Neuropeptide Y gene variants in obesity, dietary intake, blood pressure, lipid and glucose metabolism: a longitudinal birth cohort study

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ABSTRACT

Objective: Neuropeptide Y affects several physiological functions, notably appetite regulation. We analysed the association between four single nucleotide polymorphisms (SNP) in the *NPY* gene (rs5574, rs16147, rs16139, rs17149106) and measures of obesity, dietary intake, physical activity, blood pressure, glucose and lipid metabolism from adolescence to young adulthood.

Methods: The sample included both birth cohorts of the Estonian Children Personality Behaviour and Health Study at ages 15 (n = 1075 with available complete data), 18 (n = 913) and 25 (n = 926) years. Linear mixed-effects regression models were used for longitudinal association between *NPY* SNP-*s* and variables of interest. Associations at ages 15, 18 and 25 were analysed by ANOVA.

Results: Rs5574 CC-homozygotes had a greater increase per year in waist-to-hip ratio (WHR) and a smaller decrease in daily energy intake and carbohydrate intake from age 15 to 25 years; fasting glucose and cholesterol were higher in rs5574 CC-homozygotes. Rs16147 TT-homozygotes had higher body weight and a greater increase in sum of 5 skinfolds, waist circumference, WHR and waist-to-height ratio; however, they had lower carbohydrate intake throughout the observation period. Rs16147 TT-homozygotes and both rs16139 and rs17149106 heterozygotes had higher triglyceride levels. All *NPY* SNP-s were associated with blood pressure: rs5574 TT-and rs16147 CC-homozygotes had a smaller increase in diastolic blood pressure, while rs16139 and rs17149106 heterozygous had lower blood pressure throughout the study.

Conclusion: Variants of the *NPY* gene were associated with measures of obesity, dietary intake, glucose and lipid metabolism and blood pressure from adolescence to young adulthood.

Keywords: neuropeptide Y, obesity, dietary intake, blood pressure, glucose metabolism, lipid metabolism

1. INTRODUCTION

Obesity develops, when energy intake exceeds its expenditure. It was estimated that in year 2015 the prevalence of obesity was 5% among children and 12% in adults [1]. Variables

such as genetics, biology of development, psychological factors, diet and exercise, and the environment contribute to the development of obesity [2].

Neuropeptide Y (NPY), a 36-amino acid neuropeptide encoded by the *NPY* gene, was first described by Tatemoto et al. (1982) almost 40 years ago [3]. NPY is widely expressed in the human CNS with the highest concentration found in the hypothalamic arcuate nucleus [4,5] as well as in the peripheral nervous system, the adrenal medulla, endothelium, immune cells, gut [6] and adipose tissue [7].

After its discovery, several groups demonstrated that in animal models, central administration of NPY markedly increased food intake [8,9] leading to an increase in fat mass and body weight [8,10,11]. Transgenic mice overexpressing NPY under the dopamine-beta-hydroxylase promoter (OE -NPY ^{DBH}) had increased adiposity, accompanied by impaired glucose tolerance and insulin resistance [12]. Moreover, in OE -NPY ^{DBH} mice, decreased fatty acid oxidation, accelerated cholesterol synthesis, hypercholesterolemia and hepatosteatosis were observed [13]. In humans, Sitticharoon et al. (2013) described a higher *NPY* mRNA expression in both subcutaneous and visceral adipose tissue in obese women compared to normal weight subjects; greater serum NPY levels were also observed [14]. NPY has a role in numerous other physiological functions including immune homeostasis [15], mood disorders [16], vasomotion, angiogenesis and cardiac remodeling [17]. NPY exerts its multiple effects through binding and activating its different receptor types: Y1, Y2, Y4, Y5, and Y6 [18]. Both Y1 and Y5 receptors in the paraventricular nucleus of the hypothalamus are involved in the regulation of food intake [19], Y1 receptors are related to immune function regulation [15] and Y1, Y2 and Y5 are the main cardiovascular homeostasis regulators [17].

Most common type of genetic variation are single nucleotide polymorphisms (SNP). Genome wide association studies have identified SNP-s in several genes, that have an effect

on body mass index (BMI) [20] and blood pressure [21] through different pathways [22]. It was demonstrated that a *NPY* SNP rs16147, located in the promoter region of the gene, altered NPY expression in vitro and accounted for more than half of the variation in expression in vivo [23]. Rs16147 C-allele was associated with decreased expression of mRNA in post-mortem brain and lymphoblasts [23], and lower levels of plasma NPY [23,24]. Other functional NPY gene variants have been described: Kallio et al. (2001) was the first to report the functional consequences of the rs16139 (T>C) substitution, demonstrating a higher overall plasma NPY concentration and faster heart rate [25]. Mitchell et al. (2008) replicated the results, showing that SNP rs16139 (T>C) in the *NPY* gene led to an increase in the levels of prohormone and elevated NPY secretion [26].

The effect of these variations in the *NPY* gene on human obesity and glucose and lipid metabolism has however been inconclusive. Zain et al. (2015) demonstrated that rs16147 TT-homozygotes had higher odds (OR 1.27; 95% CI 1.04, 1.55) for obesity, compared to CC-homozygotes [27], but Yeung et al. (2011) did not observe any association between rs16147 and obesity among 2071 women from the Nurses' Health Study (NHS) and 1268 men from the Health Professionals Follow-Up Study (HPFS) [28].

A few other *NPY* variants have been associated with obesity. *NPY* rs5574 TT-homozygotes had lower odds (OR 0.76; 95% CI 0.61, 0.96) for obesity, compared to CC-homozygotes [27]. Nevertheless, this was not observed in another study [28]. On the other hand, this investigation reported that *NPY* rs17149106 heterozygotes had higher odds (OR 1.72; 95% CI 1.20, 2.47) for obesity, compared with GG-homozygotes, and carriers of the rs16139 C-allele had higher odds (OR 1.79; 95% CI 1.24, 2.60) for obesity, compared to TT-homozygotes [28]. Findings were similar when comparing the mean difference in body mass index (BMI) by *NPY* genotype over the follow-up period (years 1986–2006) [28]. Rs16139

(T>C) was associated with higher BMI values in both men [29] and women [29,30], while cholesterol, triglycerides and blood pressure did not differ between genotype groups [30]. Another study by Karvonen et al. (2006) did not find an association between rs16139 and body weight in children, but observed an association with triglyceride levels [31].

We have addressed the potential association of *NPY* variants with body composition in young age, paying special attention to factors leading to overweight such as diet and physical activity. The association between four SNP-s of the *NPY* gene rs16139, rs5574, rs16147 and rs17149106 of which two pairs of SNP-s (rs16147 with rs5574 and rs17149106 with rs16139) are in strong linkage disequilibrium (r^2 = 0.93 and 0.90, respectively; D' = 0.96 and 0.94, respectively) [28] and measures of obesity, dietary intake, physical activity, blood pressure and glucose and lipid metabolism was analysed in a longitudinal birth cohort study from 15 to 25 years of age.

2. MATERIAL AND METHODS

2.1 Study Sample

The study sample was originally formed for the European Youth Heart Study (EYHS) (1998/1999) and later incorporated into the Estonian Children Personality Behaviour and Health Study (ECPBHS). The rationale and procedure for the original sample formation has been described in detail elsewhere [32]. In brief, ECPBHS is a longitudinal cohort study with a population representative sample of participants, all of European descent, with school as the sampling unit (original n=1238). All schools of Tartu County, Estonia, that agreed to participate (54 of the total of 56) were included into the sampling and 25 schools were selected. All children from grades 3 (younger birth cohort, aged 9 years) and grades 9 (older birth cohort, aged 15 years) were invited to participate [33]. Follow-up studies for the

younger birth cohort took place in year 2004 at age 15 years (n = 483), in year 2007 at age 18 years (n = 454) and in year 2014 at age 25 years (n = 441) and for the older birth cohort in year 2001 at age 18 years (n = 417 + additional 62) and in year 2008 at 25 years (n = 541). The sample of this analysis comprises of non-pregnant individuals with available data on anthropometric measurements, blood pressure, metabolic biomarkers, physical activity, dietary intake and rs16139, rs5574, rs16147 and rs17149106 genotype at ages 15, 18 and 25 years. For the analysis, data from both birth cohorts of the ECPBHS was merged, with available complete data as follows: age 15 (n = 1075), 18 (n = 913) and 25 (n = 926).

Before participation, written informed consent was obtained from the subjects and, in case of subjects aged < 18 years, also from their parents. The study was approved by the Ethics Review Committee on Human Research of the University of Tartu and conducted in accordance with the Declaration of Helsinki.

2.2 Anthropometric measurements, blood pressure and metabolic biomarkers

Height and weight were measured using standardized procedures and body mass index (BMI) was calculated as weight / height squared (kg/m^2). Skinfold thickness was taken at the biceps, triceps, subscapular, suprailiac and medial calf areas on the left side of the body using a Harpenden caliper (Baty, West Sussex, England). Waist circumference (WC) was measured between the lower rib margin and the iliac crest, at the end of gentle expiration and hip circumference (HC) at the widest part of the gluteal region. Waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were calculated as waist circumference / hip circumference (units) and waist circumference / height (units), respectively. All anthropometrical measurements were taken twice and a mean value was used.

Resting systolic and diastolic blood pressure (BP) was obtained from the left arm with an automatic oscillometric method in a sitting position. A mean value of five consecutive measurements at 2 min intervals was used.

Venous blood samples were taken from the antecubital vein after an 8–12 h fast and analysed in a certified clinical laboratory.

2.3 Dietary intake

During the day(s) before the study day, the subjects were asked to complete a 24h (year 1998), 48h (years 2001, 2004, 2007) or 72h (years 2008, 2014) diet record at home. On the study day portion sizes, that were not recorded in the food diary, were elaborated using pictures of portion sizes [34] during a face-to-face interview. The procedure for the assessment of dietary intake has been described in detail elsewhere [35].

2.4 Cardiorespiratory fitness (CRF) and physical activity

CRF was determined by a cycle-ergometer test and defined as maximum power output (MPO) per kilogram of body weight (MPO/kg). The protocol for aerobic fitness originates from the European Youth Heart Study [36] and the procedure has been described in detail elsewhere [37].

Physical activity was assessed using self- and parent-reported questionnaires. Individual physical activity scores were calculated and standardized physical activity scores (z-scores) were used. The formation of physical activity score has been described in detail elsewhere [37].

2.5 Genotyping of *NPY* rs5574, rs16147, rs16139 and rs17149106

Genomic DNA was extracted from venous blood samples using Qiagen QIAamp® DNA Blood Midi Kit. The real-time polymerase chain reaction (RT-PCR) for genotyping the *NPY* rs5574, rs16147, rs16139 and rs17149106 polymorphisms was performed using a TaqMan Pre-Designed SNP Genotyping Assay (Applied Biosystems; Foster City, CA, USA) containing primers and fluorescent probes. Context sequence [VIC/FAM] for rs5574 was as follows: TTTTTTCCAGATATGGAAAACGATC[C/T]AGCCCAGAGACACTGATTTCAGACC; for rs16147: GCTTCCTACTCCGGCACCCAGTGGG[C/T]TGGTAGTCCTGTTGGCAGGAGACAA; for rs16139: CTGCAGATGCTAGGTAACAAGCGAC[C/T]GGGGCTGTCCGGACTGACCCTCGCC; for rs17149106: CCCCTGAAACCACGGGCGGGGGTGG[G/T]GTGGGGAGCGCAGCTTTGGGACCCT. Genotyping reactions were performed in a total volume of 10 µl with ~25 ng of template DNA. RT-PCR reaction components and final concentrations were as follows: 1:5 5 x HOT FIREPol® Probe qPCR Mix Plus (ROX) (Solis BioDyne) and 1:20 80 x TaqMan Primers Probe. Reactions were performed on the Applied Biosystems ViiA™ 7 Real-Time PCR System. The amplification procedure consisted of an initial denaturation step at 95 °C for 12 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Positive and negative controls were added to each reaction plate. No inconsistencies occurred. Genotyping was performed blind to all phenotypic data. Allele frequencies agreed with National Center for Biotechnology Information database and published reports.

2.6 Statistical analysis

Statistical analysis was performed using Stata 14 software (StataCorp LP, College Station, Texas, USA). Level of significance was set at 0.05.

Linear mixed-effects regression models with random intercept and random slope were used to assess the association between *NPY* rs5574, rs16147, rs16139, rs17149106 and measures of obesity, dietary intake, BP, glucose and lipid metabolism, CRF and physical activity. Mixed modelling procedure considers the correlations between repeated measurements within each subject and allows the inclusion of subjects with different number of observations, assuming that the missingness is random [38]. Measures of obesity, dietary intake, BP, glucose- and lipid metabolism, CRF and physical activity at baseline (age 15 years) and at follow up points (18 years and 25 years) were inserted as dependent variables and *NPY* rs5574, rs16147, rs16139 and rs17149106 genotypes as the independent variables. Time was treated as continuous.

Exchangeable or unstructured covariance structure and restricted maximum likelihood method was used. Likelihood-ratio test was used to assess the goodness of fit of the statistical models. Interaction with time was not included in the final model if interaction with time lacked significance and the likelihood-ratio test did not show superiority of the more complicated model. Models were not adjusted for physical activity, because the association between *NPY* SNPs and physical activity was not significant.

3. RESULTS

3.1 *NPY* rs5574, rs16147, rs16139 and rs17149106 genotype distribution

Genotype data for *NPY* rs5574, rs16147, rs16139 and rs17149106 were available at different ages as follows: 15 years n = 1075 (44.7% male), 18 years n = 913 (43.7% male), and 25 years n = 926 (45.1% male). The distribution of TT, CT and CC genotypes of the rs5574 in the studied sample at age 15 years was 19.9%, 51.1%, 29.0% and of the rs16147 was 24.1%, 51.4% and 24.5%, respectively. The distribution of the rs16139 CC, CT and TT genotypes at

age 15 years was 0.6%, 12.5%, 86.8% and rs17149106 GG, GT, TT 86.6%, 12.8% and 0.6%, respectively. Distribution of men and women did not differ between genotype groups (Supplementary Table 1). Rare homozygotes of the rs16139 and rs17149106 were not included in the statistical analysis, because of the very low prevalence and highly variable results that on average substantially differed from heterozygotes. Genotype frequencies were in Hardy–Weinberg equilibrium.

3.2 Measures of obesity and abdominal obesity

In linear mixed-effects regression models with time × rs5574 interaction a smaller increase ($p = 0.015$ for interaction) per year in WHR was observed in TT-homozygotes compared to CC-homozygotes from 15 to 25 years of age (Tables 1 and 3).

During the study period, rs16147 CC-homozygotes compared to TT-homozygotes had a significantly ($p = 0.013$) lower body weight (Table 1). In the study sample, the mean change in body weight was 1.30 kg (95% CI 1.23, 1.37) per year.

A smaller ($p < 0.05$ for interaction) increase in sum of 5 skinfolds, WHR and WHtR per year was observed in heterozygotes and CC-homozygotes, compared to TT-homozygotes in models with time × rs16147 interaction. Heterozygotes compared to TT-homozygotes had a smaller ($p = 0.034$ for interaction) increase per year in WC (Tables 1 and 3).

No longitudinal association between measures of obesity or abdominal obesity and rs16139 or rs17149106 was observed (Table 2).

A one-way ANOVA test at ages 15, 18 and 25 years revealed several associations between weight, BMI, WC, WHR and rs16147 genotype. Rs16147 CC-homozygotes had significantly lower body weight at age 15 years (by –2.45 kg; 95% CI –0.27, 4.63; $p = 0.021$), 18 years (by –2.97 kg; 95% CI –0.17, –5.78; $p = 0.033$) and 25 years (by –4.00 kg; 95% CI –

0.47, –7.54; p = 0.020), compared to TT-homozygotes. At age 25 years CC-homozygotes had significantly lower BMI (by –0.95 kg/m^2; 95% CI –0.01, –1.89; p = 0.046) and WC (by – 2.92 cm; 95% CI –0.36, –5.49; p = 0.019) and both heterozygotes and CC homozygotes had smaller WHR (by –0.01 units; 95% CI –0.001, –0.03; p = 0.033 and by –0.02 units; 95% CI –0.001, –0.003; p = 0.027) compared to TT-homozygotes, respectively (Supplementary Table 3).

An association between obesity, abdominal obesity and rs5574, rs16139 or rs17149106 was not observed by one-way ANOVA at ages 15 years, 18 years and 25 years (Supplementary Tables 2 and 4–5).

3.3 Blood pressure

In models with time × rs5574 and time × rs16147 interaction a smaller ($p < 0.05$ for interaction) increase per year in diastolic blood pressure was observed in TT-homozygotes compared to CC-homozygotes and CC-homozygotes, compared to TT-homozygotes, respectively (Tables 1 and 3).

Both rs16139 and rs17149106 heterozygotes had significantly ($p < 0.05$) lower systolic- and diastolic blood pressure from 15 to 25 years of age compared to TT-homozygotes and GG-homozygotes, respectively (Table 2). In the analysed sample, the mean change per year was 0.49 mmHg (95% CI 0.40, 0.57) in systolic blood pressure and 0.43 mmHg (95% CI 0.38, 0.49) in diastolic blood pressure.

Rs16139 heterozygotes had significantly lower diastolic blood pressure at age 18 years (by –2.41 mmHg; 95% CI –3.91, 0.91; p = 0.002), according to one-way ANOVA (Supplementary Table 4).

3.4 Metabolic biomarkers

According to the models, rs5574 heterozygotes compared to CC-homozygotes had significantly ($p = 0.011$) lower levels of fasting blood glucose and both rs16147 heterozygotes and CC-homozygotes had lower ($p < 0.05$) fasting blood glucose compared to TT-homozygotes, from age 15 to 25 years (Table 1).

Interestingly, cholesterol and HDL-cholesterol levels were higher ($p < 0.05$) in rs5574 heterozygotes compared to CC-homozygotes and in rs16147 heterozygotes, compared to TT-homozygotes from 15 to 25 years of age (Table 1).

Rs17149106 heterozygotes had higher ($p = 0.033$) cholesterol levels, compared to GG-homozygotes from age 15 to 25 years of age (Table 2). Models with time × rs16139 and time × rs17149106 interaction demonstrated a greater ($p < 0.05$ for interaction) increase per year in heterozygotes in triglyceride levels (Tables 2–3).

Mean change in fasting blood glucose was –0.005 mmol/L (95% CI –0.001, 0.009), cholesterol was 0.04 mmol/L (95% CI 0.04, 0.05) and HDL-cholesterol was 0.007 mmol/l (95% CI 0.005, 0.009) per year, in the analysed sample.

According to one-way ANOVA rs5574 heterozygotes had higher (0.016 mmol/l; 95% CI 0.003, 0.309; $p = 0.045$) cholesterol levels compared to CC-homozygotes and rs16139 heterozygotes had higher cholesterol (by 0.23 mmol/l; 95% CI 0.07, 0.40; $p = 0.006$) and LDL-cholesterol (by 0.16 mmol/l; 0.02, 0.30; $p = 0.021$) levels, compared to TT-homozygotes at age 18 years. Higher cholesterol (by 0.25 mmol/; 95% CI 0.09, 0.42; $p = 0.003$) and LDL-cholesterol (by 0.19 mmol/l, 95% CI 0.05, 0.32; $p = 0.007$) levels, at age 18 years, were also observed in rs17149106 heterozygotes compared to GG-homozygotes (Supplementary Tables 2, 4–5).

3.5 Dietary intake

Linear mixed-effects regression models with time × rs5574 interaction demonstrated a significant ($p < 0.05$) difference in the rate of change per year in daily energy intake and carbohydrate intake between CC-homozygotes and TT-homozygotes, the latter having a larger decrease per year (Tables 1 and 3).

From age 15 to 25 years, rs5574 heterozygotes had significantly ($p < 0.002$) lower protein intake (E%), compared to CC-homozygotes (Table 1).

Surprisingly, CC-homozygotes of the rs16147 had greater ($p = 0.029$) carbohydrate intake from 15 to 25 years of age, compared to TT-homozygotes. During the study period both rs16147 heterozygotes and CC-homozygotes had greater ($p < 0.05$) carbohydrate intake (E%), compared to TT-homozygotes. Lower protein (E%) intake was observed in heterozygotes, compared to TT-homozygotes (Table 1).

Rs16139 and rs17149106 heterozygotes had significantly ($p < 0.05$) lower protein intake compared to TT-homozygotes and GG-homozygotes from 15 to 25 years of age, respectively (Table 2). In models with time × rs16139 and time × rs17149106 interaction a smaller increase in the rate of change per year in protein (E%) intake was observed in heterozygotes compared to TT-homozygotes and GG-homozygotes, respectively (Tables 2–3).

In the study sample, the mean change per year in carbohydrate intake (g) was –8.73 g (95% CI –9.70, –7.77), protein (g) intake was 0.88 g (95% CI 0.63, 1.14), protein (E%) intake was 0.30 E% (95% CI 0.27, 0.34), and carbohydrate (E%) intake was –0.55 E% (95% CI 0.48, 0.62).

At age 15 years, rs5574 CC-homozygotes had lower daily energy intake (by –196.29 kcal/day; 95% CI –2.47, –390.10; $p = 0.046$) and carbohydrate intake (by –0.59 g/kg; 95% CI –

0.14, –1.05; p = 0.006), compared to TT-homozygotes, according to one-way ANOVA. Heterozygotes, compared to CC-homozygotes, had significantly lower protein intake (E%) (by 0.68 E%; 95% CI 0.12, 1.24; p = 0.011) at age 18 years (Supplementary Table 2).

Rs16147 heterozygotes had significantly lower protein intake (E%) (by –0.78 E%; –0.18, –1.37; p = 0.005), compared to TT-homozygotes and both heterozygotes (by 2.03 E%; 0.33, 3.72; p = 0.013) and CC-homozygotes (by 2.00 E%; 0.06, 3.94; p = 0.041) had larger carbohydrate intake (E%), compared to TT-homozygotes at age 18 years (Supplementary Table 3).

Rs16139 heterozygotes had lower ($p < 0.05$) protein intake (g), compared to TT-homozygotes at age 15 years (by –5.91 g; 95% CI –11.74, –0.09; p = 0.047) and 25 years (by –6.74 g; 95% CI – 13.33, – 0.15; p = 0.045) (Supplementary Table 4) and at age 25 years rs17149106 heterozygotes had lower protein intake (by –7.09 g; 95% CI –13.68, –0.50; p = 0.035 and by –1.03 E%; 95% CI –1.82, –0.23; p = 0.011) compared to GG-homozygotes (Supplementary Table 5).

3.6 MPO and physical activity

Linear mixed effects regression models did not identify statistically significant longitudinal associations between MPO, physical activity and rs5574, rs16147, rs16139 and rs17149106 genotypes.

Rs16139 heterozygotes had lower MPO (by –0.17 per kg; –0.34, 0.02; p = 0.026) compared to GG-homozygotes, at 25 years of age (Supplementary Table 4).

4. DISCUSSION

Studies describing the association between *NPY* genetic variants and weight gain or obesity have been inconclusive, some showing an association [27,29,30,39] while others demonstrating no effect [28,31]. However, studies analysing the association between variants of the *NPY* and obesity have differed regarding the age of the population under investigation, so this should be considered as a possible reason for variation.

A case-cohort study by Zain et al. (2015) recruited 1113 apparently healthy children aged 13 years and demonstrated that the frequency of the rs16147 T-allele was significantly higher in overweight and obese children compared to controls (OR 1.27; 95% CI 1.04, 1.55; p = 0.022), whereas the prevalence of the rs5574 T-allele was significantly higher in the controls (OR 0.76; 95% CI 0.61, 0.96; p = 0.020) [27]. Indeed, it has been observed that the two pairs of SNPs, rs17149106 with rs16139 (r^2 = 0.93, D' = 0.96) and rs16147 with rs5574 (r^2 = 0.90, D'= 0.94), are in strong linkage disequilibrium [28], and this fits well with the results in this study. Similarly, Olza et al. (2013) observed an association between the rs16147 T-allele and obesity (OR 1.38; 95% CI 1.08–1.78 for allelic effect) among Spanish children in a multicenter case-control study [39] that did not genotype rs5547.

In contrast, Yeung et al. (2011) did not observe any association between rs16147 or rs5574 and obesity in middle aged men and women. Instead, they demonstrated an increase in the risk of obesity among rs17149106 heterozygotes compared to GG-homozygotes and rs16139 C-allele carriers compared to TT-homozygotes [28]. Similarly, Ding et al. (2005) observed that among normal weight adults, rs16139 C-allele (n = 1246) carriers had higher BMI, with no gender disparity [30] and van Rossum et al. (2006) showed that the risk of overweight was greater among rs16139 C-allele carriers (OR 3.3; 95% CI 1.2; 8.9) [29]. In contrast, no association between rs16139 genotype and obesity has been reported in children [31].

We observed an association between rs5574 and measures of abdominal obesity, where CC-homozygotes had a greater increase per year in WHR from adolescence to young adulthood. Rs16147 TT-homozygotes had higher body weight throughout the study period and a greater increase in sum of five skinfolds, waist circumference, WHR and WHtR from age 15 to 25 years. No association between rs16139 or rs17149106 and body weight, BMI, waist and hip circumference or their ratio was observed at this age. Thus, our findings are consistent with literature with regards to the association of these *NPY* variants with obesity that have previously been positive at a young age, and the absence of association with those variants for which evidence was obtained in studies on middle-aged samples.

Genome wide association studies, including subjects of very heterogenous age groups, have not reported any association between *NPY* gene variants and obesity [20,40,41]. However, Winkler et al. (2015) demonstrated in a genome wide study the age-specificity of association of genetic variants contributing to BMI, as some had larger effects in younger (< 50 years) and other in older adults (≥ 50 years) [42]. This would be compatible with the effect of *NPY* rs5574 and rs16147 on measures of obesity being pronounced in childhood and young adulthood, while the effect of rs16139 and rs17149106 would develop later in adulthood. However, our sample is, for the time being, limited with the age 25 being the last point of observation. It should also be noted that the sample of ECPBHS consists of individuals of only European descent and therefore we cannot be sure if the associations are similar in other ethnicities.

In terms of the mechanism of increased weight gain, we have observed that the rs5574 CC-homozygotes had a smaller decrease in daily energy intake (kcal/day) and carbohydrate intake (g) per year from age 15 to 25 years. Inversely, TT-homozygotes of the rs16147 had lower carbohydrate intake at every timepoint from childhood to young

adulthood despite of their higher indicators of fat content; however, the overall energy intake was not different between genotypes. Despite suggestive results in animal studies, our present study and others [28,31] have not been able to demonstrate a clear relationship with energy intake. Thus, while some of our results appear to suggest that the NPY-mediated obesity is simply related to daily energy intake and carbohydrate intake, others are not in line with this notion. Considering the extensive physiological and homeostatic role of NPY in the mammalian organism, it is probable that in addition to food intake NPY plays a role in the development of obesity through other pathways, such as lipolysis inhibition [43], which need further study.

It has been proposed that NPY and its receptor system has a causal role in the development of type 2 diabetes and atherosclerotic cardiovascular disease [44]. Animal and *in vitro* studies have described an inhibition of glucose dependent insulin release by NPY treatment [45,46] and an increase in *NPY* mRNA expression in the arcuate nucleus in mice with gestational diabetes [47]. In humans, it has been demonstrated in middle aged and older subjects from Finnish and Dutch ancestry that regarding the rs16139 variation (T>C), the minor C-allele associated with higher NPY release was also associated with accelerated increase of the common carotid artery intima-media thickness, greater systolic and diastolic blood pressure [48], higher serum cholesterol and LDL-cholesterol levels [49] and worse glycemic control, higher triglyceride levels and higher prevalence of coronary heart disease [50].

Although we did not find any association between rs16139 and increased weight gain or higher WHR, we observed a greater increase per year in triglyceride levels in rs16139 heterozygotes compared to TT-homozygotes and rs17149106 heterozygotes compared to GG-homozygotes. In addition, we found that rs5574 CC-homozygotes and rs16147 TT-

homozygotes had higher fasting blood glucose levels. Then, our results regarding cholesterol, indicating higher levels in rs5574 heterozygotes compared to CC-homozygotes and rs16147 heterozygotes compared to TT-homozygotes remain hard to interpret, and should remain in waiting of whether they can be independently replicated.

Previously, several SNP-s in genes that encode proteins in secretory pathways and have been associated with psychiatric and metabolic diseases have also been associated with blood pressure. For prominent examples, SNP-s in the *CHGA* [51] and *CHGB* [52,53] genes have been associated with schizophrenia. Reduced levels of chromogranin A and B in the cerebrospinal fluid in patients with schizophrenia, have been noted [54]. In addition, genetic variation in *CHGA* have also been associated with blood pressure [55,56]. For example, rs7610 was linked with hypertensive renal disease [57] and rs9658667 with hypertension [58,59]. Similarly, polymorphisms rs2821 [60] and rs236140 [61] in the *CHGB* gene were linked with high blood pressure and rs1017448 in the *SCG2* gene was associated with blood pressure elevation [62]. We found associations between all the four *NPY* SNP-s and blood pressure. Rs5574 CC-homozygotes and rs16147 TT-homozygotes had a higher increase in diastolic blood pressure from 15 to 25 years of age. However, our results also demonstrated that contrary to the findings in middle aged and older adults [48,50], from adolescence to young adulthood the rs16139 heterozygotes and rs17149106 heterozygotes had lower systolic and diastolic blood pressure as compared to the TT and GG homozygotes.

It has been demonstrated that both food restriction and physical activity induced negative energy balance led to an increase in hypothalamic NPY concentration [63], yet in rats with streptozotocin induced diabetes, low intensity physical activity had a suppressive effect on the hypothalamic NPY expression [64]. We did not identify any association between NPY SNP-s and physical activity. However, rs16139 heterozygotes compared to GG-

homozygotes had a lower MPO, at 25 years of age, indicating a poorer cardiorespiratory fitness.

5. CONCLUSION

Our results confirm some of previous findings and, together with literature, suggest that the effect of *NPY* variants on measures of obesity may be age-related and thus should be further studied longitudinally over a broad age range. It is likely that at least partially, food intake mediates the NPY-associated obesity. Nevertheless, because of conflicting results, other pathways should be explored. Our results support the association between NPY and glucose- and lipid metabolism. The association between *NPY* variants and blood pressure needs further study where age, as a modifying factor, is considered.

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Table 1. Estimated main effects (mean and 95% CI) and estimated main and interaction effects (mean and 95% CI) in anthropometric measurements, dietary intake and physical activity of the ECPBHS sample from 15 to 25 years of age between *NPY* rs557*4* and rs16147 genotype according to the linear mixed-effects regression model.

	rs5574				rs16147		
	Coefficient	**95% CI**	**p value**		**Coefficient**	**95% CI**	**p value**
Body weight (kg) [a]				**Body weight (kg)** [c]			
CT genotype	–1.107	–2.518; 0.305	0.124	CT genotype	–1.318	–2.814; 0.179	0.084
TT genotype	–1.610	–3.372; 0.151	0.073	CC genotype	–2.195	–3.931; –0.459	**0.013**
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
BMI (kg/m²) [a]				**BMI (kg/m²)** [c]			
CT genotype	–0.150	–0.541; 0.242	0.454	CT genotype	–0.204	–0.619; 0.212	0.337
TT genotype	–0.402	–0.892; 0.087	0.107	CC genotype	–0.437	–0.920; 0.045	0.076
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Sum of 5 skinfolds (mm) [a]				**Sum of 5 skinfolds (mm)** [d]			
CT genotype	–0.033	–3.662; 3.595	0.986	CT genotype	8.821	–0.493; 18.135	0.063
TT genotype	–1.567	–6.096; 2.961	0.498	CC genotype	9.297	–1.473; 20.068	0.091
CT genotype × time				CT genotype × time	–0.506	–1.010; –0.001	**0.049**
TT genotype × time				CC genotype × time	–0.614	–1.196; –0.033	**0.038**
WC (cm) [a]				**WC (cm)** [d]			
CT genotype	–0.636	–1.539; 0.266	0.167	CT genotype	1.624	–0.682; 3.930	0.167
TT genotype	–0.757	–1.884; 0.370	0.188	CC genotype	1.440	–1.220; 4.101	0.289
CT genotype × time				CT genotype × time	–0.152	–0.293; –0.011	**0.034**
TT genotype × time				CC genotype × time	–0.162	–0.324; 0	0.050
HC (cm) [a]				**HC (cm)** [c]			
CT genotype	–0.146	–1.010; 0.718	0.740	CT genotype	–0.281	–1.197; 0.635	0.548
TT genotype	–0.797	–1.874; 0.281	0.147	CC genotype	–1.045	–2.107; 0.018	0.054
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
WHR (units) [b]				**WHR (units)** [d]			
CT genotype	0.0028	–0.0117; 0.0173	0.705	CT genotype	0.0086	–0.0068; 0.0241	0.272
TT genotype	0.0164	–0.0016; 0.0344	0.074	CC genotype	0.0189	0.0011; 0.0367	**0.038**
CT genotype × time	–0.0006	–0.0014; 0.0002	0.129	CT genotype × time	–0.0010	–0.0018; –0.0001	**0.026**
TT genotype × time	–0.0012	–0.0022; –0.0002	**0.015**	CC genotype × time	–0.0014	–0.0024; –0.0004	**0.004**
WHtR (units) [a]				**WHtR (units)** [d]			
CT genotype	–0.0016	–0.0064; 0.0032	0.514	CT genotype	0.0136	0.0003; 0.0268	**0.044**
TT genotype	–0.0031	–0.0091; 0.0029	0.314	CC genotype	0.0126	–0.0027; 0.0278	0.107
CT genotype × time				CT genotype × time	0.0010	–0.0018; –0.0002	**0.016**

TT genotype × time				CC genotype × time	–0.0010	–0.0019; –0.0001	**0.034**
Systolic BP (mmHg) [a]				**Systolic BP (mmHg)** [c]			
CT genotype	–0.831	–2.223; 0.561	0.242	CT genotype	–0.819	–2.295; 0.658	0.277
TT genotype	0.144	–1.592; 1.880	0.871	CC genotype	0.201	–1.511; 1.912	0.818
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Diastolic BP (mmHg) [b]				**Diastolic BP (mmHg)** [d]			
CT genotype	1.193	–1.134; 3.728	0.356	CT genotype	1.350	–1.344; 4.044	0.326
TT genotype	3.497	0.361; 6.634	**0.029**	CC genotype	3.115	0.007; 6.223	**0.049**
CT genotype × time	–0.056	–0.193; 0.081	0.425	CT genotype × time	–0.071	–0.217; 0.074	0.336
TT genotype × time	–0.193	–0.362; –0.024	**0.025**	CC genotype × time	–0.169	–0.336; –0.001	**0.049**
Glucose (mmol/L) [a]				**Glucose (mmol/L)** [c]			
CT genotype	–0.066	–0.116; –0.015	**0.011**	CT genotype	–0.060	–0.113; –0.008	**0.023**
TT genotype	–0.045	–0.107; 0.018	0.162	CC genotype	–0.071	–0.132; –0.011	**0.021**
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Insulin (mU/L) [a]				**Insulin (mU/L)** [c]			
CT genotype	–0.339	–0.892; 0.214	0.229	CT genotype	–0.509	–1.115; 0.097	0.100
TT genotype	0.146	–0.540; 0.832	0.678	CC genotype	–0.048	–0.747; 0.651	0.892
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Cholesterol (mmol/L) [a]				**Cholesterol (mmol/L)** [c]			
CT genotype	0.102	0.009; 0.194	**0.031**	CT genotype	0.112	0.014; 0.211	**0.025**
TT genotype	0.042	–0.073; 0.158	0.470	CC genotype	0.061	–0.052; 0.175	0.291
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
HDL-cholesterol (mmol/L) [a]				**HDL-cholesterol (mmol/L)** [c]			
CT genotype	0.041	0.001; 0.082	**0.046**	CT genotype	0.027	–0.016; 0.070	0.221
TT genotype	0.028	–0.023; 0.078	0.282	CC genotype	0.028	–0.022; 0.078	0.272
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
LDL-cholesterol (mmol/L) [a]				**LDL-cholesterol (mmol/L)** [c]			
CT genotype	0.045	–0.042; 0.132	0.306	CT genotype	0.068	–0.024; 0.160	0.149
TT genotype	–0.005	–0.113; 0.103	0.929	CC genotype	0.022	–0.085; 0.128	0.692
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Triglycerides (mmol/L) [a]				**Triglycerides (mmol/L)** [c]			
CT genotype	0.006	–0.044; 0.056	0.825	CT genotype	0.006	–0.047; 0.059	0.822
TT genotype	–0.020	–0.082; 0.042	0.528	CC genotype	–0.002	–0.063; 0.060	0.954
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			

Energy intake (kcal) [b1]				**Energy intake (kcal)** [c1]			
CT genotype	121.522	–178.810; 421.854	0.428	CT genotype	77.581	–241.493; 396.654	0.634
TT genotype	459.346	87.135; 831.557	**0.016**	CC genotype	355.584	–13.657; 724.825	0.059
CT genotype × time	–4.959	–18.945; 9.026	0.487	CT genotype × time	–3.384	–18.235; 11.467	0.655
TT genotype × time	–17.687	–34.921; –0.452	**0.044**	CC genotype × time	–14.721	–31.866; 2.423	0.092
Protein (g) [a1]				**Protein (g)** [c1]			
CT genotype	–1.218	–4.499; 2.063	0.467	CT genotype	–1.932	–5.416; 1.552	0.277
TT genotype	2.507	–1.565; 6.578	0.228	CC genotype	0.338	–3.695; 4.371	0.869
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Lipids (g) [a1]				**Lipids (g)** [c1]			
CT genotype	0.192	–3.764; 4.147	0.924	CT genotype	–0.915	–5.114; 3.285	0.669
TT genotype	3.704	–1.187; 8.595	0.138	CC genotype	1.555	–3.298; 6.408	0.530
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Carbohydrates (g) [b1]				**Carbohydrates (g)** [c1]			
CT genotype	15.486	–23.180; 54.152	0.432	CT genotype	6.473	–4.314; 17.261	0.240
TT genotype	64.996	17.285; 112.707	**0.008**	CC genotype	13.920	1.439; 26.402	**0.029**
CT genotype × time	–0.458	–2.468; 1.553	0.656	CT genotype × time			
TT genotype × time	–2.536	–5.005; –0.067	**0.044**	CC genotype × time			
Protein E% [a]				**Protein E%** [c]			
CT genotype	–0.489	–0.793; –0.186	**0.002**	CT genotype	–0.542	–0.864; –0.220	**0.001**
TT genotype	–0.203	–0.580; 0.173	0.290	CC genotype	–0.307	–0.679; 0.065	0.106
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Lipids E% [a]				**Lipids E%** [c]			
CT genotype	–0.280	–0.934; 0.374	0.401	CT genotype	–0.646	–1.337; 0.046	0.067
TT genotype	–0.338	–1.147; 0.470	0.412	CC genotype	–0.707	–1.504; 0.091	0.082
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
Carbohydrates E% [a]				**Carbohydrates E%** [c]			
CT genotype	0.664	–0.113; –1.441	0.094	CT genotype	1.508	0.221; 1.895	**0.013**
TT genotype	0.530	–0.432; 1.492	0.280	CC genotype	1.021	0.055; 1.987	**0.038**
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			
MPO/kg [a]				**MPO/kg** [c]			
CT genotype	–0.058	–0.144; 0.028	0.183	CT genotype	–0.051	–0.143; 0.040	0.269
TT genotype	0.036	–0.071; 0.143	0.510	CC genotype	0.009	–0.097; 0.114	0.873
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			

Standard. PA score [a]				**Standard. PA score** [c]			
CT genotype	0.045	–0.051; 0.141	0.361	CT genotype	0.012	–0.090; 0.114	0.821
TT genotype	0.030	–0.089; 0.149	0.617	CC genotype	0.007	–0.111; 0.124	0.912
CT genotype × time				CT genotype × time			
TT genotype × time				CC genotype × time			

[a] Coefficient can be interpreted as the mean difference in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between rs5574 CT and CC genotype or between TT and CC genotype at each timepoint.

[b] Difference in the rate of change in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between rs5574 CT and CC genotype or between TT and CC genotype can be calculated as the sum of main effect coefficient and time × interaction coefficient at given timepoint.

[c] Coefficient can be interpreted as the mean difference in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between rs16147 CT and TT genotype or between CC and TT genotype at each timepoint.

[d] Difference in the rate of change in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between rs16147 CT and TT genotype or between CC and TT genotype can be calculated as the sum of main effect coefficient and time × interaction coefficient at given timepoint.

[1] adjusted for body weight

BMI – body mass index; WC – waist circumference; HC – hip circumference; WHR – waist-to-hip ratio; WHtR – waist-to-height ratio; BP – blood pressure; MPO – maximum power output; PA – physical activity

Table 2. Estimated main effects (mean and 95% CI) and estimated main and interaction effects (mean and 95% CI) in anthropometric measurements, dietary intake and physical activity of the ECPBHS sample from 15 to 25 years of age between *NPY* rs16139 and *NPY* rs17149106 genotype according to the linear mixed-effects regression model.

		rs16139				rs17149106	
	Coefficient	**95% CI**	**P value**		**Coefficient**	**95% CI**	**P value**
Body weight (kg) [e]				**Body weight (kg)** [g]			
CT genotype	–1.714	–3.538; 0.110	0.066	GT genotype	–1.588	–3.402; 0.226	0.086
CT genotype × time				GT genotype × time			
BMI (kg/m²) [e]				**BMI (kg/m²)** [g]			
CT genotype	–0.183	–0.691; 0.324	0.479	GT genotype	–0.098	–0.603; 0.407	0.704
CT genotype × time				GT genotype × time			
Sum of 5 skinfolds (mm) [e]				**Sum of 5 skinfolds (mm)** [g]			
CT genotype	1.985	–2.715; 6.686	0.408	GT genotype	2.539	–2.134; 7.212	0.287
CT genotype × time				GT genotype × time			
WC (cm) [e]				**WC (cm)** [g]			
CT genotype	–0.888	–2.058; 0.283	0.137	GT genotype	–0.756	–1.920; 0.408	0.203
CT genotype × time				GT genotype × time			
HC (cm) [e]				**HC (cm)** [g]			
CT genotype	–0.896	–2.015; 0.223	0.117	GT genotype	–0.763	–1.876; 0.350	0.179
CT genotype × time				GT genotype × time			
WHR (units) [e]				**WHR (units)** [g]			
CT genotype	–0.0008	–0.0096; 0.0080	0.857	GT genotype	–0.0007	–0.0094; 0.0081	0.883
CT genotype × time				GT genotype × time			
WHtR (units) [e]				**WHtR (units)** [g]			
CT genotype	–0.0013	–0.0076; 0.0049	0.683	GT genotype	0	–0.0062; 0.0062	0.995
CT genotype × time				GT genotype × time			
Systolic BP (mmHg) [e]				**Systolic BP (mmHg)** [g]			
CT genotype	–2.167	–3.965; –0.368	**0.018**	GT genotype	–2.293	–4.081; –0.506	**0.012**
CT genotype × time				GT genotype × time			
Diastolic BP (mmHg) [e]				**Diastolic BP (mmHg)** [g]			
CT genotype	–1.078	–2.109; –0.047	**0.040**	GT genotype	–1.092	–2.117; –0.068	**0.037**
CT genotype × time				GT genotype × time			
Glucose (mmol/L) [e]				**Glucose (mmol/L)** [g]			
CT genotype	–0.0003	–0.0643; 0.0638	0.993	GT genotype	0	–0.064; 0.064	0.996
CT genotype × time				GT genotype × time			
Insulin (mU/L) [e]				**Insulin (mU/L)** [g]			
CT genotype	–0.551	–1.270; 0.169	0.134	GT genotype	–0.559	–1.274; 0.157	0.126
CT genotype × time				GT genotype × time			
Cholesterol (mmol/L) [e]				**Cholesterol (mmol/L)** [g]			

CT genotype	0.104	–0.015; 0.224	0.086	GT genotype	0.129	0.010; 0.247	**0.033**
CT genotype × time				GT genotype × time			
HDL-cholesterol (mmol/L) [e]				**HDL-cholesterol (mmol/L)** [g]			
CT genotype	0.017	–0.036; 0.069	0.537	GT genotype	0.011	–0.041; 0.063	0.673
CT genotype × time				GT genotype × time			
LDL-cholesterol (mmol/L) [e]				**LDL-cholesterol (mmol/L)** [g]			
CT genotype	0.086	–0.027; 0.199	0.136	GT genotype	0.102	–0.010; 0.215	0.075
CT genotype × time				GT genotype × time			
Triglycerides (mmol/L) [f]				**Triglycerides (mmol/L)** [h]			
CT genotype	–0.215	–0.458; 0.027	0.082	GT genotype	–0.219	–0.461; 0.023	0.076
CT genotype × time	0.013	0.001; 0.026	**0.039**	GT genotype × time	0.013	0.001; 0.026	**0.038**
Energy intake (kcal) [e1]				**Energy intake (kcal)** [g1]			
CT genotype	–91.503	–199.402; 16.395	0.096	GT genotype	–104.993	–212.279; 2.293	0.055
CT genotype × time				GT genotype × time			
Protein (g) [e1]				**Protein (g)** [g1]			
CT genotype	–4.726	–8.999; –0.453	**0.030**	GT genotype	–5.293	–9.544; –1.043	**0.015**
CT genotype × time				GT genotype × time			
Lipids (g) [e1]				**Lipids (g)** [g1]			
CT genotype	–3.249	–8.413; 1.915	0.218	GT genotype	–4.131	–9.273; 1.010	0.115
CT genotype × time				GT genotype × time			
Carbohydrates (g) [e1]				**Carbohydrates (g)** [g1]			
CT genotype	–10.685	–23.972; 2.603	0.115	GT genotype	–11.551	–24.767; 1.664	0.087
CT genotype × time				GT genotype × time			
Protein E% [f]				**Protein E%** [h]			
CT genotype	1.491	–0.258; 3.240	0.095	GT genotype	1.460	–0.284; 3.203	0.101
CT genotype × time	–0.098	–0.192; –0.004	**0.042**	GT genotype × time	–0.097	–0.191; –0.003	**0.044**
Lipids E% [e]				**Lipids E%** [g]			
CT genotype	0.008	–0.849; 0.866	0.985	GT genotype	–0.037	–0.891; 0.817	0.932
CT genotype × time				GT genotype × time			
Carbohydrates E% [e]				**Carbohydrates E%** [h]			
CT genotype	0.278	–0.742; 1.298	0.593	GT genotype	–0.418	–8.443; 0.089	0.055
CT genotype × time				GT genotype × time	0.241	0.021; 0.461	**0.032**
MPO/kg [e]				**MPO/kg** [g]			
CT genotype	–0.056	–0.159; 0.047	0.283	GT genotype	–0.064	–0.166; 0.039	0.222
CT genotype × time				GT genotype × time			
Standard. PA score [e]				**Standard. PA score** [g]			
CT genotype	0	–0.122: 0.122	0.999	GT genotype	–0.022	–0.143; 0.100	0.728
CT genotype × time				GT genotype × time			

[e] Coefficient can be interpreted as the mean difference in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between *NPY rs16139* CT and TT genotype at each timepoint.

[f] Difference in the rate of change in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between *rs16139* CT and TT genotype can be calculated as the sum of main effect coefficient and time × interaction coefficient at given timepoint.
[g] Coefficient can be interpreted as the mean difference in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between *rs17149106* GT and GG genotype at each timepoint.
[h] Difference in the rate of change in anthropometrical measurements, blood pressure, metabolic biomarkers, daily energy intake (kcal), nutrient intake (g) and nutrient intake as a percentage from daily energy intake (E%) between *rs17149106* GT and GG genotype can be calculated as the sum of main effect coefficient and time × interaction coefficient at given timepoint.
[1] adjusted for body weight
BMI – body mass index; WC – waist circumference; HC – hip circumference; WHR – waist-to-hip ratio; WHtR – waist-to-height ratio; BP – blood pressure; MPO – maximum power output; PA – physical activity

Table 3. The rate of change per year in anthropometric measurements, blood pressure and dietary intake (mean and 95% CI) of the ECPBHS sample according to the linear mixed-effects regression models with *NPY rs5574* genotype × time interaction, *rs16147* genotype × time interaction, *rs16139* × time interaction and *rs17149106* × interaction.

rs5574	**TT genotype**	**CT genotype**	**CC genotype**
WHR (units)	0.0033 (0.0025; 0.0041)[c]	0.0039 (0.0034; 0.0044)	0.0045 (0.0039; 0.0052)[c]
Diastolic BP (mmHg)	0.310 (0.181; 0.439)[c]	0.448 (0.364; 0.531)	0.503 (0.395; 0.612)[c]
Energy intake (kcal)	–51.818 (65.375; –38.261)[c]	–39.090 (–48.189; –29.991)	–34.131 (–45.692; –22.570)[c]
Carbohydrates (g)	–10.505 (12.439; –8.571)[c]	–8.426 (–9.729; –7.124)	–7.969 (–9.617; –6.320)[c]
rs16147	**TT genotype**	**CT genotype**	**CC genotype**
Sum of 5 skinfolds (mm)	2.550 (2.135; 2.964)[ac]	2.044 (1.757; 2.331)[a]	1.935 (1.527; 2.343)[c]
WC (cm)	1.197 (1.081; 1.312)[a]	1.045 (0.965; 1.125)[a]	1.035 (0.921; 1.148)
WHR (units)	0.0048 (0.0041; 0.0055)[ac]	0.0038 (0.0034; 0.0043)[a]	0.0034 (0.0027; 0.0041)[c]
WHtR (units)	0.0059 (0.0053; 0.0066)[ac]	0.0050 (0.0045; 0.0054)[a]	0.0050 (0.0043; 0.0056)[c]
Diastolic BP (mmHg)	0.514 (0.394; 0.634)[c]	0.442 (0.359; 0.526)	0.345 (0.228; 0.463)[c]
rs16139	**TT genotype**	**CT genotype**	
Triglycerides (mmol/L)	0.018 (0.014; 0.023)[d]	0.031 (0.020; 0.043)[d]	
Protein E%	0.317 (0.284; 0.350)[d]	0.219 (0.131; 0.307)[d]	
rs17149106	**GG genotype**	**GT genotype**	
Triglycerides (mmol/L)	0.018 (0.014; 0.023)[d]	0.031 (0.020; 0.043)[d]	
Protein E%	0.317 (0.284; 0.350)[d]	0.220 (0.132; 0.308)[d]	
Carbohydrates E%	–0.584 (–0.662; –0.506)[d]	–0.343 (–0.549; –0.137)[d]	

[a] $p < 0.05$ significant difference in the rate of change between rs5574 TT and CT genotypes or *rs16147* TC and CC genotypes.
[c] $p < 0.05$ significant difference in the rate of change between rs5574 TT and CC genotypes or *rs16147* TT and CC genotypes.
[d] $p < 0.05$ significant difference in the rate of change between rs16139 TT and CT genotypes or *rs17149106* GG and GT genotypes.
WC – waist circumference; WHR – waist-to-hip ratio; WHtR – waist-to-height ratio; BP – blood pressure