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Running Title

Gender Differences in Insulin Resistance

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Insulin Resistance is higher in pre-pubertal girls but switches to become higher in boys at age sixteen: a Cohort Study (EarlyBird 57)

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Abstract

Background: The risk of type 2 diabetes is increasing in teenage girls, and is associated with their greater insulin resistance (IR).

Hypothesis: We hypothesised that the adverse metabolic profile of girls (compared with boys) would persist from childhood through adolescence.

Patients and Methods: Community-based longitudinal cohort of 292 children (147 boys) studied annually from 9-16y. Measures: IR (homeostasis-model-assessment-2), HDL-cholesterol (HDL-C), triglycerides, % body-fat (dual-energy x-ray absorptiometry), pubertal stage (age at peak height velocity), physical activity (accelerometry). Multi-level modelling established the age-related trends in IR and lipids and the influence of covariates.

Results: Each year from 9-15y, girls had 21-63% higher IR than boys (girls mean IR 0.73-1.33, boys 0.51-0.89, p<0.005). At 16y the gender difference was not significant (girls IR 0.60, boys 0.56, p=0.45). Girls had lower HDL-C from 9-12y, higher triglycerides 9-14y, greater adiposity throughout, and earlier puberty, but boys were more active than girls (all p<0.05).

After adjustment for %-fat, puberty and activity, the gender difference in IR between girls and boys aged 9-15y became non-significant: (IR girls 0.66-1.01, boys 0.65-1.04, p>0.07). However, after adjustment at 16y, girls' IR was 25% lower than boys' (girls 0.44, boys 0.63, p=0.001), and they had 22% higher HDL-C (p<0.001) and 20% lower triglycerides (p=0.003).

Conclusions: The higher IR of pre- and early pubertal girls diminishes during late puberty, and boys begin to exhibit greater metabolic risk. Despite being leaner and more active, boys at 16y have higher IR than girls, suggesting future higher risk for diabetes, thus we reject our hypothesis.

Abbreviations

APHV	Age at Peak Height Velocity
BMI	Body Mass Index
CV	Coefficient of Variance
DEXA	Dual Energy X-ray Absorptiometry
HDL-C	High Density Lipoprotein Cholesterol
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
IR	Insulin Resistance
MVPA	Moderate to Vigorous Physical Activity
SDS	Standard Deviation Score
T2D	Type 2 Diabetes

Introduction

Type 2 diabetes (T2D) is presenting at increasingly younger ages, and the diagnosis is often made in the teenage years (1). T2D in children has become a serious public health concern, and in some high-risk populations it is now more common than type 1 diabetes (2). The risk of T2D in this age group is strongly associated with obesity (2); 92% of children with T2D have been reported as overweight or obese (3).

According to a large recent study from North America, T2D prevalence was 0.58 per 1000 in girls and 0.35 per 1000 in boys (4), and other studies have reported a female excess of T2D among children and adolescents (3, 5, 6). These observations contrast with findings in adults, where men are reported to be at higher risk than women (7, 8). The explanation for the gender difference in the prevalence of T2D between adults and adolescents is uncertain, however, it has been proposed that endocrine changes leading to pubertal insulin resistance (IR) may be among the factors influencing the risk of T2D in children (9). There may be a 'cross-over' of the gender difference in IR between childhood and adulthood, with a decline in IR in post-pubertal females relative to males. These changes could reflect age- and gender-dependent changes in adiposity. Logue et al (10) reported that men develop T2D at a lower BMI than women, and suggested this was because men are more insulin resistant and have more central fat than women.

Cross-sectional studies at birth (11) and throughout the prepubertal years (12, 13) have consistently shown higher fasting insulin concentrations in females. These have also been observed in longitudinal studies during adolescence (14, 15). The EarlyBird longitudinal study has previously reported higher levels of IR in five-year-old girls compared with boys, even after accounting for differences in adiposity and physical activity (16). Moreover, it is well established that puberty is associated with a substantial increase in IR (17, 18). Gender differences in IR

have been attributed to differences in adiposity, fat distribution, sex hormones and pubertal timing (19, 20). The temporal influence of different factors can only be investigated with longitudinal studies. EarlyBird is a unique longitudinal study of the same children over a period of 12 years. Gender differences from 5-8y have been reported previously(16). The aim of this analysis was to investigate gender differences in IR between 9-16y. We hypothesised that the adverse metabolic profile of girls (compared with boys) would persist from childhood through adolescence.

Patients and Methods

Design, setting and participants: EarlyBird is a non-intervention longitudinal cohort study of 347 children (174 boys, 173 girls) recruited from 53 primary schools in Plymouth, UK. Exclusion criteria were diabetes, pathological states likely to affect growth or body composition, moderate or severe physical disability, and long term use of oral steroids. Three hundred and seven children were recruited at 5y in 2000-01 and 40 more at age 9y in 2004-05 to redress a gender imbalance. Most were white Caucasian (n=342) and five children (three girls, two boys,1.5% of the cohort) were of mixed race, reflecting the racial mix of the city (98.4% white Caucasian according to the 2001 Census) (21). Children were seen annually (mean interval 1.0 year, SD 0.1). Assent from the child at each visit and written consent from the parent were obtained. Ethical approval was granted by the Local Research Ethics Committee in 1999 and updated regularly. The study has been reported in detail elsewhere (22).

Main outcome measure: Insulin resistance was measured by Homeostasis Model Assessment-2 method using the HOMA-IR program, downloaded from the University of Oxford Diabetes Trials Unit website (www.dtu.ox.ac.uk)) (23). A venous blood sample was taken at 9am after an overnight fast. Serum insulin was measured by Immulite analyser (Diagnostic Products Corporation, USA), cross-reactivity with proinsulin <1%, inter-assay coefficient-of-variation (CV) 9%, detection limit of the assay 2.0 mU/l (14 pmol/l). Glucose was measured using Cobas Integra 700 analyser (Roche Diagnostics, UK; inter-assay CV 2%). HOMA-IR has been validated against the euglycemic clamp (r=0.91) in healthy youths (24).

Secondary outcome measures: HDL-cholesterol and triglycerides measured using Cobas Integra 700 analyser (Roche Diagnostics, UK), inter-assay CV 2%.

Predictor variables: Gender; age; height measured to nearest 1mm (Leicester Height Measure, Child Growth Foundation, London); weight measured to nearest 200g (Tanita Solar 1632W electronic scales, West Drayton, Middx; body mass index (BMI) standard-deviation-scores (sds) were calculated (25); waist circumference measured by metal tape, Chasmors Ltd, London); body fat (% fat) measured annually by dual energy x-ray absorptiometry (DEXA) by Prodigy Advance fan beam densitometer (GE Healthcare).

Pubertal status measured by age at peak height velocity (APHV), determined as the tangential velocity at the middle time-point of three consecutive height measurements taken six months apart. APHV, an objective measure of puberty, allows comparison of pubertal development between genders (26, 27).

Physical activity was measured by Actigraph accelerometers (Model 7164, Fort Walton Beach, FL), having good technical reproducibility (28). Accelerometers were worn around the waist and recorded continuously for 7-days each year. The children wore the accelerometer every day from getting up until going to bed, removing it only for water-based activities, and keeping a diary of any activity undertaken while not wearing the accelerometer. Only recordings capturing at least four days of monitoring (each incorporating at least nine hours' wear time, and including at least one weekend day) were included in the analyses. Time spent in moderate-and-vigorous intensity physical activities (MVPA; minutes/week) was calculated against the threshold specific to three metabolic equivalents of task (METs), deemed by the UK Government to be the lower limit of moderate activity (29).

Children's MVPA varied little from 9 to 16y (30), and in order to characterise the overall long-term physical activity level of each child, their MVPA was averaged across all eight time points. In doing so, the children were ranked according to their activity levels throughout the whole period with 95% reliability, compared with 70% reliability when using one annual measure of PA (30).

Statistical analysis: The distributions of HOMA-IR, triglycerides, % fat and MVPA, were skewed in both genders and were log-transformed for analysis and back-transformed for presentation. Cross-sectional analyses were performed using IBM SPSS version 22 (IBM Corp., Armonk,NY).The gender difference between mean values was expressed as a percentage. Differences between individuals were assessed by independent t-tests. Univariate regression explored the association between the outcome variable and predictor variables.

Longitudinal data were analysed using linear mixed-effects modelling taking into account all available data at each time-point (R software (31) using lmer function in lme4 (32)). The effect of covariates on the gender difference in IR over time was examined. Random intercepts were included as well as age (categorized to allow for non-linear change in IR over time), gender, % fat, APHV and MVPA as fixed effects. Two-way interactions between the covariates, age and gender were examined and retained in the model if significant at the 5% level or of particular interest. Interpretation of the model coefficients was facilitated by plotting.

Results

292 children (147 boys) had measures of IR at 9y (84% of the cohort), and these subjects were used for this analysis. Descriptive characteristics are shown in Table 1. The girls attained peak height velocity 1.64y before boys. Girls had significantly higher % body fat at each age, larger waist circumferences from 10y onwards and a significantly higher BMIsds at 14y (p=0.003).

IR rose and fell through puberty in both genders, with boys reaching peak IR at 14y, and girls at 12y. IR was significantly higher in girls than boys at each age up to 15y, with the biggest gender difference at 12y. Girls had 13-18% higher triglycerides than boys from 9-14y (p<0.032) but the difference was no longer significant at 15 and 16y. Boys had 3-7% higher HDL-cholesterol than girls from 9-12y (p<0.02), whereas by 15y girls had 7% higher HDL-cholesterol (p=0.02), and by 16y girls were 9% higher (p<0.001).

Univariate Regression (Supplementary Table 1)

Predictor variables were added separately at each age to assess their contribution to IR. The variable having the greatest effect on the gender difference in IR was % fat. After adjusting for fat there was no significant gender difference in IR at ages 9-11, 14 and 15y. At 12 and 13y, girls were still more insulin resistant than boys after accounting for fat (p<0.001 and 0.018 respectively), and at 16y, girls were 40% less insulin resistant than boys (p<0.001). Waist circumference, BMI, APHV and MVPA each had more modest effects in attenuating the gender difference in IR. When these variables were included in the models, girls remained more insulin resistant than boys throughout.

Adjusting for %fat, APHV and MVPA increased the gender difference in HDL-C levels at 16y, with %fat having the greatest effect (girls 25% higher than boys; p<0.001)). The only predictor

variable which influenced the gender difference in triglycerides at 16y was %fat, adjusting for which led to girls having 20% lower levels than boys (p<0.001).

Fasting glucose levels were significantly higher in the boys than girls at 10y and 13-16y. The gender difference in glucose was explained by APHV at 14 and 15y, but adjusting for adiposity and MVPA made little difference.

Longitudinal modelling

The measure of adiposity explaining the greatest proportion of the gender difference in IR was total % fat, therefore this was included as a covariate in the longitudinal models along with objective measures of APHV and MVPA, since these are also known to influence IR.

Figure 1 shows mean (95% CI) IR in boys and girls from 9-16y. Figure 1a shows the unadjusted IR (Model estimates are given in Supplementary Table 2). Figure 1b shows IR adjusted for APHV alone, Figure 1c shows IR adjusted for APHV and MVPA, Figure 1d shows IR adjusted for APHV, MVPA and % fat. The inclusion of all three covariates in the model affected the gender difference in IR such that there was a cross-over between 13y and 14y so that by 16y, IR in girls was ~25% lower than in boys.

Figure 2 shows the mean (95% CI) HDL-C and triglycerides in boys and girls from 9-16y. Figure 2a (raw data) shows that HDL-Cholesterol fell from 9-16y, with the fall more marked in boys. After adjustment (Figure 2b), HDL-C became significantly higher in the girls from 12y onwards such that by 16y, HDL-C was ~22% higher in girls than boys (Supplementary Table 3). Triglycerides increased in both genders, girls having 13-18% higher levels than boys from 9-14y (raw data, Figure 2c). However, after adjustment for % fat, APHV and MVPA, there was no gender difference in triglycerides until 16y when the girls had ~20% lower levels than boys (Figure 2d; Supplementary Table 4).

Discussion

The principal finding of this study was that IR is significantly higher in adolescent girls than boys, and this gender difference narrows and reverses by age 16y, at which age boys become more insulin resistant than girls. After adjusting for factors known to influence IR, the gender difference in IR between 9-15y was largely attributable to differences in adiposity. Furthermore, these findings were largely mirrored by changes in levels of triglycerides and HDL-cholesterol levels, which support the likely biological significance of the temporal changes in IR.

Fasting glucose levels were slightly higher in boys, and this gender difference was attenuated to some extent by pubertal stage, and not by adiposity or activity. Relatively higher glucose in boys at 16y (by 4%) could have contributed to their higher adjusted IR at 16y, but is unlikely to have affected their lipids.

This analysis extends the findings of Moran et al (14), who studied 350 children aged 11-15y at baseline, with 55% cohort retention at 18-19y. In that study IR was higher in girls at 11y, and higher in boys at 19y. However, the authors did not find any association between pubertal development and IR. Our longitudinal dataset with eight measurements at annual time points, 93% cohort retention for measures of IR and the small age range of the participants has allowed us to identify more precisely the age at which this gender reversal in IR occurs. The use of linear mixed-effects models allowed incorporation of all covariates and repeated measures simultaneously.

Moran et al (14) did not examine the influence on IR of age-dependent changes in adiposity. Changes in IR occur against a background of body fat levels that are declining in boys and increasing in girls. These changes reflect increasing lean body mass and falling body fat as boys develop adult male body composition. It appears contradictory that IR falls in girls while they continue to gain fat mass. Despite these different patterns, the association between IR and

percentage fat remained stronger in girls. This may be of pathophysiological significance, with relevance to the excess risk of T2D in adolescent females.

The raw data results for triglycerides and HDL-cholesterol support the observations of Dai et al (33) who studied three cohorts of children measured three times in one year. For both measures, girls' triglycerides were higher and HDL-C lower between 8-11y, but had reversed by 14y. Our fully-adjusted trends over eight annual time-points are able to pinpoint the ages at which this cross-over occurs. There is clear divergence of HDL-cholesterol from 12y onwards and of triglycerides from 15y onwards. The HDL-cholesterol data are in line with the normative data collected by the Caliper Study (34), showing slightly higher levels in girls from 13-19y, and no gender difference in triglyceride levels.

The gender differences observed in metabolic variables could be explained by changes in fat distribution, with boys accumulating more central adiposity, known to be associated with increased IR and diabetes risk, and girls accumulating more subcutaneous and/or gluteal adiposity. However, waist circumference-sds increased significantly in the girls from 9-16 years but did not change in the boys, suggesting that the girls were accumulating excess abdominal fat, while boys were growing at the expected rate. Despite this potential masking effect of excess weight gain in girls, we report a cross-over in the gender difference in IR and lipids. Therefore, we are unable to explain the observed gender differences in IR and lipids by changes in body composition. Further studies using direct measures of visceral adiposity could clarify this issue.

Another possibility is that peri-pubertal changes in IR are influenced by changing sex-steroid concentrations. The post-pubertal decline in IR in girls might be related to rising estrogen concentrations; estrogen has several insulin sensitising effects (35). Alternatively, rising androgen levels in both genders could affect IR. Testosterone has been shown to decrease

insulin sensitivity in both men and women (36). However, levels of sex hormone-binding globulin, which are strongly influenced by adiposity and IR in childhood (37), modulate the bioavailability of estradiol and testosterone so that increasing adiposity augments free concentrations of sex steroids. Therefore, while sex steroids may influence IR, there is a complex and probably bidirectional interrelationship.

Growth hormone and the growth axis are also important determinants of insulin resistance during puberty. The association plays an important physiological role, since the acceleration of growth during puberty is achieved in an energy-efficient manner mediated, at least in part, through hyperinsulinaemia/insulin resistance (38, 39). The different reproductive destinies of each sex place different biological imperatives – with different tempos – on vertical growth and the acquisition of fat.

Physical activity is a potential factor that could influence metabolic health in adolescence, and may be highly relevant to diabetes and cardiovascular disease prevention strategies in young people. Physical activity declines during adolescence (40), and while there was considerable inter-individual variation in activity levels at each time point, intra-individual activity remained remarkably stable. Ranking the participants according to their eight-year activity levels allowed maximum precision, yet the overall contribution of activity to the gender difference in IR and lipids was modest.

We have previously shown that physical activity attenuates the mid-adolescent peak in IR and that this effect disappears by 16 years, independent of adiposity and pubertal stage (30). In our final model, however, physical activity explained only a small proportion of the gender difference in IR. Whether or not a modest increase in physical activity in high risk teenagers reduces their risk of T2D remains uncertain.

These findings may have practical relevance for early interventions designed to prevent or delay the onset of T2D in young people. It can be suggested that the excess risk of T2D in girls may be explained by their greater IR, although it is unclear how this is associated with beta cell failure. Weight gain between 9-16y, a key influence on IR, is a potential target for intervention. Whether or not IR and other characteristics at puberty can predict subsequent hyperglycaemia will require longer term follow-up of the cohort.

This study has strengths and limitations. EarlyBird is a well-established longitudinal cohort study with high retention and criterion measures of IR, fatness and activity. The narrow age range and multiple repeated measures allow for within- and between-subject comparisons. Longitudinal modelling uses all the available data while accommodating missing data points, and our methods allowed for retention of subjects in analyses despite some missing data points. Inevitably some data is missing over an eight year study, particularly measures of MVPA at later ages, and reliability was maximised by calculating a mean value over eight years. Although pubertal stage was not directly assessed, APHV is a well-established objective surrogate for direct assessment of pubertal stage. It was unfeasible to take more than one fasting measure from the children each year, thus insulin was measured from a single sample rather than the mean of three. HOMA is a surrogate measure of IR, but, correlating strongly with results from clamp studies, it is the most appropriate measure to use in young children. Accelerometry is a robust measure of physical activity, although limitations include the potential to miss upper body movement and the fact that they must be removed for water based activities. Activity diaries reduced some of these limitations. The EarlyBird cohort is mainly Caucasian, and results may not be generalizable to populations with different ethnic composition. The current analysis terminates at 16y when the metabolic effects of puberty may persist, especially in boys. Follow-up to adulthood will confirm whether IR remains higher in post-pubertal males compared with

females, before and after adjustment for adiposity, and whether IR is associated with increased long term risk for the development of T2D.

We conclude that the higher insulin resistance of pre- and early pubertal girls appears to be largely explained by their greater fat mass, with more modest effects of physical activity and the timing of puberty. Around the age of 16 years a 'cross-over' point is reached when, despite their lower fat mass and higher activity levels, boys become more insulin resistant than girls, and have higher triglycerides and lower HDL-cholesterol levels. The changing risk factors for boys and girls will inform diabetes prevention strategies in children.

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Table 1: Cohort Characteristics

		Boys			Girls			B vs G
		n	mean	SD	n	mean	SD	p
Age at 9y visit (years)		147	8.87	0.29	145	8.83	0.32	0.495
IMD at 9y		146	24.59	13.74	145	23.39	14.85	0.475
Age at Peak Height Velocity (years)		122	13.27	0.87	127	11.63	1.17	<0.001
BMI (sds)	9y	147	0.36	1.15	145	0.59	1.09	0.075
	10y	144	0.43	1.13	141	0.63	1.17	0.135
	11y	144	0.32	1.20	140	0.57	1.21	0.078
	12y	144	0.42	1.23	138	0.66	1.20	0.087
	13y	137	0.46	1.20	127	0.74	1.23	0.059
	14y	134	0.37	1.17	128	0.80	1.17	0.003
	15y	121	0.40	1.16	114	0.65	1.09	0.097
	16y	135	0.41	0.25	128	0.75	1.13	0.019
Waist Circumference (sds)	9y	146	0.44	1.11	143	0.63	1.23	0.173
	10y	144	0.55	1.06	140	0.84	1.25	0.039
	11y	142	0.54	1.07	134	0.96	1.28	0.003
	12y	139	0.53	1.05	128	1.00	1.32	0.001
	13y	136	0.54	1.04	127	1.08	1.33	<0.001
	14y	134	0.48	0.99	128	1.15	1.30	<0.001
	15y	133	0.46	0.96	127	1.07	1.32	<0.001
	16y	135	0.48	1.08	128	1.07	1.36	<0.001
HDL Cholesterol (mg/dL) (a)	9y	147	72.70	15.08	145	68.06	14.69	0.007
	10y	143	71.93	15.85	142	67.67	14.69	0.019
	11y	141	68.45	15.47	141	63.42	13.92	0.004
	12y	139	65.35	14.69	133	60.71	13.53	0.006
	13y	139	59.17	14.31	133	57.23	13.15	0.339
	14y	134	55.68	13.15	131	56.07	11.60	0.651
	15y	134	52.20	11.21	133	55.68	11.60	0.018
	16y	139	50.27	11.21	135	54.91	11.21	<0.001
		n	median	IQR	n	median	IQR	p
Insulin Resistance (unit)	9y	147	0.54	0.33-0.81	145	0.76	0.53-1.03	<0.001
	10y	138	0.78	0.53-1.04	142	0.97	0.69-1.33	0.005
	11y	141	0.72	0.46-1.10	141	0.98	0.69-1.52	<0.001
	12y	139	0.90	0.58-1.23	132	1.36	0.92-1.96	<0.001
	13y	138	0.89	0.63-1.34	133	1.38	0.92-1.80	<0.001
	14y	134	0.93	0.57-1.37	131	1.31	0.88-1.66	<0.001
	15y	135	0.77	0.51-1.23	133	1.09	0.69-1.56	0.001
	16y	138	0.62	0.23-1.04	133	0.67	0.23-1.09	0.446

Table 1: Cohort Characteristics (continued)

		Boys			Girls			B vs G
		n	median	SD	n	median	SD	p
Triglycerides (mg/dL) (b)	9y	147	46.06	36.3-59.3	145	55.80	36.3-59.3	0.001
	10y	143	49.60	38.1-66.4	142	56.68	38.1-66.4	0.006
	11y	141	51.37	40.7-68.2	141	62.88	40.7-68.2	0.001
	12y	139	54.03	39.9-76.2	133	64.66	39.9-76.2	0.005
	13y	139	57.57	42.5-83.3	133	66.43	42.5-83.3	0.01
	14y	134	62.00	44.3-79.7	131	70.86	44.3-79.7	0.032
	15y	135	53.14	44.3-70.9	133	62.00	44.3-70.9	0.118
	16y	139	62.00	44.3-79.7	135	62.00	44.3-79.7	0.939
DEXA fat (%)	9y	141	16	11-23	143	24	19-32	<0.001
	10y	143	19	12-27	143	27	21-34	<0.001
	11y	136	20	13-29	128	28	22-36	<0.001
	12y	138	22	15-29	128	28	22-36	<0.001
	13y	138	20	14-30	132	29	23-36	<0.001
	14y	137	18	13-26	128	31	27-38	<0.001
	15y	117	16	12-23	110	33	28-38	<0.001
	16y	132	16	11-26	126	34	29-38	<0.001
MVPA (minutes per week)	9y	137	413	293-526	135	282	215-362	<0.001
	10y	132	353	243-469	133	261	193-340	<0.001
	11y	130	341	273-469	126	228	165-304	<0.001
	12y	121	380	260-506	123	237	168-306	<0.001
	13y	123	380	248-542	127	248	167-306	<0.001
	14y	128	330	222-499	115	205	151-313	<0.001
	15y	107	343	207-463	99	207	131-268	<0.001
	16y	99	314	178-436	106	194	117-285	<0.001

(a) To convert HDL-cholesterol from mg/dL to mmol/l divide by 38.67

(b) To convert triglycerides from mg/dL to mmol/l divide by 88.57

Figure Legends

Figure 1: Insulin resistance (mean, 95% CI) in children from 9y to 16y according to gender

(a) Unadjusted

(b) Adjusted for APHV

(c) Adjusted for APHV and MVPA)

(d) Adjusted for APHV, MVPA and % fat

Figure 2: Lipid levels (mean, 95% CI) in children from 9y to 16y according to gender. HDL-cholesterol unadjusted (2a), adjusted for % fat, APHV and MVPA (2b); Triglycerides unadjusted (2c), adjusted for % fat, APHV and MVPA (2d).

Supplementary Table 1: Gender differences in IR at each age. Results from cross-sectional univariate regression analyses

Supplementary Table 2: Coefficients from mixed effects model - the effects of age, gender, APHV, adiposity (% fat), and physical activity (average MVPA between 9y-16y) on **insulin resistance** (log transformed) over time.

Supplementary Table 3: Coefficients from mixed effects model - the effects of age, gender, APHV, adiposity (% fat), and physical activity (average MVPA between 9y-16y) on **HDL cholesterol** over time.

Supplementary Table 4: Coefficients from mixed effects model - the effects of age, gender, APHV, adiposity (% fat), and physical activity (average MVPA between 9y-16y) on **triglycerides** over time.