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Examining the roles of body composition, energy expenditure and substrate metabolism in the control of daily energy intake in adolescents with obesity

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Abstract

BACKGROUND: The implication of body composition and energy metabolism in the control of human energy intake (EI) has been well described in adults, remaining however unexplored in adolescents with obesity. The aim of this study was to question the role of body composition, energy expenditure (EE) and substrate metabolism in the control of EI of adolescents with obesity. METHODS: Ad libitum 24-h EI, body composition (Dual X-ray absorptiometry), Resting Metabolic Rate (RMR, indirect calorimeter) where measured and Total EE obtained during a 36-h stay in metabolic chambers in 26 adolescents (14.1 ± 1.5 years; 14 girls) with severe obesity. RESULTS: The mean body weight and Body Mass index were 92.2 ± 17.2 kg and 33.98 ± 4.14 kg.m-2 respectively. 24-h EI was positively correlated with body weight (rho = 0.597, p = 0.014), Fat Free Mass (FFM) kg (rho = 0.576, p = 0.019), 24-h Total EE (TEE (rho =0.675, p < 0.001)), RMR (rho =0.632, p = 0.005), 24-h Carbohydrate (CHO) oxidation rates (rho = 0.716, p < 0.001), and urinary nitrogen excretion (rho =0.28, p < 0.001). According to the path analysis FFM (kg) but not Fat Mass (FM) (kg) was positively correlated with RMR, with direct effects of 0.87 (p < 0.001) and 0.027 (p = 0.74) respectively. The effect of FFM on 24-h EI was mediated by RMR (96% of the effect), while the effect of FM on 24-h EI was also mediated by RMR (67% of the effect). CONCLUSIONS: The present study provides the first evidence regarding the role of RMR as a main tonic signal of appetite control mediating the effect of body composition and mainly FFM (over FM) on daily EI in adolescents with obesity. It also suggests for the first-time relationships between 24-h CHO and protein oxidation and daily EI in this population

INTRODUCTION

The continuous development of overweight, obesity and of their related complications emphasizes the importance of research developing a better understanding of the complexities of energy balance regulation. While significant attention has been given to the role of fat mass (FM) and associated signals such as leptin as key inhibitory-signals of energy intake (EI) [1–4], the ease in which excess adiposity can accumulate indicates a lack of strong negative feedback from adipose tissue on EI to prevent weight gain. Fat-free mass (FFM), through its metabolic activity, has been proposed to exert a long-term drive to eat. Indeed, as demonstrated in various samples from young to older adults, FFM appears to generate a tonic drive to eat in order to ensure the energetic needs of key tissue-organs and processes are met [5–8]. This effect of FFM on EI reinforces the idea of a relationship between energy expenditure (EE) and EI, and there are growing evidence regarding this relationship between FFM (but not FM) and individuals' daily EI, ad libitum meal intake and subjective hunger under both controlled laboratory and free-living conditions [9–13]. Importantly, this effect of FFM on EI has been shown to be mediated by the resting metabolic rate (RMR) and total EE (TEE) [14–16]. Indeed, RMR, as the main component of TEE, is strongly determined by FFM, suggesting that EE is an important factor influencing human's daily food intake. These relationships have been demonstrated in a large number of individuals including adults with leanness and obesity, new born babies [17] and elderly people retired from work [18]. The relationships may not apply during adolescence which is a time of rapid development with changes in body composition and complex endocrine adjustments. While the associations between FFM and EI have been replicated in numerous studies in adult

individuals, Cameron et al. is the only study to date to examine these associations in adolescents with obesity showing that while RMR, FM and FFM were positively correlated with the adolescents' daily EI, only FFM (and skeletal muscle mass) remained a significant predictor of food intake after adjusting for age, sex, height, and physical activity level [19]. In addition to the relationships noted above, the association between substrate oxidation and daily food intake is not resolved. In their work conducted in Pima Indians adults, Pannacciulli et al. reported 24-h respiratory quotient (RQ), which is indicative of the overall substrate oxidation not considering the oxidation of proteins, as a positive predictor of ad libitum daily EI (independent of race, sex, age, FM% and 24-h energy balance) [20]. Moreover, while non-protein RQ is often measured due to the methodological difficulties of assessing the protein contribution to TEE, the proportion or absolute amount of energy mobilized from proteins may influence appetite and energy balance regulation during weight variation periods, according to data from the seminal Minnesota study [21] and more recently from adolescents with obesity [22]. Importantly, studying such relationships between body composition, daily EE, substrate oxidation and food intake requires the use of precise and fine measurement tools. In that context, the aim of the present analysis is to examine the relationships between body composition, resting and 24-h EE, 24- h substrate oxidation and ad libitum 24-h EI in adolescents with obesity, taking advantage of the highly controlled MUST study in which whole-room calorimetry was used. An innovative aspect of the current study is the inclusion of measures of substrate oxidation to complement and extend the relationships between EI, body composition and RMR. These measures add an additional level of explanation to the relationship between physiology and eating behavior.

MATERIALS & METHODS

Participants

Twenty-six adolescents (14.1 ± 1.5 years; 14 girls) with severe obesity (according to international curves [23]), recruited from an inpatient pediatric obesity center (Child Medical Center of Romagnat, France) were enrolled in the study. To be included, the adolescents had to: (i) be aged between 12 and 16 y, (ii) present a diagnosis of obesity, in compliance with applicable national criteria, (iii) present a Tanner 2-4, (iv) be free of any contraindication to physical exercise, (v) have no history of weight loss for at least the last 12 months. None of the participants had evidence of significant disease, non-insulin-dependent diabetes mellitus or other endocrine disease, and none were taking medications regularly or any medication known to influence energy metabolism or appetite control. All experiments were conducted in full compliance with the Declaration of Helsinki, and written informed consent was obtained from all adolescents and their legal guardians. The study was approved by the University Ethical Committee on Human Research for Medical Sciences (AU # 361).

Study design

As part of the MUST (Multidisciplinary Strategy for the Treatment of obesity) study, the adolescents were part of a 9-month inpatient multidisciplinary study performed at the local Pediatric Obesity Center (Romagnat France). The present work is a secondary analysis using the baseline energetic and nutritional evaluation from the MUST project. This project has previously been described in detail elsewhere [24–26]. Briefly, baseline data from all the participants are considered in the present analysis: ad libitum EI, body composition, RMR as well as TEE and nitrogen excretion obtained during a 36-h indirect calorimetric chamber session. The Fig. 1 illustrates the design of the study.

Measurements

Anthropometric and body composition. Body weight was measured in the fasted state to the nearest 0.1 kg using a calibrated manual weighing scale (SECA 709, Germany), and body height was measured barefoot to the nearest 0.1 cm by using a wall-mountedstadiometer (SECA, Hamburg, Germany). The body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). Dual energy X-ray absorptiometry (QDR 4500 A scanner, Hologic, Waltham, MA, USA) was used to examine the adolescents' FM (%FM and kg) and FFM (kg).

Resting metabolic rate (RMR). Resting metabolic rate was assessed, in semi-supine position after an overnight fast, for 45 min using an open-circuit, indirect computerized calorimetry (Deltatrac calorimeter; InstrumentariumOy, Datex Division, Helsinki, Finland) with a rigid, transparent, ventilated canopy. Gas analysers were carefully calibrated before each test with a reference gas mixture. Oxygen consumption (VO2) and carbon dioxide production (VCO2), standardized for temperature, barometric pressure, and humidity, were measured continuously.

Total daily energy expenditure. An open-circuit indirect calorimetric chamber was used to continuously measure EE over 36-h (an evening and a night of habituation followed by a 24-h effective measurement period). During their stay, the adolescents followed the same daily routine composed of sleeping, sedentary activities (e.g., watching TV), daily activities (e.g., washing and dressing), meals and daily physical activities such as walking, as previously described [24]. Gas exchanges were computed from outlet air flow, differences in gas concentration between air entering and leaving the calorimeter, atmospheric pressure, air temperature, and hydrometry after correction for the drift and time of response of the gas analysers and the variations of the volumes of carbon dioxide and oxygen in the calorimeters, according to the RICORS 1.0 guidelines [27]. Total EE was calculated from VO2 and VCO2 using Brouwer's equation [28].

Substrate oxidation and urinary nitrogen excretion. Respiratory quotient was calculated as VCO2/VO2. Carbohydrate and fat oxidation were calculated from gas exchanges and urinary nitrogen excretion using Ferrannini's equations [29], during 24 h, sleep (between midnight and 0600 h), sedentary activities (except meals) and physical activities (walking). Urine was collected over the 24-h of effective measurement in the calorimetric chamber (starting at 0700 h) for the determination of urinary nitrogen excretion as a proxy of whole-body amino acid oxidation. The P ratio, as the fraction of energy mobilized as protein over total energy lost or gained, was then calculated as follow: urinary nitrogen loss (g) × conversion factor for nitrogen (6.25) × energy equivalent for protein (4)/TEE, where TEE is total energy expenditure over 24 h [22].

Energy and macronutrients intake. During the 24-h effective stay in the whole-body metabolic chambers, food was offered ad libitum to reflect the adolescents' food habits and food availability of free-living conditions (as previously detailed [25]). The weight of each food provided and then not eaten were determined over 24 h in the calorimeters using an accurate balance (0.1) g. The adolescents' EI and macronutrient consumption were assessed using GENI software version 4.0 (MICRO 6, Villers les Nancy, France) based on the nutritive value of foods [30].

Statistical analyses

Continuous data are expressed according to their statistical distribution as mean and standard-deviation. The assumption of normality was analyzed using the Shapiro-Wilk test. To examine

the relationships between 24 h ad libitum EI, FFM, FM and RMR, a three-step statistical analysis was performed. First, Spearman correlation coefficients (noted rho) were estimated and were interpreted according to the following classical recommendations: rho<rho0.7: high correlation (Figs. 2 and 3). Secondarily, multivariate analyses using multiple linear regression were performed with age and gender as adjustment covariates. The normality of residuals from these models were analyzed as aforementioned. When appropriate, logarithmic transformation was applied to achieve the normality of the dependent variables. Thirdly, path analysis (Fig. 4) was conducted using structural equation modeling in order to examine the associations between EI, FFM, FM and RMR. More precisely, a model adjusted for age and sex was tested examining whether the associations between body composition (FFM and FM – independent variables) and EI (dependent variable) would be mediated by RMR (mediator variable), as previously reported [14]. Panel equations were elaborated such as EI ←RMR + FFM + FM+sex+age and RMR ← FFM + FM+sex+age. Similar path analysis models (Fig. 5) were performed to examine whether the association between TEE and EI would be mediated by CHO oxidation, Fat oxidation or nitrogen excretion (separate models were conducted due to the reduced sample size). The significance of the regression coefficients and fit statistics were calculated using the maximum likelihood estimation method. The adequacy of the mediation model was based on several recommended goodness of fit indices such as Chi-square, Tucker Lewis Index, Comparative Fit Index, and Root-Mean Square Error of Approximation. The significance of the direct, indirect and total effects was assessed using Chi-Square tests. The proportion of total effect mediated was also estimated as the ratio between indirect to direct effect and total to direct effect [31]. The bootstrap resampling method was further used to test the significance of the mediational paths, using 2000 Bootstrap samples and 95% bias-corrected confidence intervals (CI) around the standardized estimates of the effects. Furthermore, additional path analyses using aforementioned statistical models were applied were also conducted to explore the respective effect of fat oxidation, CHO oxidation and nitrogen excretion on the relationship between TDEE and EI. Analyses were adjusted on age and gender but not on puberty as all the adolescents presented a somehow similar tanner stage [3, 4] and not on their physical activity level that was only assessed though interviews at inclusion and all the adolescents presented a low to very-low physical level. Statistical analyses were performed using the Stata software package (version 15, StataCorp, College Station, US). All statistical tests were carried out for a two-sided type I error at 5%.

RESULTS

The sample had a mean body weight of 92.2 ± 17.2 kg and a mean BMI of 33.98 ± 4.14 kg.m-2. The mean FM% and FFM% of the adolescents were 41.6 ± 4.2% and 58.4 ± 4.2% (mean FFM kg of 53.9 ± 11.4 kg and FM kg of 38.2 ± 7.7 kg). The Table 1 displays the characteristics of the sample. Positive correlations were found between daily EI assessed during the 24-h stay in the metabolic chambers and the adolescents' body weight (rho = 0.597, p = 0.014), FFM kg (rho = 0.576, p = 0.019), 24-h TEE (rho =0.675, p < .001), RMR (rho =0.632, p = 0.005), 24-h CHO oxidation rates (rho = 0.716, p < 0.001), and urinary nitrogen excretion (rho =0.728, p < 0.001). The Fig. 2 present the scatter plots corresponding to the correlations between 24-h EI and the adolescents' body composition, 24-h TEE and RMR as well as daily substrate oxidation and urinary nitrogen excretion. The Table 1 details the energy expenditure and energy intake of the adolescents. The heatmap (Fig. 3) illustrates the correlations between overall EI (24-h EI and the absolute and relative macronutrients' consumption) and the anthropometric, body composition and energy metabolism parameters. The rho coefficients and p values are detailed in the supplementary Table S1. The path analysis to examine the associations between EI, FFM, FM

and RMR showed that FFM (kg) but not FM (kg) was positively correlated with RMR, with direct effects of 0.87 ($p < 0.001$) and 0.027 ($p = 0.74$) respectively. Only RMR presents a direct effect on 24-h EI ($\beta = 0.67$, $p = 0.018$) (Fig. 4). The effect of FFM on 24-h EI was mediated by RMR (accounting for 96% of its effect), while the effect of FM on 24-h EI was also mediated by RMR (accounting for 67% of the effect). The detailed regression analyses (step by step) are detailed in Table S2. According to separated path analysis conducted to separately explore the mediation effect of substrate use between TEE and EI, 43% of the effect of TEE on EI was explained by CHO oxidation, 22% by Fat oxidation and 43% by Nitrogen excretion. Details of the mediation analyses are presented in Table S3. The Fig. 5 presents these mediation models.

DISCUSSION

The present study examined the relationship between body composition, RMR, 24-h EE and substrate oxidation rates, and ad libitum daily food consumption in adolescents with obesity. According to the obtained results, FFM but not FM, was positively associated with daily EI in adolescents with obesity. Importantly, this effect of FFM on daily EI was found to be mediated by RMR. The present results also indicate that both CHO oxidation and nitrogen excretion (but not fat oxidation) are positively associated with daily EI in this population. The present findings are in line with the only previous study in youth with obesity [19]. In their study, Cameron et al. measured body composition and skeletal muscle mass using magnetic resonance imaging among 304 post-pubertal adolescent boys and girls with obesity. According to the authors, body weight, FFM, skeletal muscle mass, and FM were all significantly correlated with self-reported EI (with FFM and skeletal muscle only remaining associated once adjusted for age, sex, height, and physical activity level) [19]. However, the relationships between body size and composition with EI remain debated in lean adolescents, with some studies showing a positive association between FFM and food consumption [32] while others don't [33]. The present findings are also consistent with that observed in lean and adults with overweight and obesity [7, 9, 12, 14, 18]. In particular, it has previously been shown that the effect of FFM on EI was mediated by RMR [14] and TDEE [16]. Interestingly, Piaggi et al. reported in adults that the association between FFM and EI was reduced by about 80% and became non-statistically significant after accounting for 24-h EE [16], which has been later confirmed several times in adults, with mediation models highlighting that the effects of FFM and FM on EI were fully mediated by RMR [15]. In agreement with these studies, the present paper is the first to examine these associations using a mediation model in youths with obesity, confirming that FFM (and FM to a much lesser extent) acts as a driver for daily EI through RMR. According to our results, 96% of the effect of FFM on daily EI was mediated through RMR. Together, these findings reinforce those from Blundell and colleagues proposing RMR as a strong predictor of EI that helps ensure that a sufficient amount of kilocalories is consumed to maintain basic metabolic processes and the preservation of lean body mass [34]. Interestingly, an innovative aspect of the design of the present work and especially the use of metabolic chambers, was the possibility to objectively examine novel relationships between 24- h substrate oxidation and EI in our sample. According to our results, 24-h RQ was not associated with the adolescents' ad libitum EI. While studies are limited, this contradicts with what has been previously observed in healthy adults and adults with overweight or obesity [16, 20]. Importantly however, in the present study 24-h carbohydrate but not fat oxidation was positively associated with daily ad libitum EI, which is in line with data available in adults [16, 20]. In their work conducted in healthy adults, Piaggi and colleagues reported a positive 1% shift in 24-h RQ was associated with an increase in ad libitum food intake of approximately 204 kcal/d, pointing human substrate oxidation as a drive for food intake [16]. Importantly, while energy intake was evaluated during the 24-h stay in the present work, Piaggi

et al. measured ad libitum intake over the three days that followed the 24-h stay in metabolic chamber, using a computerized vending machine system [16]. Interestingly, using Piaggi's model, a positive shift of 1% in RQ seems to be associated with an increased EI of 158 kcal/day within our sample ($p = 0.024$, data not shown). The absence of significant association between EI and both RQ and fat oxidation in the present work might, if confirmed, suggests that CHO oxidation might act as a specific signal independently of the CHO to fat oxidation balance represented by the RQ (since only CHO oxidation appear associated with EI). Such results would be in line with the "glycogenostatic theory" which proposes glycogen availability as a critical determinant of eating behavior [35–37]. According to Flatt, feeding would be designed to maintain the body's glycogen muscle and liver stores at a specific set point, with then any reduction in glycogen acting as an internal biological cue driving EI to restore glycogen levels [36]. Altogether, it highlights the critical role of the CHO metabolism in daily food consumption, likely independently of the CHO to fat utilization balance. Importantly, these potential effects of CHO oxidation on subsequent energy intake must be considered in light of the today clear association between macronutrient intake, and particularly CHO intake, and their subsequent oxidation. Then the hypothesis raised here of the existence of a potentially bidirectional association needs to be further explored. Our results show a strong association between the adolescents 24-h nitrogen excretion and ad libitum food intake. Although the effects of acute protein ingestion or diets high in protein on appetite, food intake and body weight have been studied [38], the potential impact of protein oxidation on the control of appetite and EI remains unclear. In their seminal publications, Millward and Mellinkoff proposed the existence of protein stat and aminostatic theories of appetite [39–41]. According to Millward, there is a requirement for an aminostatic component of appetite regulation in which food intake is adjusted to provide the protein needs for body protein growth or maintenance. This is based on the existence of a protein-stat mechanism [40, 41]. In the present paper positive associations between 24-h EI and the absolute protein consumption was seen. As such, it could be suggested that the positive association seen between nitrogen excretion and EI in the present study is a reflection of (rather than a driver of) protein ingestion. Indeed, it has been stated that "an increased protein intake leads to an autoregulatory increase in its own oxidation as transamination, deamination, and ureogenesis are invoked to remove the excess amino nitrogen" [42]. However, it must be kept in mind that our sample is composed of adolescents whose obesity status and growth and maturation processes will increase protein requirements [43]. According to our results, the average of daily percentage of energy ingested from proteins as well as their ingested quantity per kg, remain below or close to recommended values (13.1 ± 2.4% and 1.03 ± 0.4 g.kg-1.day-1). As such, their protein intake in the chamber was not excessive relative to their protein requirements [43]. This would then suggest that the protein oxidation of the adolescents might not (only) represent a way to auto-regulate overconsumption, but also potentially influence appetite and EI. Although definitely speculative, this could suggest that increased oxidation (reflecting overall protein turnover which is directly in line with lean body mass) could in turn serve as a messenger for proteins needs and then act as a driver for energy and protein intake to meet protein requirements [44]. This is in line with Simpson & Raubenheimer observations on protein leverage concept aiming at reaching a kind of protein target [45]. Interestingly, while both urinary nitrogen excretion and TEE were separately positively associated with daily ad libitum EI, such relation was not observed with the P ratio (which corresponds to the amino acid oxidation, likely primarily from diet protein, contribution to total energy expenditure). This may suggest that when protein oxidation is not considered isolated but related to TEE, the association with EI disappears, highlighting the tight and inter-related regulation between protein metabolism, energy expenditure, and EI. This is in line with the

pathway analysis in the present paper showing that 43% of the association between TEE and daily EI is explained by the nitrogen excretion. Importantly, these proposed mechanisms remain hypothetical and require further dedicated studies to challenge them. However, the findings raise an interesting theoretical principle of integrating physiological effects to the drive for EI or food choice, from both FFM/RMR and glycogenostatic and/or protein leverage processes. Importantly; although the data used in the present work have been collected about 2 decades ago, the quality of the methods used and the implemented design make it possible today to use these data to question more recently developed concepts and models. Indeed, while the associations between body composition compartments and the mediating role of RMR have been particularly underlined during the last 10 years, the data collected as part of the present project, also 20 years ago, remain quite unique in order to a posteriori question these associations and mediations in adolescents with obesity. Importantly, the use of metabolic chambers as a gold standard when it comes to the evaluation of EE and substrates use, composes a strength of the present work. Although stays in such metabolic chambers have been shown to favor over-consumption in adolescents with obesity [46], the 24-h EI of adolescents was found strongly correlated with an evaluation of their ad libitum food intake over a 7-day period (rho = 0.730, $p < 0.001$, data not shown). In conclusion, these exploratory results based on a relatively modest sample but taking advantage of a robust and high-quality methodology, provide the first evidence regarding the role of RMR as a main tonic signal of appetite control mediating the effect of body composition and mainly FFM (over FM) on daily EI in adolescents with obesity. Our results also suggest for the first-time relationships between 24-h CHO and protein oxidation and daily EI in this population, reinforcing the need to further study and better understand the previously proposed glycogenostatic and aminostatic theories to improve our understating but also management of energy balance in the context of pediatric obesity.

DATA AVAILABILITY Data might be available upon request.

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AUTHOR CONTRIBUTIONS YB, SL, CM designed research and conducted research; DT, LI, BP, YD, HM analyzed data; and TD, LI, MH, JB, YB, SL, HM wrote the paper. TD, LI had primary responsibility for final content. All authors read and approved the final manuscript. COMPETING INTERESTS The authors declare no competing interests.

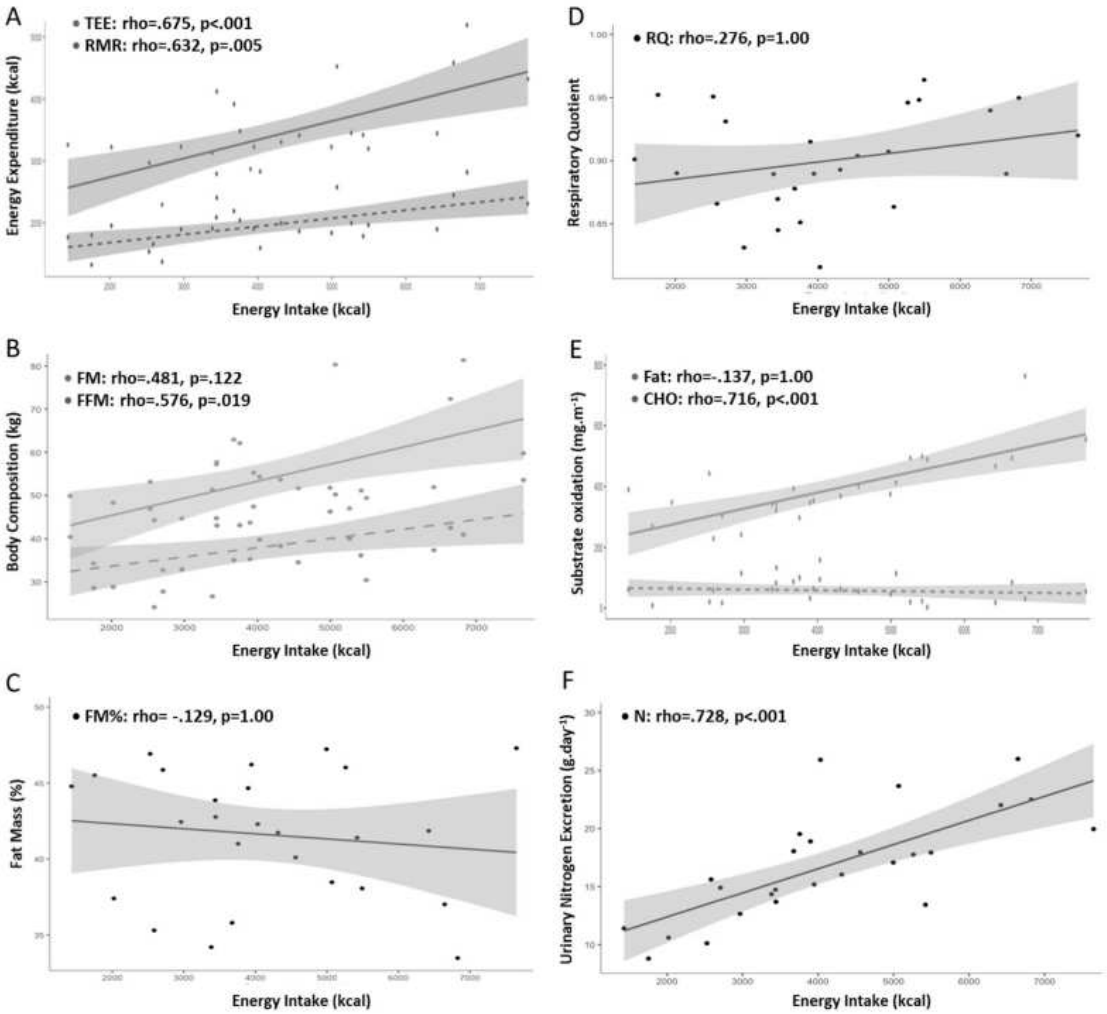

Fig. 2 24-h Energy Expenditure, substrate use and Energy Intake. Associations between total 24-h energy intake and total energy expenditure and resting metabolic rate (**A**) absolute fat mass and fat-free mass (**B**) percentage of fat mass (**C**) respiratory quotient (**D**) fat and carbohydrate oxidation (**E**) and, 24-h nitrogen excretion (**F**). TEE total energy expenditure, RMR resting metabolic rate, FM fat mass, FFM fat-free mass, RQ respiratory quotient, CHO carbohydrates, N nitrogen, p *p* value, results adjusted on age and sex.

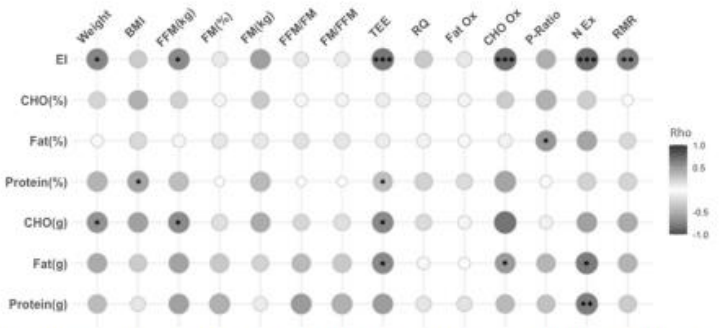

Fig. 3 Heat Map illustrating the correlations between energy intake (total and macronutrients) and the adolescents' anthropometric, body composition and energy metabolism parameters. BMI body mass index, TEE total energy expenditure, RMR resting metabolic rate, FM fat mass, FFM fat-free mass, RQ respiratory quotient, CHO carbohydrates, N nitrogen, Ox oxidation, Ex excretion, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; results adjusted on age and sex.

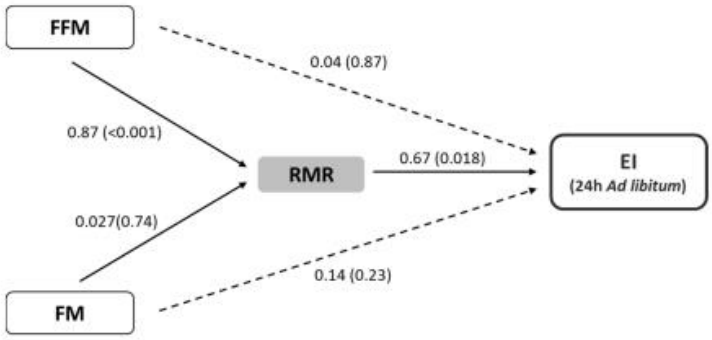

Fig. 4 Path diagram for the mediation model with the standardized parameter coefficients for the direct effects (solid lines) of fat mass and fat-free mass on resting metabolic rate and resting metabolic rate on energy intake, the indirect effect (dashed lines) of fat mass and fat-free mass on energy intake mediated by resting metabolic rate. The mediation model indicates that the effect of fat mass and fat-free mass on energy intake is fully mediated by resting metabolic rate. EI energy intake, FM fat mass, FFM fat-free mass, RMR resting metabolic rate, model adjusted on age and sex. Statistics are presented as standardized parameter coefficients (*p* values). Solid lines representing direct effects and dashed lines representing indirect effects.

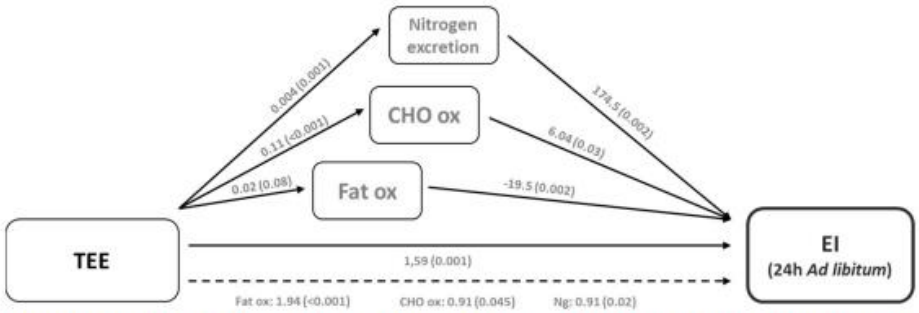

Fig. 5 Path diagram for the mediation models with the standardized parameter coefficients for the direct effects (solid lines) of Total Energy Expenditure (TEE) on Energy Intake (EI) and the respective mediation effects of Fat oxidation, Carbohydrate oxidation and Nitrogen Excretion on the effect of TEE on EI. EI energy intake, Fat ox Fat oxidation, CHO ox Carbohydrate oxidation, Ng Nitrogen, TEE Total Energy Expenditure, model adjusted on age and sex. Statistics are presented as rho coefficient (*p* values). Of note, the three mediations path have been elaborated individually as described in the methods section. Solid lines representing direct effects and dashed lines representing indirect effects.

Table 1. Characteristics of the sample, body composition, energy intake and expenditure.

Sample characteristics	Mean	SD
Age (years)	14.1	1.5
Body Weight (kg)	92.1	17.2
BMI (kg.m²)	33.9	4.1
FFM (kg)	53.9	11.4
FFM (%)	58.4	4.2
FM (kg)	38.2	7.7
FM (%)	41.5	4.2
FM (kg)	38.2	7.6
24 h Metabolic Chamber Expenditure		
24h-EE (kcal)	3409	727
24h-RQ	0.92	0.040
24h-Fat Oxidation (mg.m-1)	58.2	35.5
24h-CHO Oxidation (mg.m-1)	387.9	121.1
24h-Urinary Nitrogen excretion (g.d-1)	16.9	4.6
P ratio	0.12	0.03
RMR ($kcal.day^{-1}$)	1969	353
24 h Metabolic Chamber Intake		
24h-EI (kcal)	4162	1619
24h-CHO Intake (%)	45.5	9.0
24h-Lipid Intake (%)	41.5	8.4
24h-Protein Intake (%)	13.1	2.5
24h-CHO Intake (g)	391.8	171.6
24h-Lipid Intake (g)	132.5	50.8
24h-Protein Intake (g)	94.0	24.7

BMI Body Mass Index, *FM* Fat Mass, *FFM* Fat Free Mass, *EE* Energy Expenditure, *RMR* Resting Metabolic Rate, *EI* Energy Intake, *CHO* Carbohydrates, *SD* Standard Deviation.