828 ± 0.161; 0.743 ± 0.190, $P = 0.048$). There were no differences between men with NF1 and controls for anthropometric, strength, body composition data, and differences were observed only in women with NF1 (Table 1).

Using electrical bioimpedance to estimate total body water, there were no differences between the NF1 and control group (11.83 ± 3.03 versus 12.76 ± 2.76 L, $P = 0.254$) or between genders. Among women only, REE values were higher in the NF1 group than in the control group when adjusted by weight ($P = 0.046$), LBM ($P = 0.012$), and ALM ($P = 0.006$) (Table 2). There were no differences between men for indirect calorimetry parameters. RQ was lower in the NF1 group, showing that individuals with NF1 oxidized more lipids and fewer carbohydrates than controls (Table 2).

The main variables of anthropometry, body composition, muscle strength and food intake were evaluated to verify their correlation with REE (Table 3). REE presented a positive correlation with weight, height, BMI, WC, BSA, LBM, BMC, ALM, ALM_{BMI}, and Smax and a negative correlation with BFP.

4. Discussion

In our study, only women with NF1 had higher REE compared to health controls, when adjusted for weight, LBM and ALM. No differences were observed for men. In addition, there was a difference in the use of energy substrates (lower RQ in the NF1 group), such that individuals with NF1 oxidized more fat and less carbohydrates when compared to the control group. This study also confirmed characteristics previously reported in NF1 individuals including lower stature, muscle strength, and lean mass. For this study, we used the gold standard methods for body composition (DXA) and resting energy expenditure (indirect calorimetry).

Our initial hypothesis was that REE was lower in individuals with NF1 because are smaller in size (lower weight, shorter stature) and have lower muscle mass [4–7]. REE is influenced by factors such as muscle mass, FM, weight, age, sex, adipokine levels including leptin and adiponectin, or metabolic changes in pathological processes, including hypo- or hyperthyroidism [13–15]. This study demonstrated increased REE in individuals with NF1 after adjustment for weight, LBM, or ALM. This increase in REE is similar to that observed in the study by Leoni et al. [26] who examined patients with Costello syndrome, also a RASopathy, and observed an increase in REE in individuals affected by the disease. In Costello syndrome, the authors related increased REE with growth deficits observed in this population, when adjusted for body weight or BSA [26].

The causes for this increase in REE in NF1 need to be further investigated. Among the factors previously described, there were no differences in weight, age, sex, and FM in our study. In addition, thyroid dysfunction was an exclusion criteria for this study. Muscle mass also did not explain this finding, since the ALM_{BMI} was lower

Table 1
Demographic, anthropometric, body composition, and muscle strength characteristics.

Parameters	All (n = 52)			Male (n = 28)			Female (n = 24)		
	Control (n = 26)	NF1 (n = 26)	P value*	Control (n = 14)	NF1 (n = 14)	P value*	Control (n = 12)	NF1 (n = 12)	P value*
Age (years)	32.92 ± 6.14	34.31 ± 6.05	0.316	33.79 ± 5.19	35.50 ± 5.71	0.372	31.92 ± 7.19	32.92 ± 6.39	0.678
Weight (kg)	69.08 ± 14.11	62.54 ± 16.99	0.175	75.98 ± 13.83	70.40 ± 16.78	0.391	61.02 ± 9.74	53.37 ± 12.37	0.141
Height (m)	1.68 ± 0.08	1.61 ± 0.10	**0.003**	1.72 ± 0.08	1.67 ± 0.07	0.056	1.63 ± 0.06	1.54 ± 0.06	**0.003**
BMI (kg/m^2)	24.28 ± 3.64	23.88 ± 4.83	0.768	25.44 ± 3.92	25.00 ± 4.89	0.819	22.91 ± 2.86	22.57 ± 4.61	0.817
Waist circumference (cm)	82.37 ± 11.43	81.39 ± 14.62	0.807	87.16 ± 11.51	86.42 ± 14.64	0.894	76.78 ± 8.78	75.51 ± 12.75	0.778
Body surface area (m^2)	1.78 ± 0.21	1.65 ± 0.24	0.054	1.89 ± 0.19	1.79 ± 0.21	0.209	1.65 ± 0.15	1.49 ± 0.16	**0.040**
Body fat percentage (%)	31.97 ± 8.41	31.59 ± 8.97	0.878	27.03 ± 6.39	26.63 ± 7.28	0.886	37.73 ± 6.71	37.38 ± 7.21	0.904
Fat mass (kg)	22.19 ± 7.58	20.02 ± 8.74	0.417	21.07 ± 7.93	19.58 ± 9.39	0.691	23.50 ± 7.26	20.53 ± 8.30	0.391
Lean body mass (kg)#	44.62 (30.55–64.48)	40.80 (24.34–69.19)	0.062	53.24 (34.77–64.48)	46.90 (37.44–69.19)	0.221	35.72 (30.55–43.82)	30.56 (24.34–41.91)	**0.041**
ALM (kg)#	19.99 (13.38–28.47)	18.25 (9.88–29.74)	0.062	25.00 (15.26–28.47)	21.30 (16.85–29.74)	0.158	15.95 (13.38–20.20)	12.60 (9.88–18.99)	**0.028**
ALM adjusted for BMI	0.828 ± 0.161	0.743 ± 0.190	**0.048**	0.944 ± 0.113	0.881 ± 0.132	0.169	0.693 ± 0.083	0.584 ± 0.099	0.007
Bone mineral content (kg)	2.29 ± 0.43	2.03 ± 0.47	**0.046**	2.56 ± 0.37	2.28 ± 0.39	0.114	1.98 ± 0.25	1.73 ± 0.36	0.052
Smax (kg)	37.47 ± 10.66	31.09 ± 12.20	**0.035**	44.48 ± 9.64	40.20 ± 8.19	0.185	29.29 ± 3.64	20.46 ± 5.50	**0.001**

Note: #: values expressed as median (minimum-maximum). All others are expressed as mean ± SD; SD: standard deviation; BMI: body mass index; ALM: appendicular lean mass; Smax: maximum muscle strength; kg: kilogram; m: meter; cm: centimeter; *Means were compared using paired Student's *t*-test and medians using Wilcoxon test.
Bold represents $p < 0.05$.

Table 2
Resting energy expenditure, macronutrient oxidation, and gas exchange volumes.

Parameters	All (n = 52)			Male (n = 28)			Female (n = 24)		
	Control (n = 26)	NF1 (n = 26)	P value*	Control (n = 14)	NF1 (n = 14)	P value*	Control (n = 12)	NF1 (n = 12)	P value*
REE (kcal/d)#	1529.5 (657.2–2516.5)	1656.6 (623.8–2958.2)	0.990	1847.4 (1495.5–2516.5)	1757.6 (1539.9–2958.2)	0.683	1305.0 (657.2–1449.4)	1352.6 (623.8–1979.6)	0.754
REE adj. for weight (kcal/kg/d)#	21.9 (10.4–36.4)	26.3 (12.3–36.0)	**0.046**	24.7 (20.1–36.4)	26.3 (22.9–36.0)	0.363	19.7 (10.4–28.9)	26.5 (12.3–31.2)	**0.028**
REE adj. for LBM (kcal/kg/d)#	36.5 (19.0–45.9)	41.1 (20.1–50.4)	**0.012**	36.2 (30.4–45.9)	38.05 (33.2–45.1)	0.221	36.8 (19.0–42.2)	44.5 (20.1–50.4)	**0.023**
REE adj. for ALM (kcal/kg/d)#	82.3 (41.6–98,0)	92.4 (49.5–132.7)	**0.006**	80.6 (64.7–98.0)	90.4 (70.4–108.2)	0.177	83.0 (41.6–94.0)	102.9 (49.5–132.7)	**0.019**
VO_2 (L/min)#	0.211 (0.097–0.357)	0.236 (0.090–0.425)	0.949	0.258 (0.209–0.357)	0.254 (0.211–0.425)	0.638	0.184 (0.097–0.206)	0.194 (0.090–0.284)	0.638
VCO_2 (L/min)#	0.194 (0.067–0.308)	0.198 (0.071–0.343)	0.525	0.246 (0.180–0.308)	0.218 (0.194–0.343)	0.235	0.161 (0.067–0.182)	0.159 (0.071–0.231)	0.875
RQ	0.90 ± 0.10	0.84 ± 0.08	**0.008**	0.91 ± 0.09	0.87 ± 0.06	0.163	0.88 ± 0.10	0.82 ± 0.08	0.102
Glucose oxidation (g/min)	0.20 ± 0.09	0.15 ± 0.09	**0.029**	0.25 ± 0.08	0.20 ± 0.07	0.087	0.15 ± 0.08	0.10 ± 0.08	0.184
Fat oxidation (g/min)	0.04 ± 0.04	0.06 ± 0.03	**0.011**	0.04 ± 0.04	0.06 ± 0.03	0.255	0.03 ± 0.03	0.06 ± 0.03	**0.031**

Note: #: values expressed as median (minimum-maximum). All others are expressed as mean ± SD; SD: standard deviation; REE: resting energy expenditure; LBM: lean body mass; ALM: appendicular lean mass; VO2: oxygen uptake; VCO2: carbon dioxide output; RQ: respiratory quotient; kg: kilogram; d: day; kcal: kilocalorie; L: liter; min: minute; *Means were compared using paired Student's *t*-test and medians using Wilcoxon test.
Bold represents $p < 0.05$.

Table 3
Correlations between resting energy expenditure and study variables.

Parameters	Control (n = 26)		NF1 (n = 26)	
	R	P value	R	P value
Weight (kg)	0.672[a]	**<0.001**	0.864[a]	**<0.001**
Height (m)	0.660[a]	**<0.001**	0.653[a]	**<0.001**
BMI (kg/m^2)	0.492[a]	**0.011**	0.730[a]	**<0.001**
Waist circumference (cm)	0.622[a]	**0.001**	0.758[a]	**<0.001**
Body surface area (m^2)	0.699[a]	**<0.001**	0.857[a]	**<0.001**
Body fat percentage (%)	−0.497[a]	**0.010**	−0.124[a]	0.546
Fat mass (kg)	0.029[a]	0.889	0.514[a]	**0.007**
Lean body mass (kg)	0.881[b]	**<0.001**	0.885[a]	**<0.001**
Bone mineral content (kg)	0.758[a]	**<0.001**	0.772[a]	**<0.001**
ALM (kg)#	0.849[b]	**<0.001**	0.854[a]	**<0.001**
ALM adjusted for BMI	0.731[a]	**<0.001**	0.460[a]	**0.018**
S_{max} (kg)	0.620[a]	**0.001**	0.626[a]	**0.001**

Note: a: Pearson correlation; b: Spearman correlation; REE: resting energy expenditure; BMI: body mass index; ALM: appendicular lean mass; Smax: maximum muscle strength; kg: kilogram; m: meter; cm: centimeter.
Bold represents $p < 0.05$.

in NF1 and therefore should reduce REE, not increase it, as was observed. ALM represents skeletal muscle mass, which is different from LBM or FFM that also includes metabolically active organs and body water, respectively [22,27].

One possible explanation of these findings would be a difference in adipokine concentrations, and this association needs to be further investigated. Although measurement of adipokine levels was not performed in our study, Martins et al. [6] demonstrated higher plasma adiponectin levels in the NF1 group than in the control group, along with lower levels of leptin and vifastine. There is no consensus for the interference of leptin levels in REE, so this relationship also needs to be better investigated [15,28]. For adiponectin, an adipokine with mitochondrial action, studies are controversial. Some studies of diseases other than NF1 found a positive association between adiponectin levels and energy expenditure [28–30]. Other studies, however, did not prove this finding [31].

Obesity, metabolic syndrome, and type 2 diabetes are associated with chronic low grade inflammation and reduced insulin sensitivity. Due to inflammation, there is an increased energy cost, even though minimal, probably because of the activation of immune responses and alteration of mitochondrial activity [32]. The relationship between inflammation and increased REE may be another possible mechanism to explain the differences in REE in the NF1 group. This relationship has not yet been described in NF1 individuals. In our study, an evaluation of markers of inflammation was not performed. Liao et al. [33] evaluated the contribution of inflammation and tumor microenvironments in neurofibroma formation and suggested that preventing inflammation and possibly neural damage may be a therapeutic approach in controlling neurofibroma growth [33]. There is a possibility that the growth of benign tumors may consume more energy similar to cancer states, which needs to be better investigated [34,35].

The most likely hypothesis to explain the REE of NF1 patients may be related to the activation of RAS. Growth factors interact with receptors on the cell surface, activating guanine nucleotide exchange factors, which results in RAS activation. This activation sends intracellular signals to activate the phosphatidylinositol-3-kinase, AkT, and mTOR pathways. Normally, neurofibromin reduces the levels of activated RAS [36]. Because neurofibromin is absent or reduced in NF1, activated RAS increases signaling for all these pathways, resulting in cell proliferation and inhibition of apoptosis, which are associated with tumorigenesis and could contribute to this observed increase in energy expenditure [36]. This mechanism was also proposed in a study by Leoni et al. [26] in patients with Costello syndrome. Recently, Dard et al. [37] discussed the role of RAS in energy metabolism in rare human diseases, including NF1. According to the authors, RAS can interfere in energy control, but in NF1 it is still difficult to define the impact of the disease on energy metabolism and mitochondrial function as a function of the conflicting results in studies with different models [37].

In NF1, the differences between men and women also need to be better investigated. For the main variables of our research, men and women with NF1 showed different responses. Women with the disease presented lower stature, BSA, LBM, ALM, ALM_{BMI} and Smax when compared with control women. In addition, REE adjusted for weight, LBM and ALM was higher among women with NF1 compared to the control group. No differences were observed in these variables between men. In NF1, it is known that neurofibromas tend to start growing at puberty, and the number and size can increase during pregnancy, suggesting a possible hormonal influence [38–41]. One of our hypotheses, as previously discussed, is that neurofibroma growth may contribute to the increase in REE observed. If the hormonal profile can contribute to this growth, an area that needs to be better investigated in NF1, this relationship also needs to be evaluated in further studies.

The observed changes in the use of energy substrates also requires attention. Individuals with NF1 oxidized more lipids and less carbohydrates than healthy controls. This higher oxidation of lipids explains the lower respiratory quotient in these individuals [12,42]. Oxidation of lipids in the muscle has received attention from an Australian research group, that developed an animal model to assess muscle aspects of NF1 and observed an accumulation of intramyocellular lipids in young rats, speculating that NF1 may play a key role in the regulation of lipid metabolism [43,44]. Summers et al. [9] observed this accumulation of intramuscular lipid in muscle biopsies of six children with NF1 and in an animal model, suggesting a possible mitochondrial dysfunction that may compromise muscle strength in NF1.

These changes in lipid metabolism are usually associated with muscular changes in NF1 (weakness, reduced muscle mass, and loss of function) [9,43,44]. In the present study, the Smax was lower in the NF1 group than in the control group, confirming the findings of previous studies [8,45–47]. The observed lower ALM_{BMI} associated with reduced Smax in the NF1 group may indicate an early sarcopenia in these individuals. The definition of sarcopenia involves not only reduced lean body mass but also reduced muscle strength and/or function [22,27]. Although muscle function was not evaluated in this study, Souza et al. [48] demonstrated lower physical fitness in NF1 and shorter distances walked in the 6-min walk test. Thus, based on sarcopenia classification, individuals with NF1 need to receive special attention once they have changes in lean mass, strength and muscle function. Multidisciplinary interventions may be used in NF1 individuals because sarcopenia increases the risk of physical inactivity and poor quality of life and may be related to sedentary lifestyle, poor diet, aging, insulin resistance, mitochondrial or endocrine changes, and possibly increased morbidity and mortality [22,27].

5. Conclusions

Individuals with NF1 presented increased REE (adjusted by weight, lean body mass, or appendicular lean mass), and lower RQ when compared to controls. Furthermore, these findings were associated with lower ALM_{BMI} and Smax, which may indicate premature sarcopenia in this patient population. Body composition, strength and REE were different only among women with NF1. Further investigations, concerning energy metabolism in NF1 and gender differences, would provide promising means to explain the mechanisms involved in these findings.

Statement of authorship

SOUZA MLR (conceptualization, data curation, formal analysis, investigation, methodolody, writing – original draft, review and editing); JANSEN AK (conceptualization, data curation, formal analysis, investigation, methodolody, writing – original draft, review and editing, supervision); RODRIGUES LOC (conceptualization, methodolody, writing – original draft, review and editing); VILELA DLS (data curation, investigation, methodolody, writing – original draft), KAKEHASI AM (data curation, formal analysis, investigation, methodolody, writing – original draft), MARTINS AS (conceptualization, methodolody, writing – original draft), SOUZA JF (conceptualization, methodolody, writing – original draft), REZENDE NA (conceptualization, data curation, formal analysis, funding acquisition, investigation, methodolody, writing – original draft, review and editing, supervision).

Conflicts of interest

The authors declare no conflicts of interest in this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2019.05.006.

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