Title

Evidence for protein leverage in children and adolescents with obesity

Authors

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Author contributions

Author contributions: CS & DT conceptualized the study, did the statistical analysis and interpreted results, wrote/revised manuscript; BEH conceptualized the study, collected data and revised manuscript; KTK initial study design and data collection; ZM & EJA collected data; TO & AR analyzed accelerometer data and revised manuscript; DPB revised manuscript; SJS & DR assisted with result interpretation and revised manuscript; AMS provided statistical background for compositional data analysis; MJ revised manuscript; MAS conceptualized the study, interpreted results and revised manuscript. All authors were involved in writing the paper and had final approval of the submitted and published versions.

Bullet points

- The protein leverage hypothesis (PLH) posits that a diet low in energy from protein sources causes a compensatory increase in food intake to achieve a target protein intake (protein leverage), and that this mechanism consequently contributes to excess energy intake and obesity in humans living in modern westernised food environments. Evidence for protein leverage is based on studies in animals and adult humans, however studies investigating protein leverage or the PLH in children and adolescents are lacking.
- This study in a cohort of youth with obesity illustrates an increased total energy intake with decreasing energy intake from protein sources, irrespective of whether carbohydrates or fats were the diluents, consistent with the mechanism of protein leverage. In a physically inactive subgroup, a diet high in protein and moderate in carbohydrates was associated with decreased body-mass index z-scores.
- Modulating dietary protein may assist in reducing total energy intake in youth with obesity.

Abstract

Objective To test the protein leverage hypothesis in a cohort of youth with obesity.

Methods Retrospective study in a cohort of youth with obesity attending a tertiary weight management service. Validated food questionnaires (ACAES-FFQ) revealed total energy intake (TEI), percentage energy intake from carbohydrates (%EC), fats (%EF) and proteins (%EP). Individuals with a Goldberg cut-off >1.2 reported energy intake/basal metabolic rate from fat free mass were included. A subgroup had accelerometer data. Statistics included modelling of percentage energy from macronutrients and TEI, compositional data analysis to predict TEI from macronutrient-ratios and mixture models for sensitivity-testing.

Results 137 of 203 participants were included, mean age 11.3y (SD±2.7), 68 females, BMI z-score 2.47 (SD±0.27). Mean TEI was 10330 kJ (SD±2728), mean %EC 50.6% (SD±6.1), mean %EF 31.6% (SD±4.9) and mean %EP 18.4% (SD±3.1). The relationship between %EP and TEI followed a power function (*L* coefficient -0.48, p <0.001). TEI was inversely associated with increasing %EP. In the subgroup with <60min of moderate-to-vigorous physical activity/d (n=48), lower BMI z-scores were associated with higher %EP and moderate %EC.

Conclusions In youth with obesity, protein dilution by either carbohydrate or fat increases TEI. Assessment of dietary protein may be useful to assist in reducing TEI and BMI in youth with obesity.

Introduction

Obesity in childhood and adolescence is caused by a mismatch between energy intake and energy expenditure, whether or not an underlying genetic syndrome predominates (1). The amount and composition of macronutrients in daily nutrition (i.e. carbohydrate, fat and protein) determine total energy intake (TEI). Energy expenditure varies with age, sex, body composition, and duration and intensity of physical activity. Proteins contain less energy than the equivalent mass-amount of fats and their percentage contribution to the daily TEI is approximately 20%, compared to 50% from carbohydrates and 30% from fats in a standard diet (2). Nonetheless, daily protein intake in childhood is essential for normal development and growth, and protein requirement varies with age, sex and physical activity (3). Subtle changes in percentage intake of proteins may have a disproportionately high impact on TEI, a mechanism termed protein leverage (PL). Based on PL, *Simpson and Raubenheimer* in 2005 were the first to formulate the protein leverage hypothesis (PLH), which posits that in humans, PL contributes to weight gain and obesity (4). In a recent issue of *Obesity,* the founders have extended the theoretical foundations and updated and clarified evidence for the PLH (5).

Where diets are low in the proportion of energy from protein, PL stimulates a compensatory increase in food intake and thence TEI to attempt to achieve a certain absolute protein intake as shown in studies in mice (6). In animals such as insects, birds, fish and mammals, separate appetite systems control intake for different macronutrients, which guide animals towards an optimal intake of multiple nutrients. But under circumstances where the nutritional environment limits attaining an optimal diet, an adequate intake of proteins takes precedence over carbohydrates and fats (7, 8). Data supporting PL in adult humans come from experimental studies, systematic compilations of data from numerous trials, and analyses of cohort and population data (5). It was also shown in humans, that dilution of protein by either carbohydrates or fats had the same effect on protein leveraging (9). As originally shown by

Simpson and Raubenheimer in the 2005 paper (4), *Hall* most recently confirmed that protein leverage was a potential major contributor to the US obesity epidemic, where an increase in total energy intake of 950 kcal/capita was accompanied by a decline of 1.5% of energy from protein sources between 1961 to 2013 (10).

Studies in infants however, have shown positive associations between higher percentage of energy intake from protein sources with later development of obesity (11). There are no studies that have assessed PL or the PLH in children. In this retrospective cohort study, we aimed to assess PL and the PLH in children and adolescents with obesity, aged 6-18 years old, enrolled in a cohort study of children and youth with obesity, using a validated food questionnaire (ACAES-FFQ). Our hypothesis was that in obese children and adolescents, TEI increases with decreasing proportion of dietary energy from protein. Our secondary aim was to investigate the protein leverage hypothesis, i.e. whether protein leverage is associated with increased body mass index z-score in youth with obesity.

Methods

Study cohort

Data were collected from participants of the Childhood Overweight Biorepository of Australia (COBRA cohort), Australia's largest cohort of predominantly obese children and adolescents. Details of recruitment, sample collection protocols and methodology are described elsewhere (12). For the purpose of this retrospective cohort study, participant data on anthropometry and results from food questionnaires and accelerometry were used for analysis. Written consent by the legal guardian was obtained. The study protocol was approved by the Royal Children`s Hospital Human Research Ethics Committee, RCH, Melbourne, Australia (HREC Ref. # 28082Q, 9th of October 2017) and is in accordance with Helsinki principles.

Participant characteristics

Height was measured without shoes to the nearest 0.5 cm, using a fixed Harpenden stadiometer. Weight was assessed in light clothes to the nearest 100 g. Body mass index (BMI) was calculated according to the formula weight in kg / height in m^2 and then converted into BMI z-scores adjusted for age and sex using the US Centres for Disease Control (CDC) growth reference charts (13). Total body fat percentage was assessed by a four-point bio-impedance device (Tanita® Japan), previously validated for use in children >6 years (14), therefore children >6 years of age were included in the study. The fat free mass (ffm) was calculated according to the formula ffm = weight (kg) x (100-body fat percentage) and then used to calculate basal metabolic rate (BMR) according to the Schofield equation (15). A specialist paediatric endocrinologist or a consultant general paediatrician assessed the Tanner stage for pubertal development, where Tanner 1 was considered pre-pubertal, Tanner 2-3 peri-pubertal, Tanner 4-5 post-pubertal (16). For the analysis, we only used data from individuals aged 6-18 years with obesity (i.e. BMI > 95 centile on sex and age matched CDC growth charts).

Food Questionnaires

Data on dietary intake were collected using the Australian Child and Adolescent Eating Survey - Food Frequency Questionnaire (ACAES-FFQ) (17) between the years 2010 and 2018. As part of the Australian Eating Survey suite of food-questionnaires, these questionnaires have undergone comprehensive evaluation for validity and reproducibility and can be self-administered or completed by parents for young children. Reproducibility and comparative validity for this survey have previously been established (17). The ACAES-FFQ is a 120-item semi-quantitative FFQ with 15 supplementary questions regarding age, use of vitamin supplements, food behaviours and sedentary behaviours. The ACAES-FFQ was sent to the University of Newcastle for scanning, and nutrient analysis assessed using *FoodWorks* software to elicit total energy intake (kilojoules, kJ) and macronutrient composition as percentage energy from total energy intake (% TEI). Energy from dietary carbohydrate, fat and protein were calculated by multiplying grams of intake by 16.7 kJ/g for carbohydrate and protein and 37.7 kJ/g for fat (2).

Reporting of total energy intake in dietary assessments

Misreporting, specifically under-reporting of TEI with food questionnaires, is a recognised phenomenon in adolescence, particularly in individuals with obesity (18). In this study, we used a Goldberg cut-off of greater than ≥ 1.2 of the ratio of reported TEI to BMR to exclude under-reporters of TEI with high specificity (19). BMR was calculated from the fat free mass which previously explained 79.8% of the BMR variance in a cohort of children and adolescents aged 9.5-16.5 including obese and non-obese individuals (20).

Accelerometry data

A subgroup had physical activity measured by Respironic Actical© accelerometers (REF 198-0200-01, 109-0302-00 and 1063544) worn on the left hip on an elasticized belt continuously for 7 days, including for water-based activities and sleep. Accelerometer results were considered valid for participants with data on 3 or more weekdays and 1 or more weekend days with a wearing-time of at least 600 min and a maximum of 360 min of non-wear time in each 24 hour period, as per published guidelines regarding acceptability (21). Actical accelerometry physical activity intensity was categorized according to published intensity cut-off points (22).

Descriptive statistics

Categorical participant variables (sex, pubertal stage) are shown in absolute numbers and percentages. Continuous participant characteristics and food questionnaire data were analysed for their distribution and log-transformed if skewed. Descriptive statistics are shown as mean, standard deviation (SD) and range. Univariate linear correlation analysis between explanatory variable and response variable were performed with Pearson`s test including 95% confidence intervals (95%CI) and p values, where the condition of bivariate normality for the variable was checked. Student's t-test was used to compare associations between pubertal stages, sex and total energy intake. Linear regression models were used to analyse differences in TEI based on pubertal stage, sex, FFM and age.

Modelling of macronutrients and total energy intake using power functions

Power functions were fitted to predict strength of leverage from the relevant percentage energy intake from macronutrients towards TEI. The default formula for a power law solution adapted for protein leverage is characterised by:

$$\textit{Required food intake (in grams)} = P \times p^{L}$$

Where P is the targeted intake of proteins in gram, p is the proportion of protein from a given diet and L is the strength of leverage. P and p are derived from the analysis, the exponent L reveals the strength of leverage. Assuming full protein leverage, i.e. L = -1, small changes in p will cause substantial changes in total energy intake. Assuming no protein leverage, i.e. L=0, changes in p will not cause changes in total energy intake (5).

Compositional data analysis to explain total energy intake from macronutrient`s composition

Multiple regression with compositional predictors (e.g. percentages of energy from relevant macronutrients) may lead to inferential errors due to the covariance that exists amongst the percentages of different components; e.g. an increase in the % energy from protein must necessarily lead to a decrease in the % energy from fats and/or carbohydrates. Mixture models, also known as Scheffe's polynomials, are an analytical framework based on multiple regression that is robust to the analysis of outcomes with compositional predictors (23). To test the sensitivity of our results to the choice of analysis, we analysed the TEI and BMI z-score data as a function of dietary macronutrient content (proportion EC, proportion EF and proportion EP) in a mixture model framework, which allows us to test for effects of all three macronutrients simultaneously. We fitted five different models to each outcome. Model 1 was a null model, which assumes no effect of dietary composition on the outcomes. Models 2 through 5 were mixture models corresponding to equations 1 through 4 in *Lawson and Willden* (23) (see supplementary text S1), to test for linear additive through increasingly complex non-linear interactive effects of macronutrients on total energy intake and BMI z-score. We note here that model 2, which tests for linear effects is identical to the `partition` substation model commonly used in nutritional epidemiology (see supplementary text S1) (24, 25). To select among models we used an information theoretic approach based on Akaike Information Criterion (AIC) (26). Of the five models fitted, that with the minimal AIC was favoured, and in the event that two models where within two AIC points of one another, the simplest model was selected. Where the null model is favoured no effect of macronutrient composition on the outcome is inferred, with subsequent models suggesting more complex effects of diet composition on the outcome of interest. Mixture models were implemented using the 'MixModel' function in the R package *mixexp*. For interpretation we plotted the predictions from the AIC-favoured model as surfaces on a right-angle mixture triangle (RMT) (27). All analyses were performed using R statistics (28). A p value of <0.05 was considered significant.

Results

A total of 203 individuals reported on food frequency questionnaires. Of those, 137 fitted with the age criteria, were classified as obese, had plausible energy intake according to a Goldberg cut-off of >1.2 and were considered for further analysis. The mean age was 11.3 years (SD 2.7), 68 (50%) were females, mean BMI z-score 2.47 (SD 0.27). Mean TEI was 10330 kJ (SD 2728), mean %EC 50.6% (SD 6.1), mean %EF 31.6 (SD 4.9) and mean %EP 18.4% (SD 3.1) (see table 1 & 2 for descriptive characteristics of participants and food questionnaire related data).

Energy intake per age, pubertal stage, BMI z-score and sex

TEI increased with age for both sexes (see figure 1a) (Pearson's correlation coefficient 0.20, 95%CI 0.03 to 0.36, p=0.02). Total energy intake was associated with increasing BMI z-scores (correlation coefficient 0.20, 95% CI 0.04 to 0.36, p=0.02, see figure 1b). T-tests to compare total energy intake between pubertal stages (pre- to peri, peri- to post- and pre- to post-puberty) were non-significant when looking at both sexes (see figure 1c). Linear regression models to assess whether TEI varies independently of FFM, age and pubertal stage was not significant for females. In post-pubertal males however, TEI was significantly reduced independently of these co-factors (estimated -2577kJ in TEI post-pubertal versus pre-pubertal males, p=0,017).

Modelling of macronutrients and total energy intake

Figure 2 illustrates associations between proportion of energy derived from each macronutrient (x-axis) and the TEI (y-axis) in all individuals and in the physically inactive subgroup. In all individuals (Figure 2a), the distribution of percentage intake from macronutrients and TEI significantly followed a power function for proteins (%EP), but not for carbohydrates or fats (%EC, %EF). For protein, the strength of leverage for protein was -0.48 (p < 0,001), whereas the exponent (*L*) for carbohydrates and fats were 0.22 (p=0.23) and 0.06 (p=0.67), respectively (see figure 2a and table 3).

Compositional data analysis

To assess whether there was a relationship between the macronutrient composition of diet, TEI and BMI z-score, mixture model analysis was performed. For TEI, model 2 had the most favourable AIC (supplementary table 1 and supplementary table 2 for all model coefficients), which suggests a linear effect of dietary macronutrients on TEI. TEI was highest in those diets with the lowest %EP (see figure 3a). In this population of youths with obesity, dietary macronutrient composition was not a significant driver of BMI z-score, as the null model was favoured (see figure 3a and supplementary table 1).

Subgroup analysis for physically inactive youth with obesity

The Australian guidelines recommend 60 minutes of moderate to vigorous activity per day for children and adolescents aged 5-17 years. Of the 57 individuals with valid accelerometry data, 48 (84%) did not meet these recommendations and for the purpose of our study were considered to be physically inactive individuals (see table 1, accelerometry data) (29). Figure 2b illustrates associations between percentages of energy intake from each macronutrient (x-axis) and the total energy intake (y-axis) in the physically inactive subgroup. The percentages of energy from protein (%EP) and carbohydrate (%EC) followed a power function with respect to TEI. Relevant exponents (L) for protein were -0.57 ($p < 0.05$), for carbohydrates 0.82 ($p < 0.05$) and for fats 0.82 (p 0.30) respectively (see figure 2b and table 3).

For compositional data analysis in the physically inactive group, model 2 was favoured by AIC, suggesting linear effects of dietary macronutrients on TEI (see supplementary tables 3 and 4). For BMI z-score in physically inactive youths, compositional model 4 was favoured, suggesting effects of macronutrients composition on BMI z-score were complex and non—linear: physically inactive individuals with a diet high in %EP with moderate %EC had lower BMI z-scores, whereas individuals with a diet moderate in %EP and low %EC had higher BMI z-scores (see supplementary table 3 and 5).

Discussion

In this retrospective cohort study, we analysed macronutrient food composition data derived from the Australian Child and Adolescent Eating Survey - Food Frequency Questionnaire (ACAES-FFQ) in 137 children and adolescents with obesity, aged 6-18 years. This is the first study to provide evidence for protein leverage in youth with obesity. Specifically, we found, that with decreasing percentages of total energy intake from protein sources, total daily energy intake increases, consistent with the mechanism of protein leverage. The strength of leverage was L = -0.48 (p < 0,001), indicating partial protein leverage that is comparable to available studies from adults (5). In a physically inactive subgroup that did not meet daily recommended physical activity levels, lower percentage energy from protein sources remained the only macronutrient to increase total energy intake. Also, in the physically inactive subgroup, a diet high in protein and moderate in carbohydrate contents was associated with lower BMI z-score, whereas a diet moderate in protein and low in carbohydrates was associated with higher BMI z-scores. This is the first time that protein leverage and the protein leverage hypothesis have been tested in a cohort of youth with obesity and further studies are now required to see whether similar effects are seen in children or adolescents of all weight categories.

Protein leverage versus protein leverage hypothesis in this study

As recently explained by *Raubenheimer and Simpson*, protein leverage is necessary for the PLH, but not sufficient (5). We found evidence for PL, as seen in the negative association between dietary energy from protein and TEI. We also found a positive association between TEI and BMI z-scores. However, in our analyses we did not detect statistical support for an association between macronutrient intake and BMI z-scores in this cohort of children and adolescents with obesity. There are several possible explanations for this. Detecting relationships between diet and body composition is challenging, due to the fact that obesity result from long-term accumulation of small daily differences in intake (30). Additionally, all subjects in our study had high BMI z-scores and consequently our data provided a limited range over which to detect significance in the relationship between diet and body composition. Missing information about dietary non-macronutrient composition in our cohort might also be relevant. In particular, the content of dietary fibre has shown to attenuate PL-effects on TEI and obesity in mice (5). Also, our results have to be interpreted in the context of specific growth-characteristics for children with obesity and within the context of varying protein targets in childhood. Throughout childhood, normative BMI centiles are steadily, in a near-linear manner trending upwards from about 6 years of life and so does the difference between the 50^{th} and the 95^{th} centile increase (the latter representing the threshold to determine obesity in childhood) (13). Hence, for protein leverage in this age group to be associated with increasing BMI z-scores, the effect size must exceed the one from adulthood, where overweight and obesity are determined by static thresholds (i.e. BMI 25kg/m^2 and 30kg/m^2). Also, protein targets vary throughout life, influenced amongst other factors by age, early nutritional experience and physical activity (5). Limited data for physical activity analysis only allowed us to test the PL and PLH in a physically inactive subgroup, where we found an effect of protein leverage on total energy intake and of dietary macronutrients (protein and carbohydrate) on BMI z-scores.

Comparison with recommendations for dietary protein intake

Based on nitrogen studies in adults, the recommended dietary allowance (RDA) is 0.8 g protein per kg body weight. Due to rapid growth and development, children need a positive nitrogen balance. Therefore, the RDA of protein in children aged 4-13 and 14-18 years is slightly higher with 0.95 and 0.85 g per kg body weight, respectively, as recommended by the *Institute of Medicine* (IOM) (31). Yet importantly these RDA-measures aim to provide the minimum need of most of an investigated group to avoid adverse effects from undersupply. The true requirement for protein intake is affected by protein quality (plant-based versus meat protein), individual physiological characteristics (age, fitness), individual health (e.g. chronic disease, obesity) and environmental factors (temperature) (3). Comparing with the Nutrient Reference Values for Australia and New Zealand, the protein intake in this cohort is considered moderate (between 1-1.5gr/kg) and well below reported long-term intake of up to 2.8g/kg for male athletes without adverse health consequences (2). Similar to the RDAs from the US, the Australian National Health and Medical Research Council published acceptable macronutrient distribution ranges (AMDR) associated with reduced risk for chronic disease while guaranteeing adequate intake: for children aged 4-18y, the relevant AMDR's per total energy intake for carbohydrate, fat and protein are 45-65%, 20-35% and 15-25% (2). The average intake of protein in this study (1.42 g protein per kg body weight) was above the RDA and the percentage range of energy intake from protein sources (11-30%) spanned the above-mentioned AMDR by roughly 5% in either direction. The percentage intake of fat exceeded AMDR (ranging up to 51% in this study), whereas the percentage intake of carbohydrates at the lower end of the range meeting only 32%, is considerably lower than recommended. This may in part explain the complex interactions between macronutrients intake and BMI z-scores in the physically inactive subgroup, where BMI z-scores were higher for those on a diet moderate in proteins and low in carbohydrates.

Dietary proteins related to body weight in different age groups

In infants and toddlers, several longitudinal studies have revealed positive associations between higher protein-content formula and later elevated adiposity markers (32). In the Childhood Obesity Project (CHOP) study, a European multicentre, double-blind, randomised clinical trial, healthy infants were randomly assigned to receive a high versus lower protein-content formula, during the first year of life. At age 6 years, the high-protein formula fed children had higher BMI (0.51; 95%CI 0.13 - 0.90, p = 0.009) and an odds ratio of 2.43 (95% CI 1.12-5.27, p value 0.024) to develop obesity compared to the low-protein formula fed children (11). This may be explained by two mechanisms: i) if the protein intake target during infancy was set higher than normal due higher protein intake, protein leverage may drive an increased total energy intake later in life to achieve this higher protein set point, therefore contributing to obesity (5) and ii) a mechanism termed 'the early protein hypothesis' assumes that higher protein intake in infancy triggers elevated levels of insulin-like growth factor 1 (IGF-1) and insulin (33) and therefore stimulates early weight gain. However, a recent systematic review (including the CHOP trial) has revealed insufficient evidence to urge a reduction in the protein content in infant formulae to avoid later weight gain (34).

Systematic reviews of studies in late childhood and early adolescence that investigate the effects of higher intake of protein on later body mass index and cardiometabolic risk factors (including hypertension, dyslipidaemia and insulin resistance) found limited and inconclusive results for adverse effects (35, 36).

In adults, diets with low carbohydrate and high protein contents have been shown to be effective in weight management (37). However, there is growing evidence from longitudinal studies that high-protein, low-carbohydrate diets are associated with increased mortality and cancer. A detailed discussion about macronutrient composition and health related outcomes in animals and humans are reviewed elsewhere (38).

Implications of protein content in foods

Population-based studies investigating macronutrient composition trends over the last 4 decades in the US (10) have shown a decrease in the percentage intake of energy from protein sources accompanied by an increase in the overall intake of total energy, correlating with increasing trends in BMI. A recent analysis of National Health and Nutrition Examination Surveys (NHANES) from 2009-2010, identified the most likely causative food product for this trend as being ultra-processed foods (UPF). UPF is a group of food products including soft drinks, industrialized desserts, reconstituted meat products, "ready to consume", representing almost 60% of all energy intake in the US diet but containing just around 9.5% energy from protein sources (39). On a population level, the mechanism of the PLH may well contribute to an overall obesity epidemic as recently commented by *Hall*, investigating data from the US food supply since 1973 (10).

Strengths of this study are the availability of validated food questionnaire data in a reasonably large cohort of children and adolescents with obesity, including data on all three relevant macronutrients. We addressed the problem of under-reporting by excluding those reporting a total energy intake below a Goldberg cut-off of 1.2. This sanctioned the total number of participants, but also increases validity of the data. Accelerometry data was available from 57 individuals with 42 of those not meeting physical activity recommendations, allowing for analysis in a what was considered a physically inactive subgroup.

This study does have important limiting factors. Whereas we addressed the effects from under-reporting of total energy intake, we could not do so for misreporting of relative macronutrients content. However, when we looked at the whole cohort (n=203) – hence including under-reporters of total energy intake – we found consistent effects from protein leverage on total energy intake (data not shown). This allows the assumption that misreporting of overall energy intake should apply equally to protein, carbohydrate and fat intakes in this cohort. Further limiting factors are the cross-sectional study design which does not allow extrapolation to causality. In addition, the available food questionnaire data unfortunately do not allow investigation into the quality of protein, the specific amino acids or the fibre content.

This study was undertaken in a cohort of obese children and adolescents, because the majority of them are estimated to remain obese at the age of 35y (40). For a child aged 6 years with obesity there is a 21.8% chance to reduce their weight classification to non-obese (BMI $<30kg/m^2$) at the age of 35 years and at 18 years, this chance is further reduced to 14.2%. Identifying modifiable mechanisms such as changing the nutrients composition of diets provides an opportunity to decrease the total energy intake and positively affect this risk trajectory. Further studies are now warranted in much larger cohorts of population-based groups of children and adolescents, ideally assessing dietary composition with subsequent weight changes. However, these studies will require extensive data collection relating to potential confounders.

Conclusion

This is the first study investigating protein leverage and the protein leverage hypothesis in a cohort of obese children and adolescents aged 6-18 years. We identified decreased proportion of dietary protein to be the only macronutrient associated with an increased overall energy intake. Importantly, dietary macronutrient composition may assist to reduce total energy intake in children and adolescents with obesity seeking weight management counselling.

Table 1, Characteristics of the study cohort

Variable		n	Mean (SD)	Range	%
Age (y)		137	11.3 (2.7)	6-18	
Sex (female)		68			50
Pubertal stage					
	Pre-pubertal	66			48.2
	Peri-pubertal	40			29.2
	Post-pubertal	31			22.6
Weight (kg)		137	77.6 (26.3)	30.7-175.9	
Height (m)		137	1.52 (0.15)	1.10-1.90	
BMI (kg/m^2)		137	32.5 (6.0)	21.9-58.8	
BMI z-score		137	2.47 (0.27)	1.86-3.09	
Accelerometry		57			42
	Ave. counts/d		249`734 (97`904)	96`841-493`728	
	MVPA (min/d)		43.4 (24.1)	3.5-110-8	
	<60min MVPA/d	48			84

BMI: body mass index; BMI z-score according to Centres for Disease Control (CDC) growth charts;
Accelerometry; Ave. counts/d: number of signals reflecting deceleration and acceleration forces counted by the accelerometer, averaged per day. MVPA: time in minutes spent with moderate to vigorous physical activity, derived from Ave. counts/d according to published intensity cut-off points (22).

Table 2, Energy intake and macronutrients composition

Variable	Mean (SD)	Range (min–max)
Total energy intake (TEI in kJ)	10`330 (2728)	5467-17`886
Total daily protein intake (g)	110 (30)	56-213
Energy intake from proteins (kJ)	1831 (497)	933-3565
% TEI protein	18.4 (3.1)	11.0-30.0
Total daily carbohydrate intake (g)	307 (95)	154-611
Energy intake from carbohydrates (kJ)	5119 (1293)	2563-10`202
% TEI carbohydrate	50.6 (6.1)	32.0-68.0
Total daily fat intake (g)	84 (27)	37-195
Energy intake from fats (kJ)	3173 (1013)	1408-7354
% TEI fat	31.6 (4.9)	21.0-51.0

Total energy intake as calculated from Australian Child and Adolescent Eating Survey - Food Frequency Questionnaire (ACAES-FFQ), given as total energy intake (TEI) and macronutrients (protein, carbohydrate and fat) in gram, kilojoules and percentages from TEI

Table 3, Results from power functions fitted between macronutrients and TEI

All individuals, n=137

Macronutrient	*Strength of leverage (L)*	*p value*
Protein	-0.48	<0.001
Carbohydrate	0.22	0.23
Fat	0.06	0.67

Physically inactive subgroup, n=47

Macronutrient	*Strength of leverage (L)*	*p value*
Protein	-0.57	<0.05
Carbohydrate	0.82	<0.05
Fat	-0.31	0.30

Model coefficients for fitted power functions for the whole cohort and for the physically inactive subgroup. L indicating strength of leverage for each macronutrient (-1 signifies complete leverage, 0 means no leverage). In the whole cohort, leverage on total energy intake was only significant from the proportion of protein. In the physically inactive group, leverage on total energy intake was significant from proportions of protein and carbohydrate.

Supplementary Text S1

Following *Lawson and Willden* (2016), mixture models for the 2 through 5 equated to equations S1 through S4. Model 2, which tests for linear effects of diet composition on outcome *y* was:

$$y = \sum_{i=1}^{q} \beta_i x_i + \varepsilon, \qquad \text{(S1)}$$

where x_i is the proportion of total energy that is the *i*th macronutrient (i = 1 … *q*; *q* = 3), β_i is the effect of predictor x_i on the outcome and ε is the residual. Model 3, which tests for quadratic effects on *y* was:

$$y = \sum_{i=1}^{q} \beta_i x_i + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} x_i x_j + \varepsilon, \qquad \text{(S2)}$$

where β_{ij} is a coefficient for quadratic curvature for binary mixtures of nutrients x_i and x_j, and all other notation is as above.

Models 3 and 4 test cubic effects of diet composition on *y*, as in equations S3 and S4:

$$y = \sum_{i=1}^{q} \beta_i x_i + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} x_i x_j + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \delta_{ij} x_i x_j (x_i - x_j) + \sum_{i=1}^{q-2} \sum_{j=i+1}^{q-1} \sum_{k=j-1}^{q} \beta_{ijk} x_i x_j x_k + \varepsilon, \qquad \text{(S3)}$$

$$y = \sum_{i=1}^{q} \beta_i x_i + \sum_{i=1}^{q-1} \sum_{j=i+1}^{q} \beta_{ij} x_i x_j + \sum_{i=1}^{q-2} \sum_{j=i+1}^{q-1} \sum_{k=j-1}^{q} \beta_{ijk} x_i x_j x_k + \varepsilon, \qquad \text{(S4)}$$

where equation S3 is the formula for the third-degree polynomial and equation S4 is a special case where $\delta x_i x_j (x_i - x_j)$ is not considered.

It is notable that the three-macronutrient 'partitioning model' that is commonly used for isocaloric substitution analysis in nutritional epidemiology is written as equation S5:

$$y = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3, \qquad \text{(S5)}$$

where, β_1, β_2 and β_3 are the coefficients for effect for macronutrients, and x_1, x_2 and x_3 are the % of energy coming from each macronutrient (*Song and Giovannucci, 2018*). S5 is equivalent to S1.

Supplementary Table S1, AIC scores for five mixture-models in all individuals

Model	Energy intake (kJ)	BMI z-score
1	2559.47	37.83
2	2549.87	36.84
3	2553.16	37.11
4	2554.39	43.52
5	2554.87	38.4

AIC scores to test for effects of percentage energy from protein, carbohydrates and fats on energy intake and BMI z-scores. Models with minimal AIC scores are favoured. In the event that two models where within two AIC points of one another, the simplest model was selected. Model 1 refers to the Null model, indicating no effect from macronutrient ratios.

Supplementary Table S2, Coefficients for effects of macronutrients on TEI as estimated by models 1 through 5 for the complete dataset

Model	Coefficient	Estimate	LCL	UCL
1	Overall Mean	10330	9874	10787
2	P	-13451	-25954	-949
	C	13232	9631	16832
	F	19564	12099	27029
3	P	150048	-104831	404926
	C	23040	3871	42209
	F	44361	-29665	118387
	P:C	-233020	-557064	91023
	P:F	-288538	-828894	251817
	C:F	-31035	-159564	97494
4	P	-1741482	-7944686	4461722
	C	93955	-10567	198477
	F	-391499	-1105216	322217
	cubic(P, C)	1665960	-2482517	5814436
	cubic(P, F)	1451894	-8234293	11138081
	cubic(C, F)	-936709	-1802283	-71135
	P:C	3228458	-6960723	13417639
	P:F	4048574	-7300807	15397955
	C:F	831748	-366318	2029815
	P:C:F	-5852421	-17945501	6240659
5	P	256684	-218073	731440
	C	44931	-39422	129285
	F	85147	-84933	255227
	P:C	-498954	-1548288	550379
	P:F	-668294	-2192652	856063
	C:F	-160104	-661215	341006
	P:C:F	808325	-2224416	3841066

AIC favoured model 2 .

Coefficient`s estimates for all models and their lower and upper (LCL and UCL) 95% confidence limits are given.

Supplementary Table S3, AIC scores for five mixture-models in the physically inactive subgroup

Model	Energy intake (kJ)	BMI z-score
1	904.52	12.37
2	901.28	16.12
3	904.09	15.8
4	911	8.76
5	905.74	15.95

AIC scores to test for effects of percentage energy from protein, carbohydrates and fats on energy intake and BMI z-scores in the physically inactive subgroup. Models with minimal AIC scores are favoured. In the event that two models where within two AIC points of one another, the simplest model was selected.

Supplementary Table S4, Coefficients for effects of macronutrients on TEI as estimated by models 1 through 5 for the physically inactive subgroup

Model	Coefficient	Estimate	LCL	UCL
1	Overall Mean	10435	9615	11255
2	P	-16796	-40568	6976
	C	18594	11253	25935
	F	12343	-2316	27002
3	P	92645	-430390	615680
	C	28903	-15218	73024
	F	233028	-25630	491686
	P:C	42520	-647483	732522
	P:F	-729124	-1952487	494239
	C:F	-361128	-800248	77992
4	P	-1649591	-13634417	10335235
	C	121794	-128434	372023
	F	-90799	-4620790	4439192
	cubic(P, C)	150040	-10802419	11102499
	cubic(P, F)	4259852	-16900884	25420588
	cubic(C, F)	-1263825	-6762573	4234923
	P:C	2204105	-18508319	22916530
	P:F	3572937	-19205348	26351221
	C:F	287580	-7960052	8535213
	P:C:F	-3476868	-32548728	25594992
5	P	357001	-719741	1433743
	C	82347	-112576	277271
	F	393280	-232701	1019261
	P:C	-591191	-2946601	1764219
	P:F	-1956969	-6488185	2574247
	C:F	-787022	-2362855	788811
	P:C:F	2446140	-6240065	11132346

AIC favoured model 2.
Coefficient`s estimates for all models and their lower and upper (LCL and UCL) 95% confidence limits are given.

Supplementary Table S5, Coefficients for effects of macronutrients on BMI *z*-score as estimated by models 1 through 5 for the physically inactive subgroup

Model	Coefficient	Estimate	LCL	UCL
1	Overall Mean	2.54	2.47	2.62
2	P	2.86	0.51	5.21
	C	2.37	1.64	3.09
	F	2.65	1.20	4.10
3	P	-50.60	-100.71	-0.48
	C	3.86	-0.37	8.09
	F	1.19	-23.60	25.97
	P:C	64.77	-1.34	130.89
	P:F	95.49	-21.73	212.71
	C:F	-12.20	-54.28	29.87
4	P	-898.00	-1891.10	95.10
	C	0.02	-20.72	20.75
	F	-367.65	-743.02	7.72
	cubic(P, C)	1312.43	404.88	2219.99
	cubic(P, F)	-1006.34	-2759.78	747.11
	cubic(C, F)	-211.55	-667.19	244.10
	P:C	2046.05	329.75	3762.34
	P:F	2169.65	282.17	4057.13
	C:F	690.17	6.74	1373.59
	P:C:F	-3539.23	-5948.22	-1130.25
5	P	-108.04	-209.61	-6.46
	C	-7.75	-26.14	10.63
	F	-33.63	-92.68	25.42
	P:C	202.47	-19.73	424.66
	P:F	362.28	-65.17	789.72
	C:F	80.33	-68.32	228.99
	P:C:F	-531.50	-1350.90	287.90

AIC favoured model 4.
Coefficient`s estimates for all models and their lower and upper (LCL and UCL) 95% confidence limits are given.

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Figure titles and captions

Title figure 1

Total energy intake according to age, BMI z-score and pubertal stage

Caption Figure 1

1a) Total energy intake (TEI), given in kilojoule (kJ) per age (in years), classified by sex. Pearson's correlation coefficient 0.20, 95%CI 0.02 to 0.37, p-value 0.03. 1b) Positive correlation between Increasing total energy intake (TEI) given in kilojoule (kJ) and increasing BMI z-score (using CDC BMI z-scores). Pearson's correlation coefficient 0.28, 95%CI 0.10 to 0.44, p-value 0.002. 1c) No difference in total energy intake (TEI in kilojoule, kJ) related to pubertal stage was found for the whole cohort. Open diamonds indicate mean levels of TEI. Closed dots are outliers. Tanner stage 1 was considered pre-pubertal, Tanner stage 2 &3 peri-pubertal and Tanner stage 4&5 post-pubertal.

Title figure 2

Figure 2, Power functions between total energy intake and relevant percentages energy from macronutrients

Caption Figure 2

Scatterplot revealing associations between proportions of each macronutrient and total energy intake that followed the law of a power function (see methods). Series above illustrating results for all individuals (n=137), series below for the physically inactive subgroup (n=48).

For all individuals (series a): Proportion of proteins and total energy intake followed a power function with strength of leverage L = -0.48 ($p<0.001$). Small changes in proportion of energy from protein sources result in substantial changes in total energy intake. Power functions between proportions of carbohydrate or fat with energy intake did not meet significance.

For physically inactive subgroup (series b): Proportions of proteins and carbohydrates followed a power function with respect to the total energy intake with strength of leverage L = -0.57 ($p<0.05$) for proteins and L = 0.82 ($p<0.05$) for carbohydrates (see table 3).

Title figure 3

Figure 3, Effects from macronutrients composition on total energy intake and BMI z-scores

Caption Figure 3

Right-angle mixture triangles (RMTs) illustrating energy intake (kJ) and BMI z-score as a function of percentage total energy from protein (x-axis), carbohydrates (y-axis) and fats (implicit-axis) in series a) for all individuals (n=137) and series b) for physically inactive subgroup (n=48). Values shown are as predicted by AIC-favoured mixture models. Areas of red and blue space correspond to high and low outcomes, respectively.

Figure 1, Total energy intake according to Age, BMI z-score and pubertal status

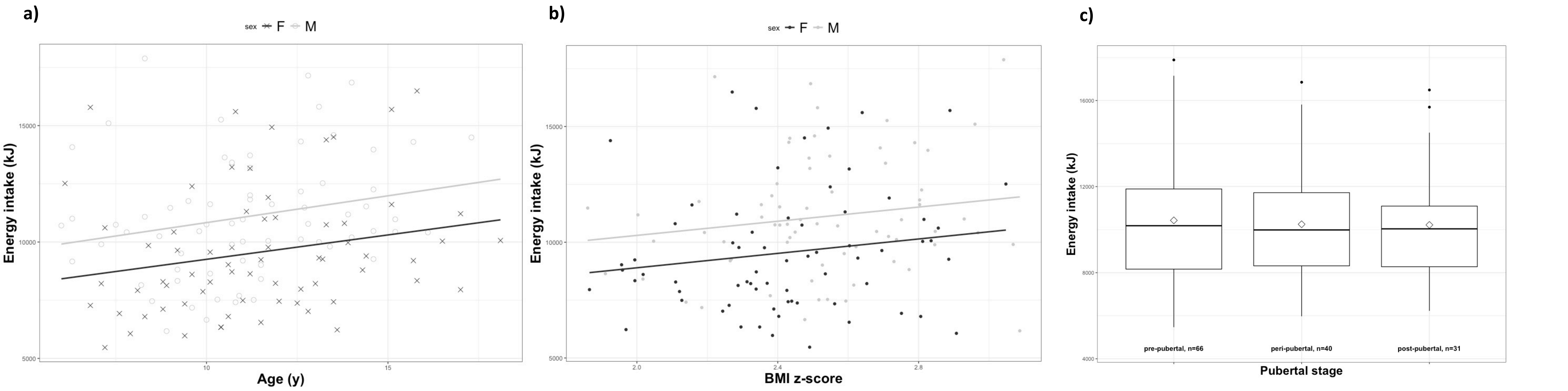
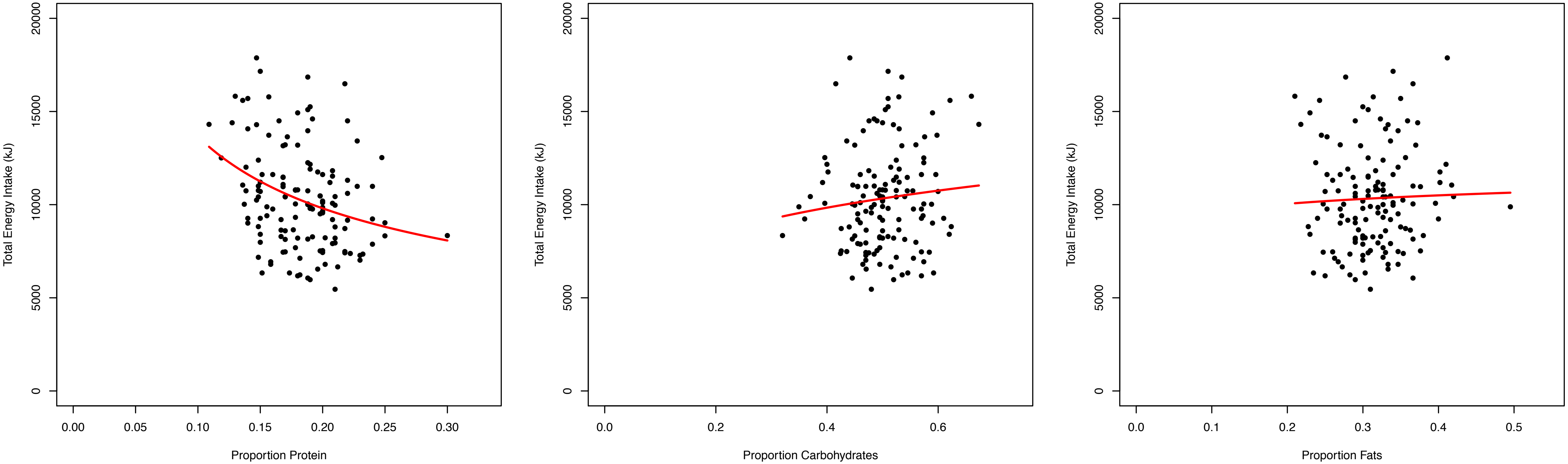


Figure 2: Power functions between percentages energy from macronutrients and total energy intake

a) All individuals



b) Physically inactive subgroup

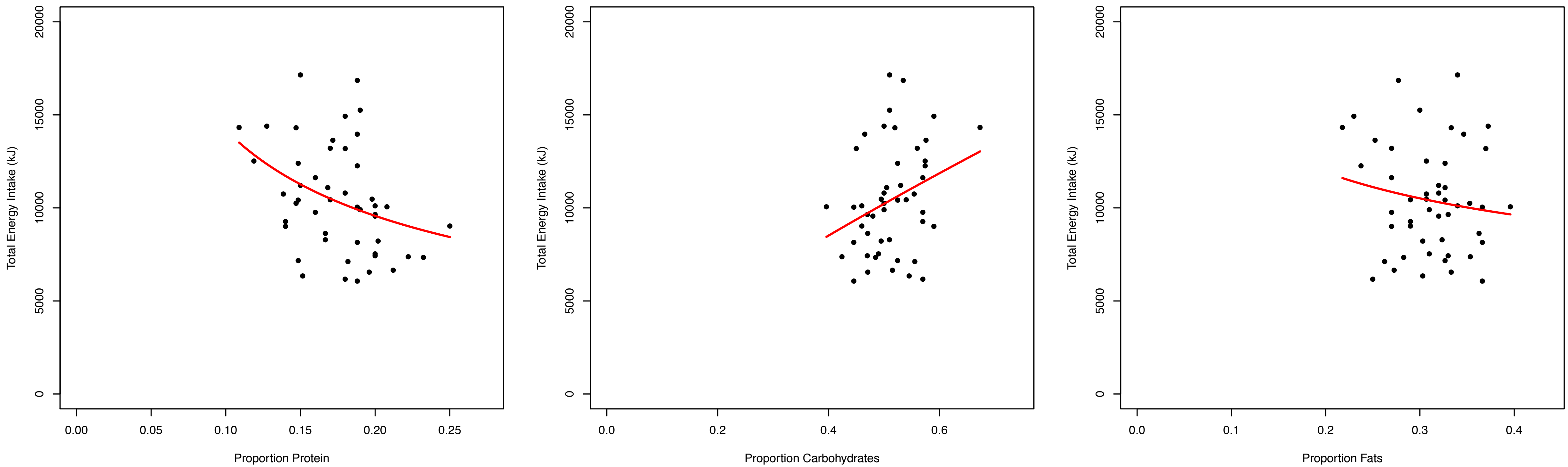
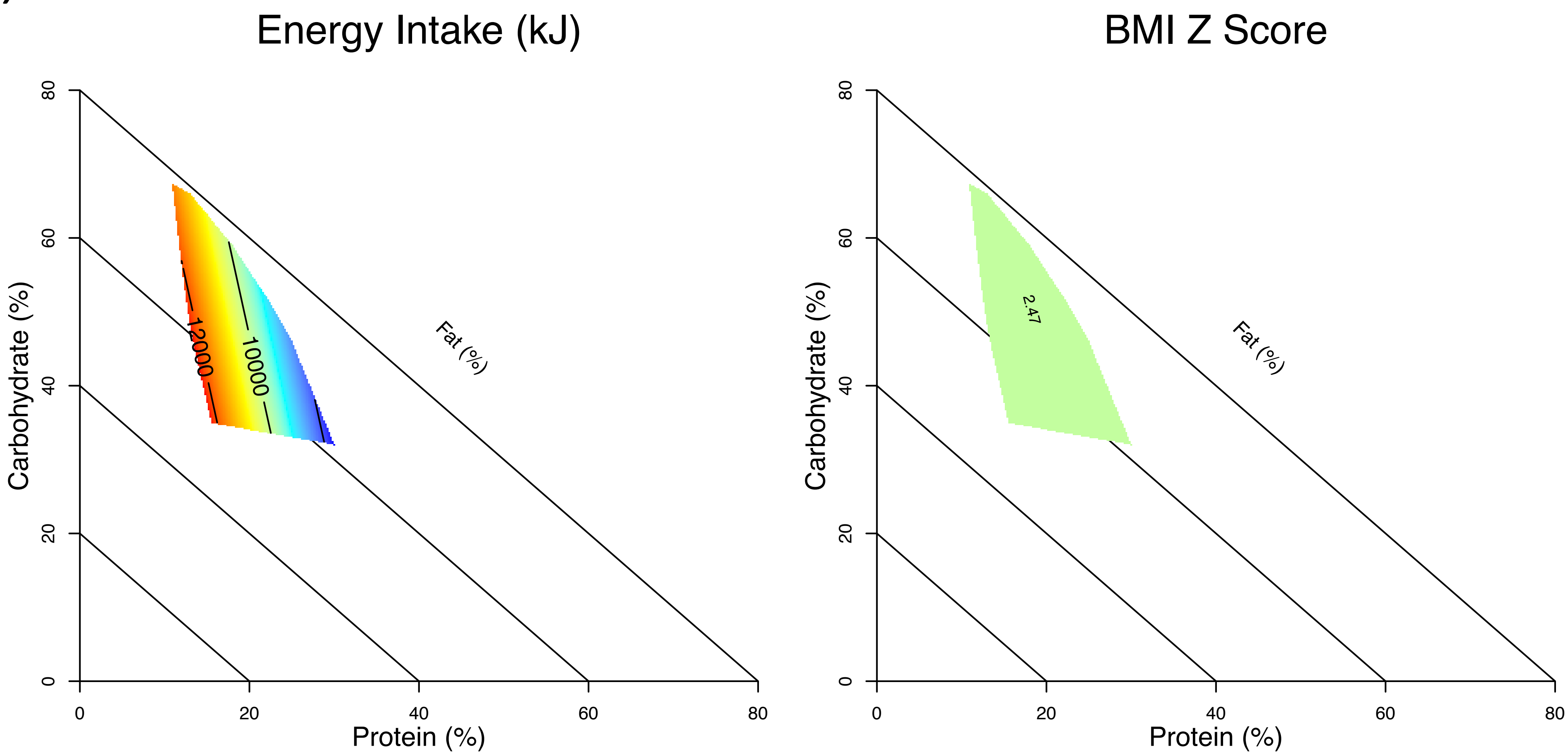


Figure 3: Effects from macronutrients composition on total energy intake and BMI z-scores

a) All individuals



b) Physically inactive subgroup