Dietary fat intake modifies the influence of the *FTO* rs9939609 polymorphism on adiposity in adolescents; the HELENA cross-sectional study

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ABSTRACT

Background and aims: The fat mass and obesity associated gene (*FTO*) has been associated with obesity and dietary intake. The aims were: (i) To assess whether energy and macronutrient intakes were different across the *FTO* rs9939609 genotypes in adolescents, and (ii) to explore whether dietary fat intake modified the association of the rs9939609 polymorphism with adiposity.

Methods and Results: The *FTO* rs9939609 polymorphism was genotyped in 652 adolescents (53% females, 14.8±1.2 years, TT=246, TA=296, AA=110). Energy and macronutrient intake were assessed by two non-consecutive 24h-recalls. Weight, height, waist circumference and skinfold thicknesses were measured and body fat percent was calculated. Energy and macronutrient intake were similar across the *FTO*rs9939609 genotypes (P>0.2). There were significant interactions between the *FTO* polymorphism and fat intake on adiposity estimates (P<0.05). In adolescents whose fat intake was below 30% (N=203), the A allele of rs9939609 was not associated with adiposity indices. In contrast, in adolescents whose fat intake was between 30% and 35% of energy (N=190), the rs9939609 polymorphism was associated with a 1.9 % higher body fat per risk allele (95%CI: 0.39, 3.33; P<0.05), and in those whose fat intake was higher than 35% (N=259), it was associated with a 2.8 % higher body fat per risk allele (95%CI: 1.27, 4.43; P<0.001).

Conclusions: These findings support the concept that the deleterious effect of the *FTO* rs9939609 polymorphism on adiposity is exacerbated in adolescents consuming high fat diets. In contrast, the consumption of low fat diets (<30% of energy) may attenuate the genetic predisposition to obesity in risk allele carriers.

KEY WORDS: *FTO* gene, diet composition, fat intake, obesity, adolescents

INTRODUCTION

Obesity is the result of complex interactions between genetic and lifestyle factors such as diet and physical activity[1]. The fat mass and obesity associated (*FTO*) rs9939609 polymorphism has been consistently associated with excess adiposity in youth[2]. We showed that each copy of the *FTO*rs9939609 polymorphism A allele is associated with 1.03% higher body fat percentage (BF%) in European adolescents[3]. The frequency of the minor A allele in European or European ancestry populations ranges from 0.38 to 0.49[4, 5]. The *FTO* gene seems to play a role in the regulation of energy balance[4, 6, 7], yet the exact mechanism remains unknown. It seems, however, that the *FTO* gene is involved in the control of energy expenditure[7] and/or energy intake[8].

Several authors reported that children carrying the A allele have higher energy intake[9], particularly derived from dietary fat[8, 10, 11], than non-allele carriers of the *FTO*rs9939609; while others found no influence of the *FTO*rs9939609 on energy or fat intake[12]. Furthermore, the influence of the *FTO* gene on adiposity may be modified by physical activity[3] and diet[2]. Likewise, it has also been suggested that the influence of the *FTO*rs9939609 on adiposity may be greater in an "obesogenic" environment, such as high fat diets[13], or low levels of physical activity[3, 14]. Studies in adults reported that dietary macronutrient distribution modified the influence of the *FTO*rs9939609 on adiposity[13, 15] and insulin sensitivity[16], though contradictory findings have also been reported[17]. Given that dietary fat content has been previously associated with increased adiposity in adolescents[18, 19], and that low energy and low fat diets have been proposed as effective strategies to avoid excess adiposity in youth carrying the A risk allele[20], the aims of the present study were (i) to examine the association between the *FTO*rs9939609 genotypes and dietary energy and macronutrient intake , and (ii) to explore whether dietary fat intake modifies the association of the

95 *FTO*rs9939609 with adiposity in European adolescents participating in the Healthy
96 Lifestyle in Europe by Nutrition in Adolescence (HELENA) cross sectional study.
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METHODS

Study participants and design

The HELENA study was conducted between 2006 and 2007 in ten European cities (Athens, Greece; Heraklion, Greece; Dortmund, Germany; Ghent, Belgium; Lille, France; Pecs, Hungary; Rome, Italy; Stockholm, Sweden; Vienna, Austria; Zaragoza, Spain) (hereafter called centers). A detailed description of the HELENA study sampling and recruitment approaches, standardization and harmonization processes, data collection, analysis strategies and quality control activities has been published elsewhere[21]. The present study comprises 649 adolescents with valid data on the *FTO*rs9939609, BMI and two 24h non-consecutive dietary recalls (**Supplemental Figure 1**). All procedures involving human participants were approved by the Ethics Committee of each involved center. Written informed consent and assent were obtained from both adolescents and their parents before being enrolled in the study.

Genotyping

The *FTO*rs9939609 genotyping was done by an Illumina system, using the GoldenGate technology. The genotyping success rate was 100%. Genotyping was performed once for each sample. Genotype frequencies did not differ significantly (χ^2=1.74, *P*=0.19) from Hardy-Weinberg equilibrium expectations.

Adiposity

The anthropometric methods followed in the HELENA project have been described elsewhere[22]. In brief, we measured in triplicate height, weight, waist circumference, and 6 skinfold thicknesses (triceps, biceps, subscapular, suprailiac, thigh and calf) using standard protocols. BMI was calculated and then transformed to an age- and sex-specific z-score BMI. The corresponding BMI z-scores, relative to the British 1990 Growth Chart References, were determined to obtain comparable values across both

genders and all ages. The BMI z-score is the number of standard deviation units that a person's BMI deviates from a mean or reference value. BMI was categorized into non-overweight and overweight (including obesity) according to the age- and sex-specific BMI international cut-offs proposed by the International Obesity Task Force[23]. BF% was calculated from the triceps and subscapular skinfolds using the Slaughter's equation[24]. Fat mass index (FMI) was calculated dividing FM by height squared (in meters). The sum of 6 skinfolds (mm), BF% and FMI were used as general adiposity estimates and waist circumference as central adiposity estimate.

Dietary assessment

The HELENA-DIAT (Dietary Assessment Tool) 24-h dietary recall software was used to obtain dietary intake data. To calculate energy and nutrient intake, data of the HELENA-DIAT were linked to the German Food Code and Nutrient Database (BLS (Bundeslebensmittelschlussel) version II.3.1, 2005)[25]. The Multiple Source Method (MSM)The MSM was used to calculate individual usual dietary intake, adjusting for between person variability [26]. The MSM calculates dietary intake for individuals first and, then, constructs the population distribution based on the individual data. With this method dietary data was corrected for between and within person variability. For the purposes of this study total energy intake (kcal/day), fat intake (g/day and percent of energy), carbohydrate intake (g/day and percent of energy), protein intake (g/day and percent of energy) and fiber intake (g/day) were analysed. Thereafter, study participants were categorized into three categories according to their fat intake as follows: low <30%, moderate (between 30% and 35%), and high fat content (>35%)[27]. Under-reporters were considered as individuals with a ratio of energy intake over estimated basal metabolic rate lower than 0.96, calculated with the equation of Schofiled [28], according to the Goldberg cut-offs[29-31].

Statistical analyses

Statistical analyses were performed using commercially available software (SPSS, version 21.0; SPSS, Inc, Chicago, Illinois), and the level of significance was set at 0.05. Variables with skewed distribution were transformed in a natural logarithmic basis (Ln) to obtain a more symmetrical distribution.

The differences in body fat estimates (z-score BMI, BF%, FMI, the sum of 6 skinfolds, and waist circumference) and in dietary energy, macronutrient and fiber intake among the three *FTO*rs9939609 genotypes were analyzed using ANCOVA with the genotypes as fixed factor (TT, TA, AA), center as random variable, and age and sex as covariates. All the analyses were repeated after further adjustment for body fat estimates. To examine the existence of an interaction between the *FTO*rs9939609 and the percent of energy derived from fat intake as continuous variable and categorized variable (<30%, between 30% and 35%, and ≥35%) on body fat estimates, we used the same model as above but we added a cross-product term *FTO**fat intake into the model. Finally, we determined the increase in adiposity estimates per A risk allele of the *FTO*rs9939609 using the additive model after controlling for confounders (i.e., sex, age, and center as dummies variables) with the analyses stratified by fat intake categories (<30%, between 30% and 35%, and ≥35%). Trend tests were performed by adding genotype categories in the regression analysis as ordinal variables (0=TT, 1=TA, 2=AA). As under-reporting of energy intake is common, particularly in overweight participants, all the analyses were repeated after excluding under-reporters.

RESULTS

The descriptive characteristics of HELENA participants across the *FTO* genotypes are shown in **Table 1**. The minor A allele frequency was 0.39 in the study. The results showed that adiposity estimates (i.e. BMI z-score, FMI, body fat percent, the sum of 6 skinfolds, waist circumference) and the percentage of overweight were higher in adolescents carrying the A allele of rs9939609. There were no statistically significant differences in the number of under-reporters across the *FTO* genotypes.

There were no statistically significant differences in energy, carbohydrate, protein and fiber intake across the *FTO*rs9939609 genotypes (Table 1). These relationships did not differ after further adjustment for BMI (all P>0.1). Moreover, the exclusion of the under-reporters from the analyses did not substantially change the results (P=0.429 for energy intake, P=0.971 for the percent of energy derived from carbohydrate intake, P=0.227 for the percent of energy derived from protein intake and P=0.342 for fiber intake in g/100kcal, respectively).

A trend to higher percent of energy derived from fat intake was observed in adolescents carrying the A risk allele (Table 1, P=0.08). This weak (borderline non-significant) association of the *FTO*rs9939609 with fat intake (% of energy) persisted after the exclusion of under-reporters from the analyses (32.9±0.38, 33.4±0.35, 34.5±0.60, for the TT, TA and AA genotypes, respectively; P=0.06). However, the strength of the association of the A allele with fat intake (percent of energy) was diminished and became non-significant after further adjustment for adiposity estimates such as BMI (P=0.161), BF% (P=0.164) or FMI (P=0.162).

There were significant interactions between the *FTO* polymorphism and fat intake derived energy percent (continuous variable and categorized variable as <30%, from 30% to 35%, and >35%) when considering Z-score BMI (P<0.001 and P=0.013,

for continuous and categorized variables, respectively), BF% (P<0.001 and P=0.006 for continuous and categorized variables, respectively), FMI (P<0.001 and P=0.002 for continuous and categorized variables, respectively) and waist circumference (P=0.002 and P=0.005 for continuous and categorized variables, respectively) (**Figure 1**). There were no significant differences in the distribution of the three *FTO* genotypes among the fat intake categories (χ=6.44; P=0.168).

In those adolescents whose energy derived from fat intake was below 30%, the A allele of the *FTO*rs9939609 did not show any significant association with Z-score BMI, body fat percent, FMI and waist circumference (all P>0.5). In contrast, in adolescents whose energy derived from fat intake was between 30% and 35%, the A allele of the *FTO* polymorphism was significantly associated with higher z-score BMI [+0.20 kg/m^2 per risk allele (95%CI: 0.004, 0.39); P<0.05], higher BF% [+1.9 % per risk allele (95%CI: 0.39, 3.33); P<0.05], higher FMI [+0.6 kg/m^2 per risk allele (95%CI: 0.13, 1.09); P<0.05] and higher waist circumference [+1.6 cm per risk allele (95%CI: 0.21, 2.92); P<0.005] after adjusting for center, sex and age (Figure 1).

The strongest influence of the A risk allele of the *FTO*rs9939609 was observed in adolescents with the highest percent of energy derived from fat intake (>35%, Figure 1). Indeed, the A allele of the *FTO*rs9939609 was significantly associated with higher z-score BMI [+0.30 kg/m^2 per risk allele (95%CI: 0.009, 0.50); P<0.01], BF% [+2.8 % per risk allele (95%CI: 1.27, 4.43); P<0.001], FMI [+0.9 kg/m^2 per risk allele (95%CI: 0.50, 1.56); P<0.001] and waist circumference [+2.1 cm per risk allele (95%CI: 0.50, 3.80); P<0.05] after adjusting for center, sex and age (Figure 1).

DISCUSSION

In the current study, we investigated the association of the common rs9939609 variant of the *FTO* gene with energy and macronutrient intake, as well as the interaction between dietary fat intake and *FTO*rs9939609 on adiposity estimates in adolescents. There were two main findings: firstly, there were no significant associations of rs9939609 with total energy intake and the percent of energy derived from fat, carbohydrate, protein and fiber intake and irrespective of the exclusion of under-reporters from the analyses; secondly, we observed that the percent of energy derived from fat intake modified the influence of the *FTO*rs9939609 on adiposity in European adolescents. It was observed that the A risk allele of the *FTO*rs9939609 had no significant deleterious influence on any adiposity in adolescents whose dietary fat intake was below 30% of energy intake. In contrast, the effect of the A risk allele on total and central adiposity increased proportionally to the dietary fat content. These findings have important public health implications, and indicate that having a dietary fat intake below 30% of energy may offset the genetic predisposition to obesity associated with the *FTO*rs9939609 in adolescents.

To our knowledge, this the first report examining the influence of dietary macronutrient distribution on the effect of the *FTO* gene on adiposity conducted in a relatively diverse sample of European adolescents. The current study adds to the current knowledge two interesting aspects: (i) the measurement of body composition and body fat distribution instead of using BMI as adiposity proxy, which cannot distinguish fat and fat free mass and that does not give any indication about body fat distribution[13, 32], and (ii) the categorization of the percent of energy derived fat intake into categories usually used to classify dietary fat content by scientific societies and public health institutions (<30%: low fat diets, 30-35%: fat intake recommendation for healthy

242 individuals in Mediterranean countries, >35%: typical Western diets or high fat diets)
243 instead of tertiles or quartiles, as in previous studies.

244 Findings of the present study showed that the *FTO*rs9939609 was not
245 significantly associated with energy intake. Moreover, the removal of under-reporter
246 adolescents did not substantially affect the results. These results concur with a previous
247 study on 14-18 year old adolescents[33] and with another reporting similar findings in
248 children and adolescents aged 6-19 years[11]. In contrast, other studies reported higher
249 energy intake in children carrying the A allele[2, 9, 10]. The influence of the
250 *FTO*rs9939609 on energy intake was also examined in adults, showing no long-term
251 effect [32], or a lowering effect of the A allele on energy intake[8, 12]. The inverse
252 association of the *FTO* variant with energy intake observed in adults has been
253 previously explained by the under-reporting of energy intake[2]. However, in our study
254 the results were consistent even when the analysis was restricted to non-under-reporter
255 adolescents.

256 Conflicting data exist in the literature regarding the influence of *FTO* on fat
257 intake. Several studies observed higher energy derived from fat intake in risk allele
258 carriers[8], particularly among *FTO*rs9939609 AA genotype carriers[9, 10] and obese
259 individuals[13], while others observed no significant association of the *FTO*rs9939609
260 and the percent of energy derived from fat intake[2]. In our study, there was a trend
261 towards higher energy-adjusted fat intake in A allele carriers. However, this relationship
262 disappeared after controlling for adiposity. These findings suggest to some extent that
263 the association of the *FTO* gene with higher percent of energy derived from fat intake in
264 adolescents was mediated by the influence of adiposity on fat intake percent, and that
265 adolescents with higher FM are more likely to consume high fat diets, in line with
266 recent findings in this cohort[18] and in other reports[20].

The most relevant finding of our study is the interaction between the *FTO*rs9939609 and the percent of energy derived from fat intake on total and central adiposity in European adolescents. This *FTO**dietary fat content interaction was robust regardless the adiposity estimate considered in the analyses (i.e., BMI, z-score BMI, BF%, FMI, waist circumference). In the stratified analyses, we observed that the A risk allele was not associated with BMI, z-score BMI, BF%, FMI and waist circumference in adolescents whose energy derived from dietary fat intake was below 30%. These results indicate that consuming low fat diets may offset the genetic predisposition to obesity associated with the *FTO*rs9939609 in adolescents. Moreover, it was observed that the effect size of the adiposity increasing influence of the A allele was higher in adolescents consuming higher percent of energy derived from fat intake. Thus, the increase per risk allele on BF% was +1.9% in those adolescents consuming diets whose fat percent of energy was between 30% and 35%, while the effect of the A allele on BF% increased up to +2.8% in those youths whose energy derived from fat intake was above 35%. The present findings are consistent with previous studies in which the influence of the *FTO* gene was restricted to individuals consuming high fat diets[13, 32]. Sonestedt et al.,[13] in a cross sectional study and Lappalainen et al.,[34] in a longitudinal study observed that the association of the *FTO*rs9939609 with BMI was significant only in individuals whose dietary fat content was within the upper tertile (mean fat intake in the upper tertile 44.7% and 43.8%, respectively). In contrast, our results do not concur with previous observational studies in which no significant interaction effect was found between the *FTO* variant and macronutrient distribution in association with BMI in adults [12, 35]. It is worth noting that in the study of Gustavsson et al.,[35] in which no interaction effect was found, dietary intake was assessed by means of a single semi-quantitative food frequency questionnaire.

Moreover, in the above mentioned combined analysis of adults[12], the percent of fat intake was dichotomized as low (below median) or high fat diets (above median) and dietary intake was evaluated by means of different methods. In contrast, the studies in which significant interaction effects were observed, dietary intake was estimated using 3 days food records[34] or estimated from seven days menu books and food frequency questionnaires[13].

Genetic susceptibility to obesity may be modified by environmental factors such as physical activity and dietary habits. Previous studies observed that physical activity can attenuate, and even offset, the deleterious effect of the A risk allele on adiposity[3, 36]. Our findings support the hypothesis that avoiding an obesogenic environment characterized by the consumption of high fat diets and sedentary lifestyle[3] overcomes the effect of the *FTO*rs9939609 on obesity risk. Given the growing interest in the development of personalized advice based on genetic make-up of individuals, findings from the HELENA study suggest that meeting physical activity recommendations[3]and consuming low fat diets (<30% of energy from fat intake) may be particularly beneficial for European adolescents carrying the A allele of the *FTO*rs9939609.

There are several limitations of the current study that should be considered. Findings from our study should be taken with caution due to i ts cross-sectional design. One of the main pitfalls in dietary assessment by self-reported dietary recall is under-reporting energy intake in youth overweight participants[37, 38]. To account for this, we conducted sensitivity analyses and excluded under-reporters from the analyses and the results did not change. Fat intake was calculated based on two self-administered, computer-assisted, non-consecutive 24-h recalls, according to the recommendations of the European Food Consumption Survey Method study [39]. Collection of dietary data for more than two days or the use of a food frequency questionnaire would have been

desirable to compensate for day-to-day variability. The 24-h dietary recall method does not allow the quantification of proportions of non-consumers of particular food items, a fortiori for infrequently consumed foods. To mitigate this limitation, dietary intake was corrected for within-person variability by applying the MSM methods. The thorough standardization of the methods and collection of data throughout all the cities involved in the HELENA study and the large battery of lifestyle, socioeconomic and health indicators collected available for adjustment should be mentioned as strengths. However, further studies with bigger sample sizes, in children and adults, and in other ethnicities are needed to confirm our findings.

In summary, the present study strengthens the role of diet composition in the association of the *FTO*rs9939609 with adiposity, and shows that the deleterious effect of the rs9939609 risk allele on adiposity is higher in adolescents consuming high fat diets (>35%). In contrast, the consumption of low fat diets (<30% of energy derived from fat intake) is particularly beneficial for adolescents carrying the A allele risk of the *FTO*rs9939609 who are genetically susceptible for obesity.

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Contribution of authors: IL conceived the hypothesis, conducted the statistical analyses and drafted the manuscript, JRR, FOB, AM, IH and LAM critically revised the drafted manuscript. AL, MGG, KW, DM, YM, and SDH collected the data and critically revised the manuscript.

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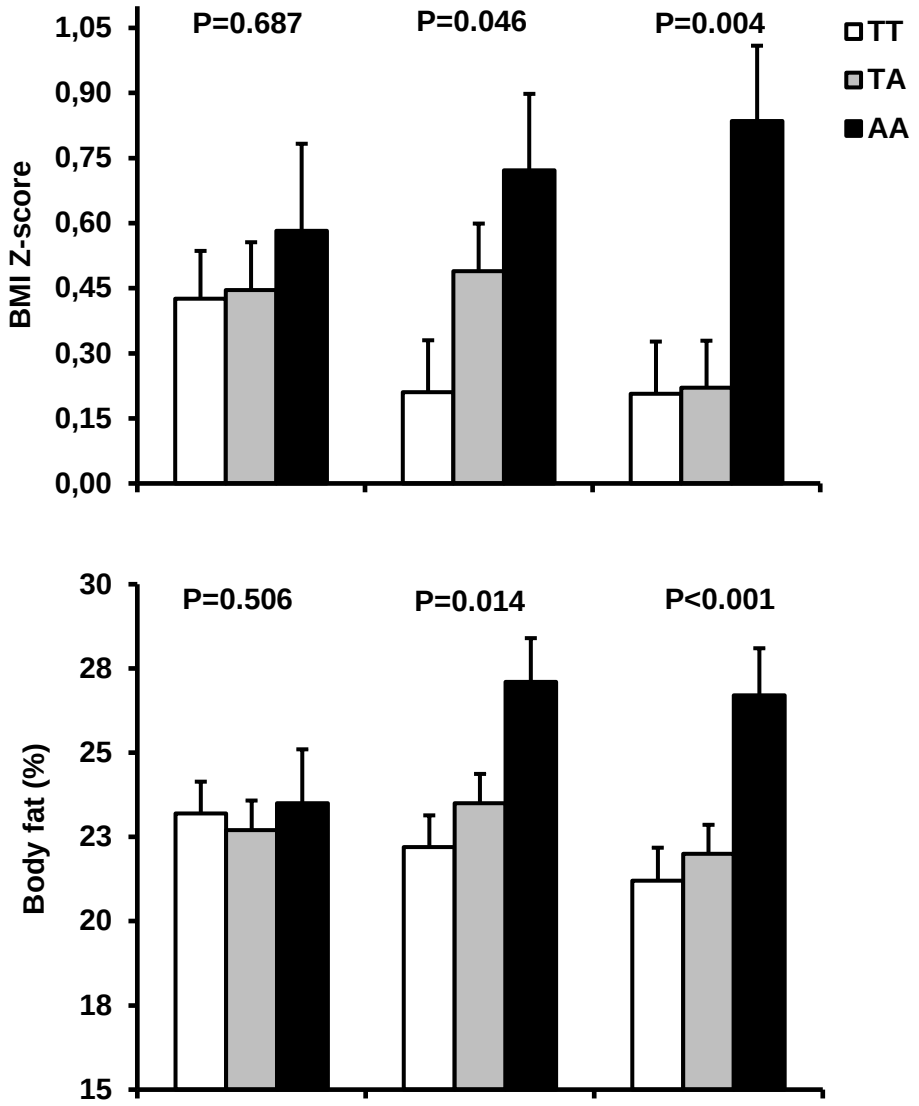
475

476 **FIGURE LEGENDS**

477 **Figure 1**. Influence of *FTO* rs9939609 polymorphism on body mass index z-score
478 (BMI Z-score), body fat percent, fat mass index and waist circumference of adolescents
479 categorized into three groups according to the percentage of energy derived from fat
480 intake (<30%, from 30% to 35%, and >35%). Interaction effect between *FTO*
481 rs9939609 and fat intake for BMI Z-score P=0.013, for body fat percent P=0.006, for
482 FMI P=0.002 and for waist circumference P=0.005. Values are means and standard
483 errors adjusted with age, sex and center (dummy variable). Sample size between
484 brackets.

485


P=0.687
P=0.046
P=0.004
TT
TA
AA
BMI Z-score
1,05
0,90
0,75
0,60
0,45
0,30
0,15
0,00
P=0.506
P=0.014
P<0.001
Body fat (%)
30
28
25
23
20
18
15

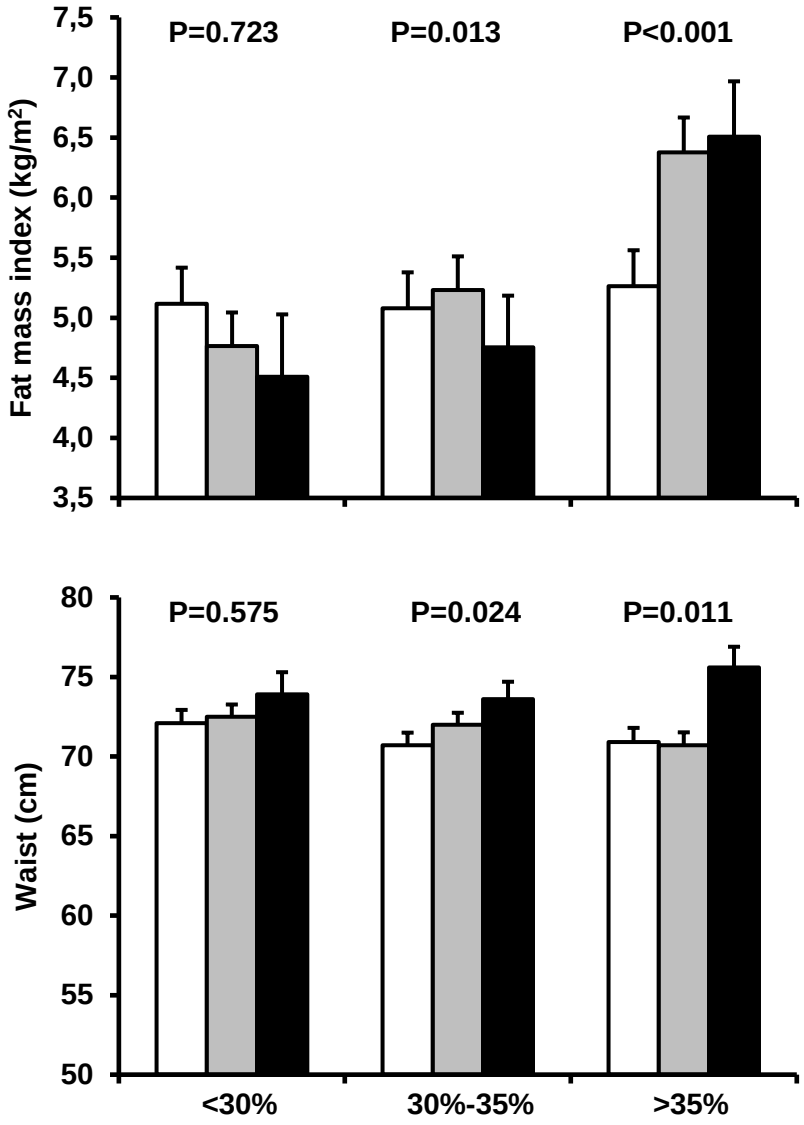
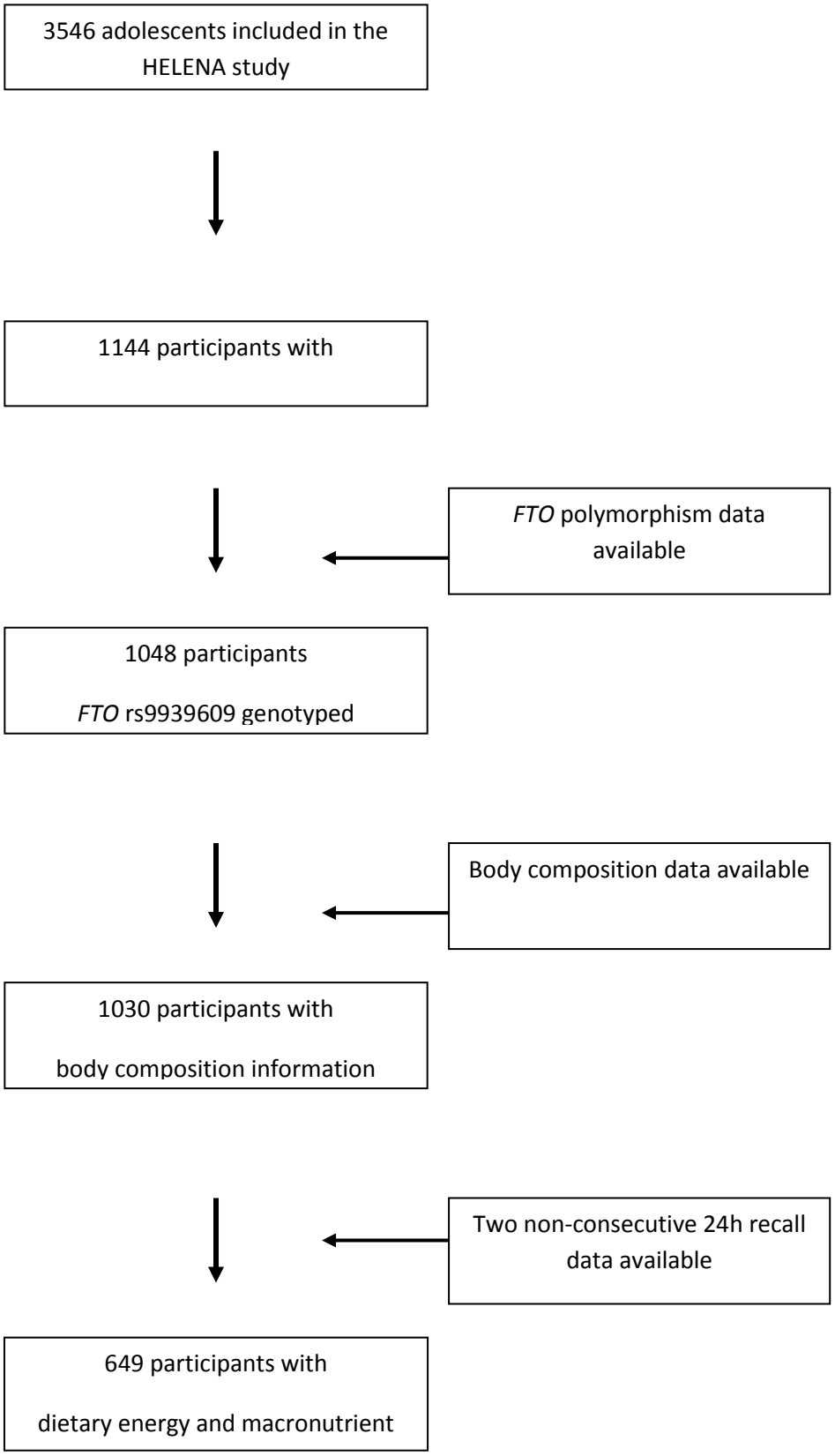

P=0.723
P=0.013
P<0.001
Fat mass index (kg/m²)
7,5
7,0
6,5
6,0
5,5
5,0
4,5
4,0
3,5
P=0.575
P=0.024
P=0.011
Waist (cm)
80
75
70
65
60
55
50
<30%
30%-35%
>35%

Figure 1.



Supplemental Figure 1. Flow diagram of participants.