Association of Physical Activity with Metabolic Profile from Adolescence to Adulthood

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Keywords physical exercise, metabolomics, metabolic profiling, longitudinal, childhood, primordial prevention

Funding
This work was supported by the Academy of Finland (grant numbers: 206374, 294834, 251360, 275595, 307996, and 322112); the Juho Vainio Foundation; the Finnish Foundation for Cardiovascular Research; the Finnish Ministry of Education and Culture; the Finnish Cultural Foundation; the Sigrid Jusélius Foundation; Special Governmental grants for Health Sciences Research, Turku University Hospital; the Yrjö Jahnsson Foundation; the Finnish Medical Foundation; and the Turku University Foundation. MAK has a research grant from the Sigrid Jusélius Foundation.

Conflict of interest
Authors have no conflicts of interest relevant to this article to disclose.

Clinical trial registration
The STRIP study is registered at ClinicalTrials.gov, NCT00223600, https://www.clinicaltrials.gov

Short title Physical Activity and Metabolic Profile

Data sharing statement
The dataset supporting the conclusions of this article were obtained from the STRIP study. The STRIP dataset comprises health related participant data and their use is therefore restricted under the regulations on professional secrecy (Act on the Openness of Government Activities, 612/1999) and on sensitive personal data (Personal Data Act, 523/1999, implementing the EU data protection directive 95/46/EC). Due to these legal restrictions, the data from this study cannot be stored in public repositories or otherwise made publicly available. However, data access may be permitted on a case by case basis upon request only. Data sharing outside the group is done in collaboration with STRIP group and requires a data-sharing agreement. Investigators can submit an expression of interest to the chairman of the STRIP steering group (Prof Olli Raitakari, University of Turku, Turku, Finland).

Ethics
The study was approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital. Written informed consent was obtained from the parents in the beginning of the study and from the adolescents at the age of 15 and 18 years.

Section speciality area
III Health, disease and physical activity

ABSTRACT

Objective Physical activity benefits cardiometabolic health, but little is known about its detailed links with serum lipoproteins, amino acids, and glucose metabolism at young age. We therefore studied the association of physical activity with a comprehensive metabolic profile measured repeatedly in adolescence.

Methods The cohort is derived from the longitudinal Special Turku Coronary Risk Factor Intervention Project. At ages 13, 15, 17, and 19 years, data on physical activity was collected by a questionnaire, and circulating metabolic measures were quantified by nuclear magnetic resonance metabolomics from repeatedly assessed serum samples (age 13:n=503, 15:n=472, 17:n=466, and 19:n=361).

Results Leisure-time physical activity (LTPA;MET h/wk) was directly associated with concentrations of polyunsaturated fatty acids, and inversely with the ratio of monounsaturated fatty acids to total fatty acids (-0.006SD; [-0.008, -0.003]; $p<0.0001$). LTPA was inversely associated with very-low-density lipoprotein (VLDL) particle concentration (-0.003SD; [-0.005, -0.001]; $p=0.002$) and VLDL particle size (-0.005SD; [-0.007, -0.003]; $p<0.0001$). LTPA showed direct association with the particle concentration and size of high-density lipoprotein (HDL), and HDL cholesterol concentration (0.004SD; [0.002, 0.006]; $p<0.0001$). Inverse associations of LTPA with triglyceride and total lipid concentrations in large to small sized VLDL subclasses were found. Weaker associations were seen for other metabolic measures including inverse associations with concentrations of lactate, isoleucine, glycoprotein acetylation, and a direct association with creatinine concentration. The results remained after adjusting for body mass index and proportions of energy intakes from macronutrients.

Conclusions Physical activity during adolescence is beneficially associated with the metabolic profile including novel markers. The results support recommendations on physical activity during adolescence to promote health and possibly reduce future disease risks.

List of Abbreviations

BMI = body mass index

CVD = cardiovascular disease

DHA = docosahexaenoic acid

HDL = high-density lipoprotein

HOMA-IR = homeostatic model assessment for insulin resistance

LDL = low-density lipoprotein

LTPA = Leisure-time physical activity

MET = metabolic equivalent

MUFA = monounsaturated fatty acid

NMR = nuclear magnetic resonance

PUFA = polyunsaturated fatty acid

SAFA = saturated fatty acid

SD = standard deviation

STRIP = Special Turku Coronary Risk Factor Intervention Project

VLDL = very-low-density lipoprotein

INTRODUCTION

Physical activity affects health through multiple mechanisms. Assessment of serum metabolic profile offers a window to study a wide range of metabolic measures possibly related to physical activity[1]. Serum metabolic profile can be feasibly studied with high-throughput serum nuclear magnetic resonance (NMR) spectroscopy and has been used in large epidemiological studies[1-3]. With NMR, large data sets reflecting e.g. serum lipoprotein subclasses, amino acids, and metabolic substrate concentrations are obtained. Physically active adults have been shown to present a favorable metabolic profile with e.g. lower concentration of saturated fatty acids when compared to inactive peers[4]. Still, studies investigating the association of physical activity with a comprehensive serum metabolic profile are scarce, particularly in children and adolescents. The prior studies in youth have observed associations of physical activity with e.g., HDL and VLDL particle size, and additionally with an inflammatory marker, glycoprotein acetylation[5,6].

The Special Turku Coronary Risk Factor Intervention Project (STRIP), established in 1989, was launched to reduce the children's exposure to environmental cardiovascular risk factors from infancy to early adulthood[7]. Physically active lifestyle was encouraged but it was not an active part of the intervention focusing mainly on the quality of dietary fat and prevention of smoking, and no intervention effect on physical activity was observed[8]. We have previously shown in the cohort that physical activity among adolescents is associated with cardiometabolic health benefits[9], including better endothelial function and lower intima-media thickness[8].

To increase understanding on the complex links between physical activity and cardiometabolic health in early life, the aim of this study was to elucidate the associations of physical activity with a detailed metabolic profile measured repeatedly during adolescence. Furthermore, the comprehensive dietary data from the STRIP study was used to gain more

insight on how dietary factors affected the results. Possible sex differences were also studied. Thus, this study provides novel data on how physical activity, independent of diet, is reflected on vast serum metabolic profile measured at four time points at ages 13, 15, 17, and 19 years in 503 participants.

METHODS

Study Design and Participants

The here applied study cohort is derived from the STRIP study. The STRIP study is prospective, randomized trial beginning in infancy and aiming to prevent atherosclerosis[7,10]. In brief, families of 5-month-old infants, born between July 1989 and December 1991, were recruited at well-baby clinics in Turku, Finland by nurses. At the age of seven months, 1062 infants (56.2% of the eligible age-cohort) were randomly allocated to a dietary intervention (n=540) or control (n=522) group. Additionally, a group of 45 children, born from March to July 1989, was recruited and randomized (intervention n=22, control n=23) to first test the study protocols and thus serve as a 'pilot' group. The intervention group received individualized dietary and subsequently antismoking counseling at least biannually until the age of 20 years[7,11,12]. A physically active lifestyle was encouraged but it was not a structured, continuous part of the counseling. The children in the control group were met biannually until 7 years of age and annually thereafter. The control families did not routinely receive any detailed intervention focused on the prevention of atherosclerosis risk factors. In this study, the participants were treated as a single cohort, irrespective of their STRIP study group allocation (intervention/control). For the present analyses, longitudinal data on both physical activity and metabolic measures at the four time points were applied. We included children who provided physical activity data and having metabolic biomarkers quantified by high-

throughput NMR metabolomics at the age of 13 (n=503), 15 (n=472), 17 (n=466) or 19 (n=361) years, representing 81% - 96% of the total study participants.

The study was approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital. Written informed consent was obtained from the parents in the beginning of the study and from the adolescents at the age of 15 and 18 years.

Physical activity

Leisure-time physical activity (LTPA) comprising recreational and organized physical activity/sports outside school hours was assessed with a self-administered questionnaire, where the frequency, duration, and intensity of habitual LTPA were reported. LTPA was calculated by multiplying mean frequency, duration and intensity (multiple of the resting metabolic rate; MET) of weekly LTPA and expressed as MET h/wk[8]. The questionnaire has been widely used in studies involving children, adolescents and adults[13]. It correlates weakly to moderately with objectively assessed physical activity (accelometers: r=0.26-0.40; pedometers: r=0.30-0.39)[13] in young adults, and with maximal exercise capacity (r=0.49-0.53)[14]. One hour of e.g. brisk walking per week corresponds approximately to a level of 5 MET h/wk, and a level of 30 MET h/wk corresponds to about an hour of moderate intensity exercise every day of the week or e.g. running three hours per week[15]. Current physical activity guidelines for school-aged children and adolescents recommend an hour of moderate or moderate-to-vigorous physical activity daily[16].

Metabolomics

A high-throughput NMR metabolomics platform was used to quantify 75 serum lipid and metabolic measures from fasted samples. This metabolomics platform provides simultaneous measurement of clinical lipoprotein measures, as well as total lipid, cholesterol, esterified cholesterol, free cholesterol, triglyceride, phospholipid, and particle concentrations of 14

lipoprotein subclasses, and further quantifies abundant fatty acids, amino acids, ketone bodies, and gluconeogenesis-related metabolic measures in absolute concentration units[2,17]. The NMR metabolomics platform is widely used in various epidemiological studies and details of the experimentation have been described[3,17].

Anthropometric, pubertal, additional biochemical, and dietary data

Height was measured by a Harpenden stadiometer (Holtain, Crymych, Great Britain) and weight with an electronic scale (Soehnle S10; Soehnle, Murrhardt, Germany). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Pubertal status (breast tissue diameter/testicular length measured with a ruler) was recorded according to well-established criteria[10,18].

In addition to the metabolic measures, fasting venous blood samples were applied to provide data on triglyceride, total and high-density lipoprotein (HDL) cholesterol (reported in **Table 1**/characteristics of the participants) as well as on insulin concentrations. The analyses were performed at the laboratory of the National Public Health Institute in Turku, Finland, and details of the analyses have been described previously[11,12]. Serum total cholesterol and triglyceride concentrations were analyzed with a fully enzymatic cholesterol oxidase-p-aminophenazone method (Merck, Darmstadt, Germany), with an automatic AU400 analyzer (Olympus, Hamburg, Germany). Serum HDL cholesterol concentration was measured after precipitation of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) with dextran sulfate 500,000. LDL cholesterol concentration was obtained by using the Friedewald formula. Serum insulin was measured with a microparticle enzyme immunoassay (insulin IMX system reagent; Abbott, Chicago, IL), or chemiluminescent microparticle immunoassay (ARCHITECT insulin assay; Abbott) at 13 years of age. From 15 years onwards, serum insulin was measured by radioimmunoassay (Pharmacia Diagnostics,

Uppsala, Sweden). As an estimation of insulin resistance, HOMA-IR (fasting insulin mU/mL x [fasting glucose (mmol/L)/22.5]) measures were used[19].

Dietary information was obtained by using a 4-day food record (consecutive days; at least one weekend day included)[7], considered as golden standard methodology. Parents or other caregivers were responsible for filling out food records during early childhood. As the children aged, they were given more responsibility in completing the records while caregivers were advised to still check them. During the study, children and their caregivers received verbal and written instructions on filling the food records. From the age of 13 years onward, a detailed food booklet was used to assist in estimating the amounts of food or drink consumed. A dietitian checked the food records for accuracy and completeness during the study visits, and, if necessary, added missing details after discussion with the child or parents. The food and nutrient intakes were analyzed with a Micro-Nutrica® program (Research Center of the Social Insurance Institution, Turku, Finland)[20]. The program calculates 66 nutrients of over 4000 foods and dishes. A single dietary technician analysed all food records and updated the data bank throughout the study.

Statistical analyses

Metabolic measures with skewed distributions were log(x+1)-transformed prior to analyses. For each measure of metabolite concentration, a linear mixed-effects model for repeated measures was fitted with continuous physical activity variable (MET h/wk), sex and age as fixed effects and subject as a random effect (model 1). This analysis applies longitudinal data on both physical activity and metabolic measures at the four time points. The analysis allows for missing observations. To gain more insight on whether diet or BMI confounded the results, a second model adjusted additionally for BMI, and dietary intakes of protein, carbohydrates, and fatty acids (saturated, monounsaturated, and polyunsaturated) as

percentage of daily energy intake was performed (model 2). The STRIP study has previously reported differing results between the sexes on physical activity and metabolic risk factors in adolescents[9], thus sex-stratified analyses with similar adjustments were conducted to study possible differences in the associations of physical activity with metabolic profile between males and females in adolescence.

To facilitate comparison of effect sizes, all metabolic measures were scaled to standard deviation (SD) units. Reported metabolic measures are from pooled analysis across 4 time points, and the effect sizes reported thus correspond to the average difference in SD-scaled metabolic concentration due to one unit increase (= 1 MET h/wk) in physical activity during adolescence. Therefore, to obtain the difference of e.g. 30 MET h/wk on metabolic measures, multiplication of the effect size by 30 is required. Multiple testing correction with Bonferroni adjustment for 75 independent tests gives p-value threshold of 0.0007. Since the metabolic measures in part correlate, a principal component analysis was additionally performed showing 23 metabolic measures explaining 95% of the observed variance in dataset, and thus p-value < 0.002 gives evidence of an association. Statistical analyses were conducted using R 3.6.1 software[21].

RESULTS

This study investigated the association of physical activity with metabolic profile of 361 – 503 participants assessed at four time points between 13 and 19 years of age. Clinical characteristics of study participants are shown in **Table 1**.

Association of physical activity with serum metabolic profile

The results on the associations of physical activity with metabolic profile are shown in **Figures 1 to 3** with sex-stratified results shown as online figures (**Figures 4 to 6, online**). To ease comparison, results adjusted additionally for BMI and proportions of energy from

macronutrients are shown in the same Figures. After additional adjustments for pubertal status and study group, the results remained essentially unchanged (data not shown). The strongest inverse associations between LTPA and metabolic measures were found for the ratio of monounsaturated fatty acids to total fatty acids, serum triglyceride concentration, VLDL particle size, and total lipid concentration of VLDL subclasses. The strongest direct associations between LTPA and metabolic measures were found for the ratio of polyunsaturated fatty acids to total fatty acids, HDL particle size, HDL cholesterol concentration, and total lipid concentration of very large to large HDL subclasses.

Circulating serum fatty acids

Figure 1 shows the associations between LTPA, measured as MET h/wk, and circulating serum fatty acid concentrations. LTPA was directly associated with concentrations of omega-3 fatty acids with no association found for docosahexaenoic acid (DHA). Most prominent direct associations were seen for the ratios of circulating omega-6 fatty acids and polyunsaturated fatty acids (PUFAs) to total fatty acids. There was a weak inverse association with monounsaturated fatty acids (MUFAs) while the ratio of MUFA to total fatty acids was distinctly inversely associated with LTPA. No association was found between LTPA and serum saturated fatty acid (SAFA) concentrations. LTPA was directly associated with the number of double bonds per fatty acid while no association was found in the total concentrations of fatty acids. These associations mostly remained when adjusting for BMI and macronutrient intakes.

Sex-stratified results for fatty acids are shown in **Figure 4**, online. There was no association between LTPA and the number of double bonds per fatty acid or the amount of total fatty acids in females whereas for males LTPA was directly associated with the number of double bonds per fatty acid and a weaker direct association was observed with the number of total

fatty acids. DHA was weakly and directly associated with LTPA in males, with no association found when DHA to total fatty acid ratio was considered. Other PUFA-related measures were directly associated in males while in females a similar pattern was found only with metabolic measures evaluating their ratio to total fatty acids. LTPA was inversely associated with concentrations of MUFAs in women, and for both sexes, strongly inversely associated with the circulating ratio of MUFAs to total fatty acids. No associations were found between LTPA and SAFAs in males or females. The associations remained similar when adjusted for BMI and macronutrients.

Lipoproteins

The associations of LTPA with a broad panel of cholesterol and lipoprotein lipid measurements are shown in **Figure 2**. Of conventional lipid measures, LTPA was directly associated with HDL cholesterol, apolipoprotein A1 (apo A1) and inversely associated with serum concentration of triglycerides.

LTPA was inversely associated with VLDL particle concentration and directly associated with HDL particle concentration. LTPA was also inversely associated VLDL particle size and directly associated with HDL particle size whereas no association was found for LDL particle concentration or size. LTPA was inversely associated with VLDL lipoprotein subclass total lipid concentrations except for very small VLDL lipoprotein total lipid concentration, where no association with LTPA was found. There was a direct association between LTPA and lipid concentration of very large and large HDL subclasses. Additionally, an inverse association between LTPA and the triglyceride concentration of VLDL particles, specifically the triglyceride concentration of chylomicrons and extremely large VLDL subclass was observed. LTPA was directly associated with cholesterol concentration in large HDL subclass.

All found associations remained virtually unchanged after additional adjustments for BMI and with proportions of energy intake from macronutrients were made.

Sex-stratified results for lipid and lipoprotein measures (**Figure 5, online**), mostly followed a similar pattern for males and females. In males, LTPA was directly associated with HDL cholesterol, whereas females showed a weaker direct association on this metabolic measure.

Other metabolic measures

The association between LTPA and serum glycolysis related metabolic measures, amino acids, ketone bodies, metabolic waste products and other metabolic measures are shown in **Figure 3**. LTPA was strongly inversely associated with insulin and HOMA-IR measures whereas glucose showed only a similar tendency. LTPA was also inversely associated with the serum concentrations of lactate, isoleucine, and glycoprotein acetylation, while a direct association was found with the concentration of creatinine. There was a weak inverse association of LTPA with serum concentration of glycerol, and a direct association with serum concentrations of citrate, and phospholipid-related metabolic measures. The results mostly remained after adjusting for BMI and macronutrients.

The sex-stratified results for these metabolic measures (**Figure 6, online**), followed a similar pattern with the sex-combined results except for phospholipid metabolic measures which were directly associated with LTPA in males while no association were found in females.

DISCUSSION

This study demonstrates that physical activity in adolescence is beneficially associated with several metabolic measures predictive of CVD risk and type 2 diabetes, and those associations largely remain when adjusting for BMI and the proportions of energy intakes from protein, carbohydrate, and saturated, monounsaturated and polyunsaturated fatty acids.

We for instance found that physical activity was directly associated with serum concentrations of omega-6 PUFAs and inversely associated with MUFA to total fatty acids ratio, associations linked with reduced CVD risk[22]. Physical activity also showed inverse association with the concentrations of triglycerides, and direct association with serum concentration of HDL cholesterol – indicators of reduced CVD risk[23,24]. For other metabolic measures, physical activity was inversely associated with serum concentrations of lactate, isoleucine, and glycoprotein acetylation, and directly associated with concentration of creatinine. Higher serum concentrations of branched-chain amino acids, isoleucine included, have been associated with risk for type 2 diabetes[3], and serum concentration of glycoprotein acetylation has been shown to be higher in individuals with type 2 diabetes[25]. Physical activity has been previously positively associated with kidney function[26] and more days of aerobic or strength exercise have been associated with higher concentrations of creatinine in young adults[27]. Additionally, physical activity was inversely associated with insulin and HOMA-IR, a measure of insulin resistance. Our findings thus support the current recommendations on physical activity in adolescence to possibly reduce future CVD and type 2 diabetes risk.

Studies assessing the associations of long-term physical activity with metabolomics are scarce[28]. In adults, an elegant study by Kujala et al.[4] found that individuals who were persistently physically active had a favorable metabolic profile described by lower concentrations of isoleucine, α1-acid glycoprotein, glucose, very-low-density lipoprotein particles, and fatty acid profile shifted towards less saturated compared to those who were inactive. Another targeted metabolic profiling study on Japanese men showed higher levels of total physical activity, assessed by a questionnaire evaluating the average time spent in various physical activities during the last 12 months, to be inversely associated with e.g., isoleucine and triglycerides, and directly with HDL cholesterol[29]. Our study corroborates

these results on healthy adolescents, and with similar direction of association between physical activity and glucose. Partly in line with our results, an untargeted metabolomics study using accelerometer-measured physical activity data on Chinese adults observed higher level of physical activity to be inversely associated with branched chain amino acids and carbohydrates in glucose metabolism[30]. Another study conducted on four large adult cohorts found associations between habitual physical activity and e.g. certain phospholipids[31], a tendency seen in our study as well, with stronger associations seen in males. An accelerometer-measured moderate-to-vigorous physical activity tended to associate with variables related to HDL metabolism in a recent metabolome study performed on young men[32]. In our study, physical activity was directly associated with most HDL-related metabolites, and this was seen especially in males.

In children and adolescents, only two cross-sectional studies have assessed the association of physical activity with metabolic profile[5,6]. In the Active Smarter Kids study[6], accelometer measured moderate- to vigorous physical activity was associated with larger HDL subclasses, HDL cholesterol and particle size, while an inverse association was found for most apolipoprotein B -containing lipoprotein subclasses and triglyceride measures. A study in the Avon Longitudinal Study of Parents and Children cohort examined relations of physical activity with metabolic traits[5] with repeat measures of accelometry data from multiple time points and metabolic profile assessed at the age of 15. Higher total activity was associated widely across metabolic traits, e.g. with higher concentration of HDL cholesterol, and lower cholesterol and triglycerides in VLDL particles, and also lower concentration of glycoprotein acetyls, though the associations were small in magnitude. Our results are in line with findings from these studies and benefiting from the repeated measures of both physical activity and NMR measured metabolomics, partly showed stronger associations. In addition to the prior

studies, we were able to include dietary data in the analyses, thus controlling for its potential to confound the results.

Regarding more conventional lipid and glucose metabolism measures, previous studies in children and youth lend evidence that physical activity, especially more vigorous activity, is beneficially associated with traditional cardiometabolic biomarkers such as triglycerides, total cholesterol, and fasting glucose[33]. A recent longitudinal study conducted on 6-8 year old children with objective physical activity measurements at baseline and during 2-year follow-up, found that increase in vigorous and moderate-to-vigorous physical activity was associated with reduced cardiometabolic risk score (comprising of e.g. insulin, glucose, triglycerides, and HDL cholesterol concentrations) and increased HDL cholesterol[34]. Simultaneously, increased vigorous physical activity was additionally related to decreased plasma triglyceride concentration. Our current results show similar associations with physical activity during adolescence.

To make these reported associations more tangible, it is helpful to compare them to our previously reported associations of achieving the dietary targets of the STRIP study intervention with metabolic profile[35]. For example, compared to achieving none of the four dietary targets achieving at least two of them, associated with serum proportion of PUFA relative to total fatty acids ($\beta = 0.126$ SD [95% confidence interval (CI): 0.040, 0.212], corresponding to 0.5% higher PUFA ratio). This association corresponds to the association of physical activity of ~32 MET h/wk, as the association of physical activity with serum proportion of PUFA observed in this study was $\beta = 0.004$ SD [0.002, 0.006]. As previously mentioned, this roughly equals to the current physical activity recommendations for adolescents. Based on the results it can also be deduced that the 30 MET h/wk of physical

activity is associated with 0.033 mmol/l [0.018, 0.048] (p<0.0001) higher HDL cholesterol concentration.

The sex-specific analyses revealed some differences between males and females regarding the associations of physical activity and the studied metabolic measures. Our study shows strong direct macronutrient and BMI adjusted associations in males on most PUFA-related measures, whereas in females, only similar pattern for these metabolic measures as proportions of total fatty acids were observed. Males also showed stronger association of LTPA with HDL cholesterol, and phospholipids than females. Simultaneously, males showed stronger association between LTPA and HDL particle concentration, which might reflect the fact that phospholipids are the main constituents of HDL particles. Leveraging the STRIP data, we have previously shown an increase in physical activity at the age of 13 to be associated with a decreased clustered metabolic risk (cluster included BMI, HDL cholesterol, triglycerides, and blood pressure) in females[9], and in males similarly for sedentary and highly active adolescents. Collectively, few studies have reported sex differences for the comprehensive metabolic response of physical activity[28]. However, these data are still scarce and most studies either do not report sex-stratified results or have all-male study populations[33,36]. Thus, knowledge on the sex-specific effects of physical activity remains incomplete. Based on our data it may be hypothesized that physical activity associates with metabolic markers nearly similarly in males and females.

Although physical activity links beneficially with various health risks and metabolic changes[6], and physical activity recommendations to prevent or treat health conditions have been given[16,37], it is still unclear whether physical activity is causally related to health outcomes or with longevity. For example, in observational studies physical activity is associated with reduced all-cause mortality[38], while in a controlled intervention study

physical activity was not associated with the risk of CVD death[39]. Observational studies may be susceptible to biases and a variety of confounding factors whereas randomized controlled trials are lacking possibly due to required long-follow up times making them unfeasible to conduct. Mendelian randomization has been used to tackle the problems of observational studies, with some studies finding no causal role of physical activity for the risk of type 2 diabetes or CVD[40,41], and one suggesting a possible reverse causation for physical activity and childhood adiposity[42]. Furthermore, it has been suggested that same genetic factors influence physical activity levels, and risk of death, which might partly explain associations between higher physical activity and reduced mortality[43, 44]. Thus, the exact ways through which physical activity positively impacts health are likely complicated and not fully understood.

The main strength of our study is the unique STRIP trial providing a large number of study participants with detailed metabolic profile, physical activity and e.g. meticulously collected dietary data measured at multiple time points. As this is the first study to report the association of physical activity with several serum metabolic measures assessed repeatedly with high throughput NMR in healthy children, comparison to prior studies is limited. We acknowledge that the use of self-reported physical activity is a limitation of the study, and future studies should preferably use objectively measured physical activity (e.g. apply accelerometers). There might also be selection bias in the initial recruitment as the families who took part in the trial might have been more health-conscious and motivated towards healthy lifestyle habits. In addition, all participants were White and as with all single country studies, the findings may not be generalizable to other areas of the world. Lastly, some participants were lost to follow-up, as inherent to any longitudinal studies. However, the current study sample is likely representative of the original cohort given that we have previously shown no systematic differences in the key characteristics such as weight, total

cholesterol, blood pressure, saturated fat intake, or physical activity between the participants who were lost to follow-up and those who continued in the study[7, 45].

Perspective

This study shows that the beneficial associations of physical activity with metabolic profile, exceeding the conventional lipid and glucose metabolism measures, are evident already in adolescence. Importantly, the associations seem to be independent of body mass index and diet. Some associations were more robust in males than in females, especially in PUFA-related measures. These results complement and add to the prior data among links between physical activity and metabolic profile at young age[5,6]. Our findings thus emphasize and support efforts and guidelines to promote a physically active lifestyle among adolescents to improve current cardiometabolic health and to reduce the future risk of cardiometabolic health concerns.

Conflict of interest

Authors have no conflicts of interest relevant to this article to disclose.

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Figure Legends

Figure 1. Association of physical activity with fatty acid metabolic measures. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk). Statistical model 1, adjusted for age and sex, is denoted by black dot. Statistical model 2, adjusted with age, sex, body mass index and dietary factors, is denoted by black triangle. Error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the 4 time-points. n-3 fatty acids/total FA denotes the ratio of omega-3 fatty acids to total fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids; DHA, docosahexaenoic acid; LA, linoleic acid.

Figure 2. Association of physical activity with serum lipid measures. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk). Statistical model 1, adjusted for age and sex, is denoted by black dot. Statistical model 2, adjusted with age, sex, body mass index and dietary factors, is denoted by black triangle. Error bars indicate 95% confidence intervals. Lipid measures are from pooled analyses across the 4 time-points and those with skewed distributions were log(x+1)-transformed prior to analyses. C, cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

Figure 3. Association of physical activity with other serum metabolic measures. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk). Statistical model 1, adjusted for age and sex, is denoted by black dot. Statistical model 2, adjusted with age, sex, body mass index and dietary factors, is denoted by black triangle. Error bars indicate 95% confidence intervals. Metabolic

measures are from pooled analyses across the 4 time-points and those with skewed distributions were log(x+1)-transformed prior to analyses. HOMA-IR, homeostatic model assessment for insulin resistance.

Figure 4, online. Association of physical activity with fatty acid metabolic measures stratified by sex. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk) with females denoted by black dot and males by black triangle. Results from statistical model 1, adjusted for age, are shown left, and results from statistical model 2, adjusted with age, body mass index and dietary factors, are shown right. Error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the 4 time-points. n-3 fatty acids/total FA denotes the ratio of omega-3 fatty acids to total fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids; DHA, docosahexaenoic acid; LA, linoleic acid

Figure 5, online. Association of physical activity with serum lipid measures stratified by sex. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk) with females denoted by black dot and males by black triangle. Results from statistical model 1, adjusted for age, are shown left, and results from statistical model 2, adjusted for age, body mass index and dietary factors, are shown right. Error bars indicate 95% confidence intervals. Lipid measures are from pooled analyses across the 4 time-points and those with skewed distributions were log(x+1)-transformed prior to analyses. C, cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

Figure 6, online. Association of physical activity with other serum metabolic measures stratified by sex. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk) with females denoted by black dot and males by black triangle. Results from statistical model 1, adjusted for age, are shown left, and results from statistical model 2, adjusted for age, body mass index and dietary factors, are shown right. Error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the 4 time-points and those with skewed distributions were log(x+1)-transformed prior to analyses. HOMA-IR, homeostatic model assessment for insulin resistance.

Figure 1.

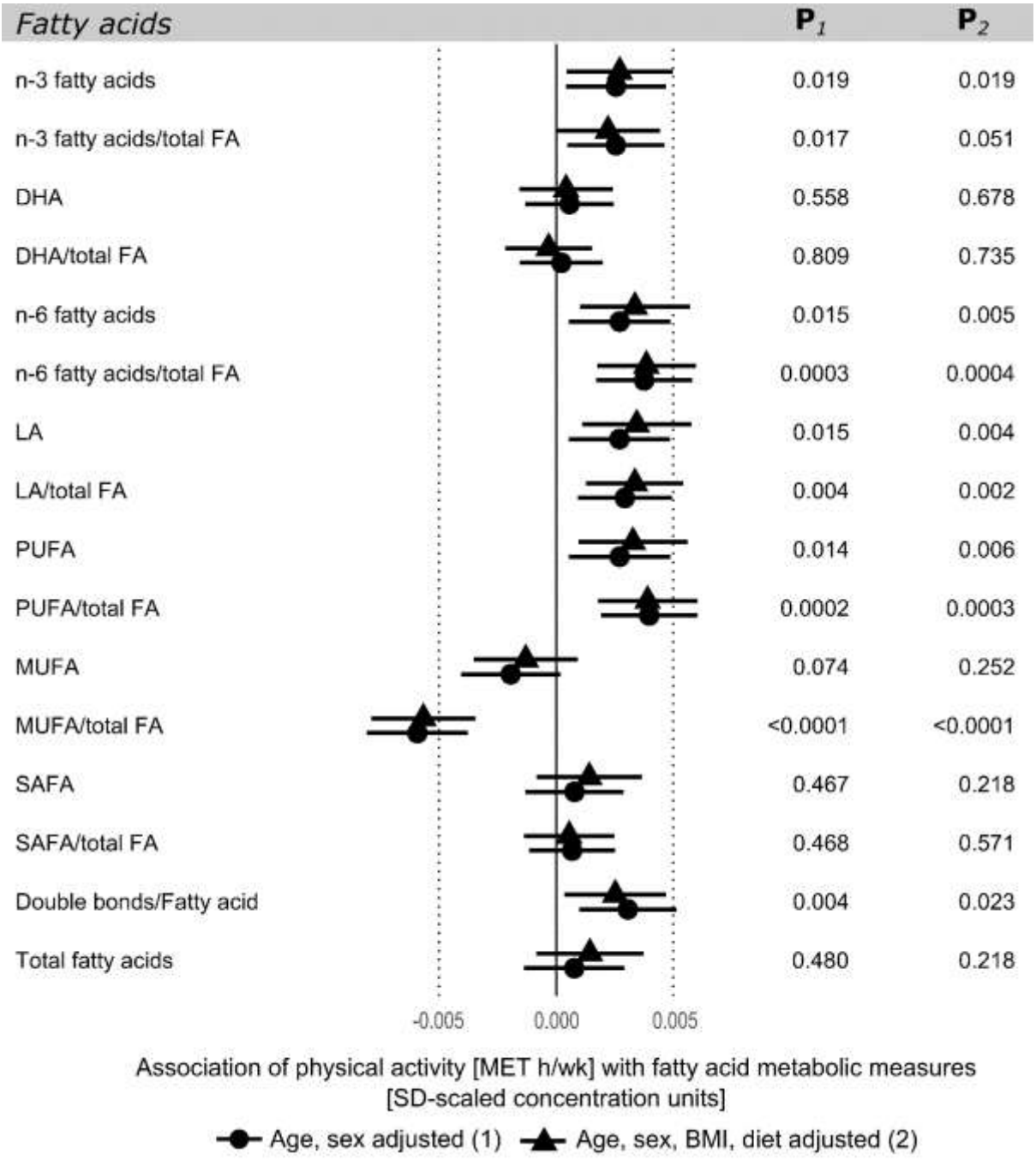
Fatty acids
P1
P2
n-3 fatty acids
0.019
0.019
n-3 fatty acids/total FA
0.017
0.051
DHA
0.558
0.678
DHA/total FA
0.809
0.735
n-6 fatty acids
0.015
0.005
n-6 fatty acids/total FA
0.0003
0.0004
LA
0.015
0.004
LA/total FA
0.004
0.002
PUFA
0.014
0.006
PUFA/total FA
0.0002
0.0003
MUFA
0.074
0.252
MUFA/total FA
<0.0001
<0.0001
SAFA
0.467
0.218
SAFA/total FA
0.468
0.571
Double bonds/Fatty acid
0.004
0.023
Total fatty acids
0.480
0.218
-0.005
0.000
0.005
Association of physical activity [MET h/wk] with fatty acid metabolic measures
[SD-scaled concentration units]
Age, sex adjusted (1)
Age, sex, BMI, diet adjusted (2)

Figure 2.

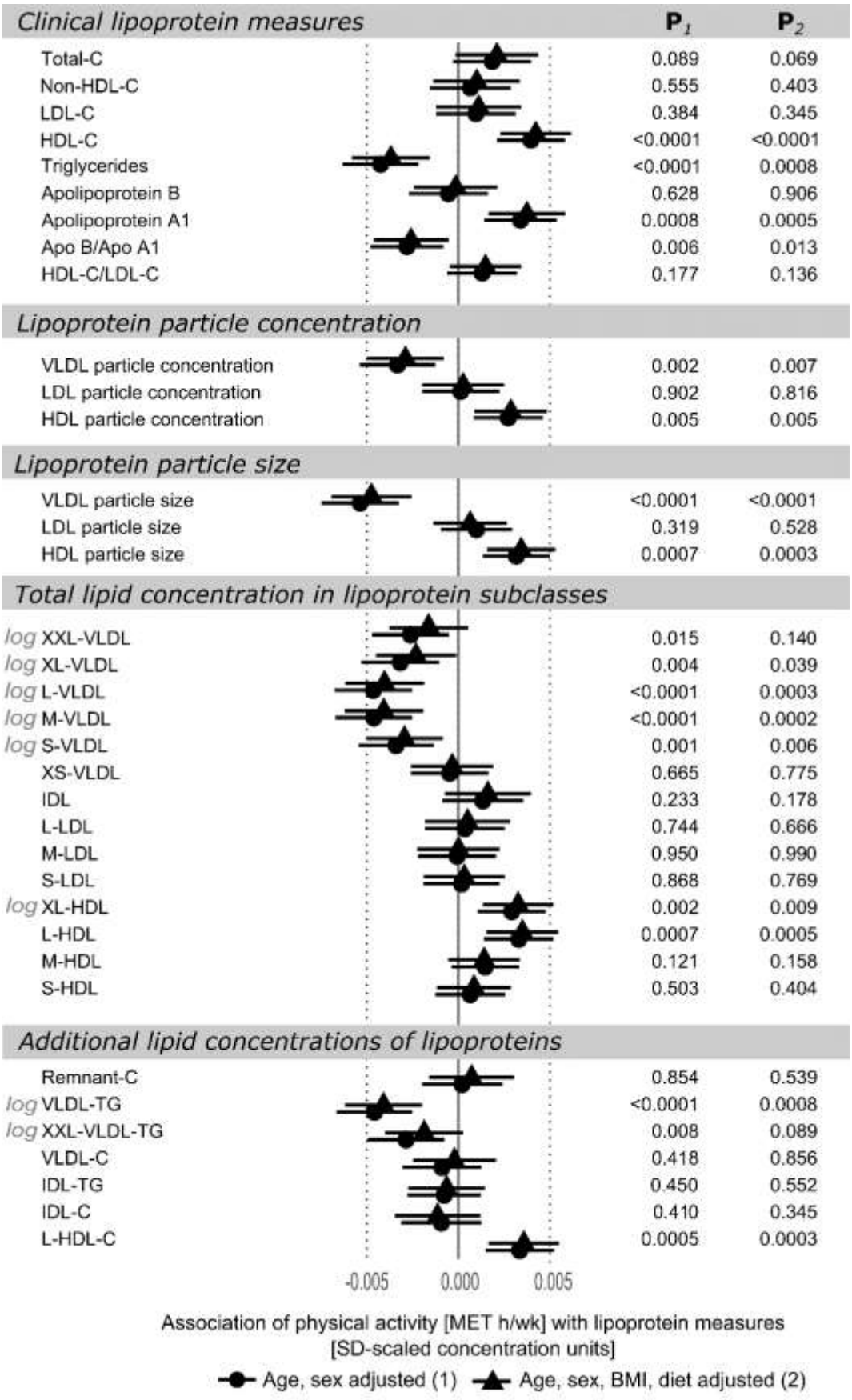
Clinical lipoprotein measures
P1
P2
Total-C 0.089 0.069
Non-HDL-C 0.555 0.403
LDL-C 0.384 0.345
HDL-C <0.0001 <0.0001
Triglycerides <0.0001 0.0008
Apolipoprotein B 0.628 0.906
Apolipoprotein A1 0.0008 0.0005
Apo B/Apo A1 0.006 0.013
HDL-C/LDL-C 0.177 0.136
Lipoprotein particle concentration
VLDL particle concentration 0.002 0.007
LDL particle concentration 0.902 0.816
HDL particle concentration 0.005 0.005
Lipoprotein particle size
VLDL particle size <0.0001 <0.0001
LDL particle size 0.319 0.528
HDL particle size 0.0007 0.0003
Total lipid concentration in lipoprotein subclasses
log XXL-VLDL 0.015 0.140
log XL-VLDL 0.004 0.039
log L-VLDL <0.0001 0.0003
log M-VLDL <0.0001 0.0002
log S-VLDL 0.001 0.006
XS-VLDL 0.665 0.775
IDL 0.233 0.178
L-LDL 0.744 0.666
M-LDL 0.950 0.990
S-LDL 0.868 0.769
log XL-HDL 0.002 0.009
L-HDL 0.0007 0.0005
M-HDL 0.121 0.158
S-HDL 0.503 0.404
Additional lipid concentrations of lipoproteins
Remnant-C 0.854 0.539
log VLDL-TG <0.0001 0.0008
log XXL-VLDL-TG 0.008 0.089
VLDL-C 0.418 0.856
IDL-TG 0.450 0.552
IDL-C 0.410 0.345
L-HDL-C 0.0005 0.0003
-0.005
0.000
0.005
Association of physical activity [MET h/wk] with lipoprotein measures
[SD-scaled concentration units]
Age, sex adjusted (1)
Age, sex, BMI, diet adjusted (2)

Figure 3.

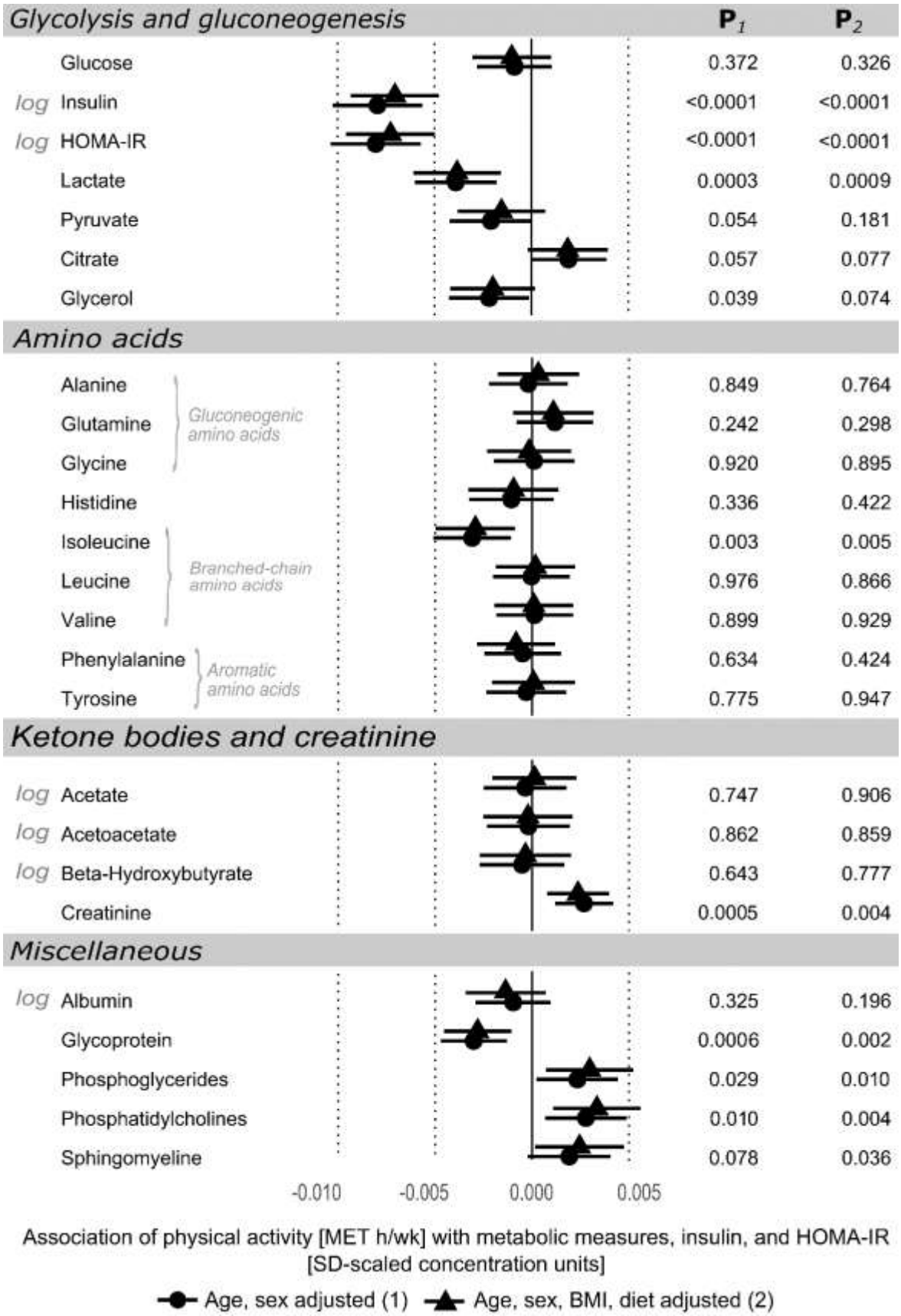
Glycolysis and gluconeogenesis
P1
P2
Glucose
0.372
0.326
log Insulin
<0.0001
<0.0001
log HOMA-IR
<0.0001
<0.0001
Lactate
0.0003
0.0009
Pyruvate
0.054
0.181
Citrate
0.057
0.077
Glycerol
0.039
0.074
Amino acids
Alanine
Glutamine
Glycine
Gluconeogenic amino acids
0.849
0.764
0.242
0.298
0.920
0.895
Histidine
0.336
0.422
Isoleucine
Leucine
Valine
Branched-chain amino acids
0.003
0.005
0.976
0.866
0.899
0.929
Phenylalanine
Tyrosine
Aromatic amino acids
0.634
0.424
0.775
0.947
Ketone bodies and creatinine
log Acetate
0.747
0.906
log Acetoacetate
0.862
0.859
log Beta-Hydroxybutyrate
0.643
0.777
Creatinine
0.0005
0.004
Miscellaneous
log Albumin
0.325
0.196
Glycoprotein
0.0006
0.002
Phosphoglycerides
0.029
0.010
Phosphatidylcholines
0.010
0.004
Sphingomyeline
0.078
0.036
-0.010
-0.005
0.000
0.005
Association of physical activity [MET h/wk] with metabolic measures, insulin, and HOMA-IR [SD-scaled concentration units]
Age, sex adjusted (1)
Age, sex, BMI, diet adjusted (2)

Table 1. Basic characteristics of the study participants at different time points, sex combined and stratified by sex. The values shown are mean (SD).

Variable \ Age	13 n = 503		15 n = 472		17 n = 466		19 n = 361	
	Females n=241	Males n=262	Females n=232	Males n=240	Females n=236	Males n=230	Females n=188	Males n=173
MET [h/wk]	27.3 (22.5)		28.3 (23.0)		26.9 (22.7)		24.1 (22.5)	
	23.4 (20.9)	30.9 (23.2)	25.2 (21.4)	31.3 (24.1)	25.3 (19.2)	28.7 (25.6)	21.6 (20.1)	26.7 (24.7)
BMI [kg/m^2]	19.3 (3.1)		20.5 (3.1)		21.6 (3.4)		22.5 (3.7)	
	19.5 (3.2)	19.0 (3.0)	20.7 (3.1)	20.3 (3.1)	21.7 (3.5)	21.5 (3.3)	22.4 (3.9)	22.6 (3.4)
Waist [cm]	69.9 (8.6)		73.3 (8.4)		74.6 (8.2)		77.4 (9.6)	
	69.5 (8.7)	70.2 (8.6)	71.5 (7.6)	75.0 (8.8)	72.1 (8.0)	77.0 (7.8)	74.3 (9.7)	80.7 (8.3)
Protein [E%]	16.69 (2.88)		17.46 (3.00)		18.10 (3.17)		18.28 (3.63)	
	16.56 (2.98)	16.81 (2.80)	17.08 (2.83)	17.79 (3.11)	17.33 (2.83)	18.90 (3.32)	17.60 (3.45)	19.08 (3.70)
Carbohydrates [E%]	51.90 (5.57)		51.57 (5.88)		50.11 (6.65)		48.30 (6.36)	
	52.36 (5.80)	51.48 (5.35)	52.50 (6.07)	50.74 (5.59)	52.09 (5.82)	48.03 (6.84)	49.91 (5.55)	46.45 (6.75)
Saturated fats [E%]	12.34 (2.84)		12.03 (2.86)		12.22 (2.94)		12.11 (2.78)	
	12.30 (3.07)	12.38 (2.61)	11.92 (2.96)	12.12 (2.77)	11.92 (2.91)	12.53 (2.95)	11.83 (2.60)	12.44 (2.96)
Monounsaturated fats [E%]	10.93 (2.10)		10.63 (2.16)		10.75 (2.53)		10.92 (2.50)	
	10.72 (2.11)	11.13 (2.07)	10.31 (2.24)	10.92 (2.05)	10.14 (2.25)	11.39 (2.64)	10.66 (2.45)	11.19 (2.54)
Polyunsaturated fats [E%]	5.57 (1.48)		5.63 (1.59)		5.76 (1.72)		6.18 (1.98)	
	5.53 (1.49)	5.61 (1.47)	5.48 (1.64)	5.76 (1.53)	5.48 (1.68)	6.06 (1.72)	6.07 (1.82)	6.30 (2.14)
Serum total cholesterol [mmol/l]	4.21 (0.71)		3.96 (0.74)		4.09 (0.76)		4.32 (0.80)	
	4.29 (0.71)	4.15 (0.71)	4.17 (0.72)	3.77 (0.70)	4.35 (0.76)	3.82 (0.65)	4.56 (0.76)	4.07 (0.76)
LDL cholesterol [mmol/l]	2.63 (0.62)		2.41 (0.64)		2.44 (0.62)		2.51 (0.66)	
	2.68 (0.63)	2.59 (0.60)	2.54 (0.63)	2.28 (0.62)	2.57 (0.63)	2.30 (0.59)	2.60 (0.65)	2.42 (0.66)
HDL cholesterol [mmol/l]	1.20 (0.24)		1.15 (0.23)		1.20 (0.27)		1.32 (0.31)	
	1.22 (0.23)	1.19 (0.24)	1.22 (0.22)	1.08 (0.22)	1.33 (0.27)	1.07 (0.21)	1.46 (0.30)	1.17 (0.24)
Serum total triglycerides [mmol/l]	0.79 (0.39)		0.85 (0.43)		0.95 (0.44)		1.09 (0.55)	
	0.82 (0.37)	0.77 (0.40)	0.85 (0.37)	0.85 (0.48)	0.97 (0.42)	0.94 (0.46)	1.12 (0.49)	1.07 (0.61)

MET = metabolic equivalent, BMI = body mass index, E% = percent of daily energy intake, LDL = low-density lipoprotein, HDL = high-density lipoprotein.

Supplementary Material

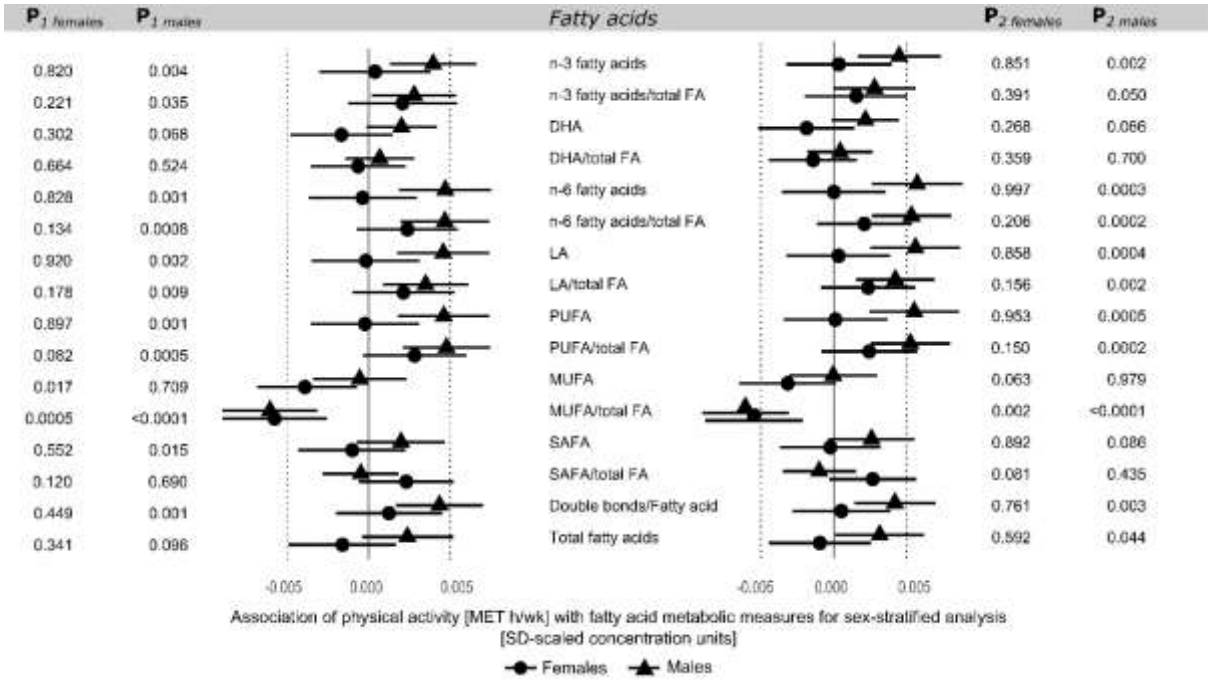

Figure 4, online. Association of physical activity with fatty acid metabolic measures stratified by sex. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk) with females denoted by black dot and males by black triangle. Results from statistical model 1, adjusted for age, are shown left, and results from statistical model 2, adjusted with age, body mass index and dietary factors, are shown right. Error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the 4 time-points. n-3 fatty acids/total FA denotes the ratio of omega-3 fatty acids to total fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids; DHA, docosahexaenoic acid; LA, linoleic acid

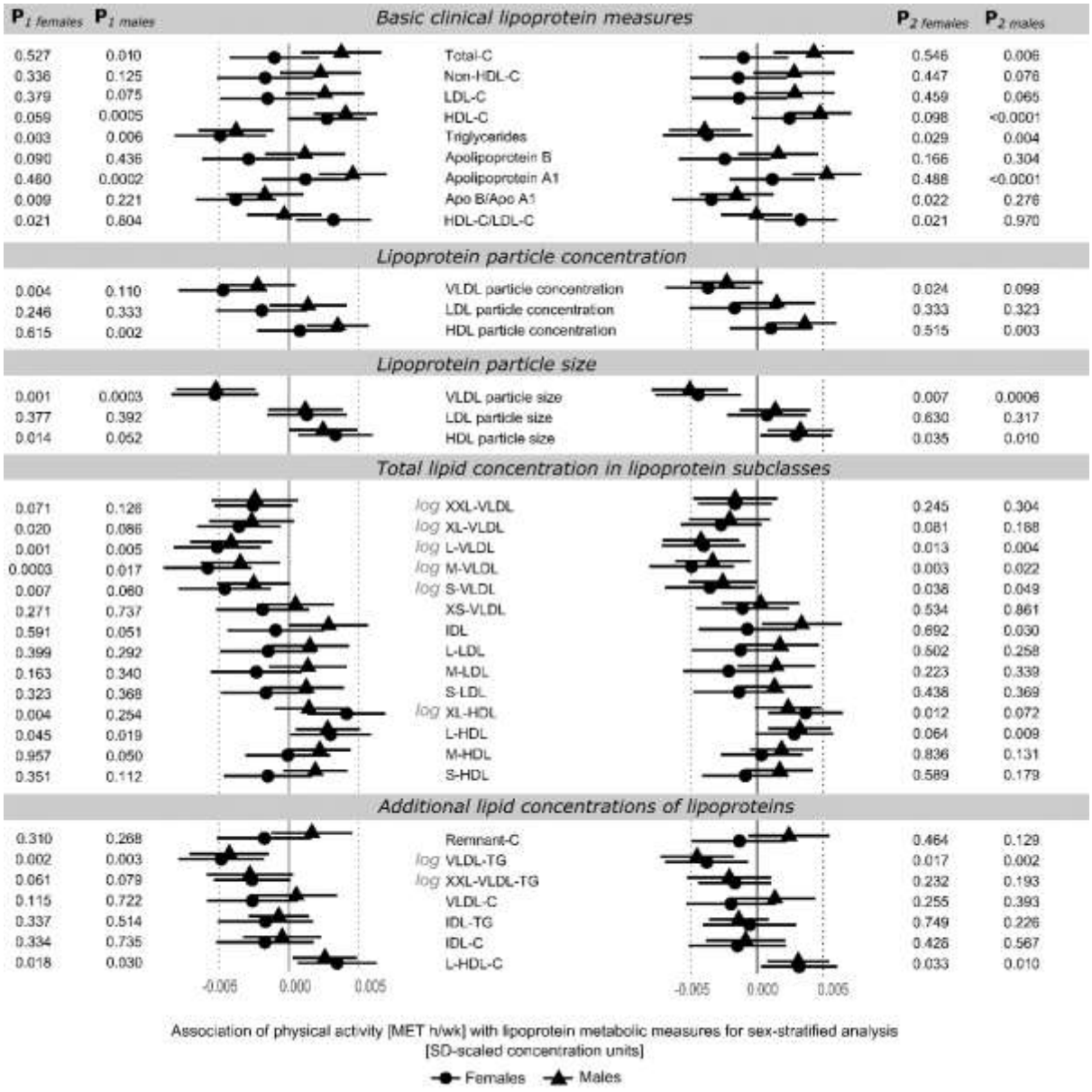

Figure 5, online. Association of physical activity with serum lipid measures stratified by sex. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk) with females denoted by black dot and males by black triangle. Results from statistical model 1, adjusted for age, are shown left, and results from statistical model 2, adjusted for age, body mass index and dietary factors, are shown right. Error bars indicate 95% confidence intervals. Lipid measures are from pooled analyses across the 4 time-points and those with skewed distributions were log(x+1)-transformed prior to analyses. C, cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-

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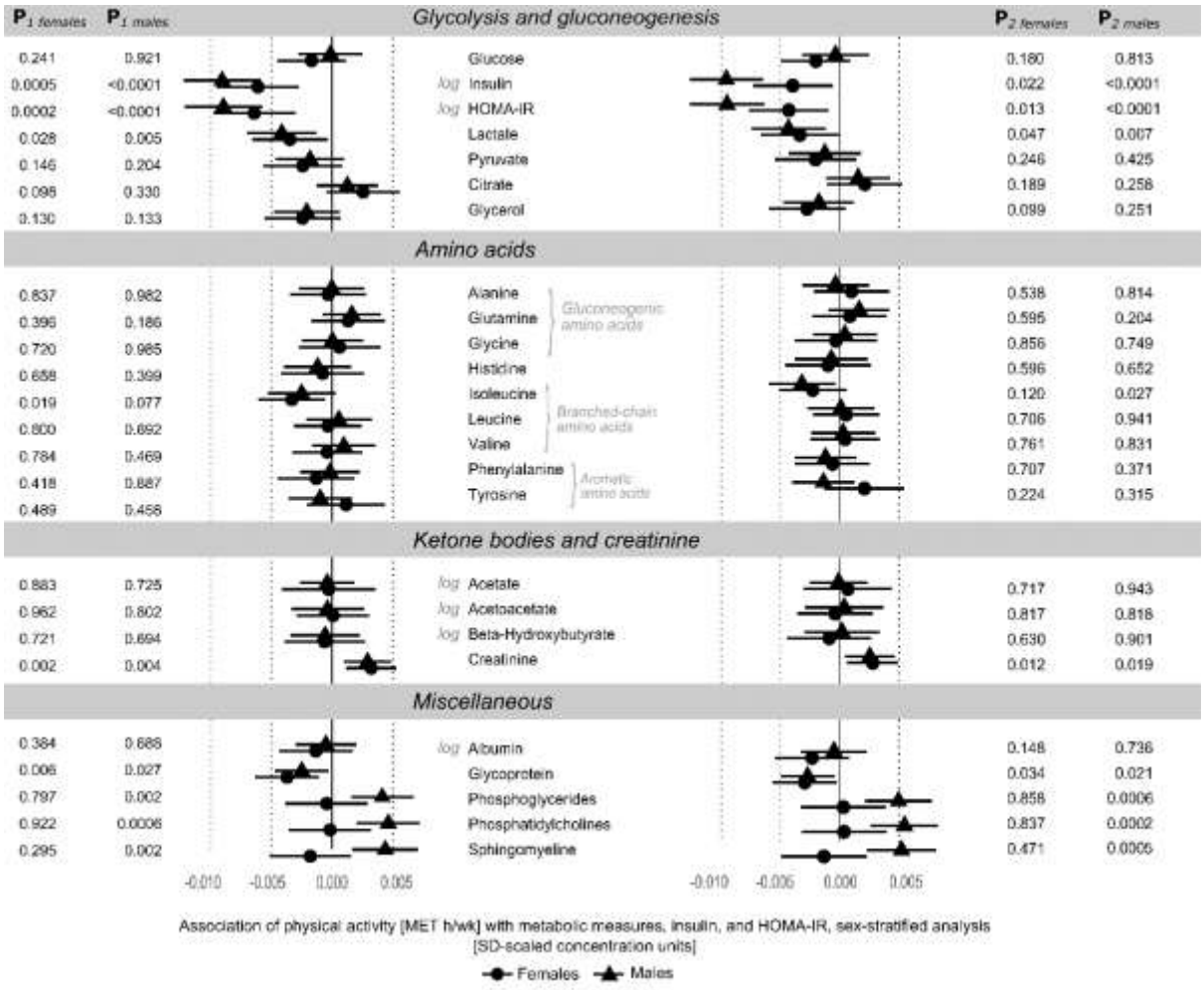

Figure 6, online. Association of physical activity with other serum metabolic measures stratified by sex. Effect estimates are standard deviation scaled differences in metabolite measures for one unit increase in physical activity (MET h/wk) with females denoted by black dot and males by black triangle. Results from statistical model 1, adjusted for age, are shown left, and results from statistical model 2, adjusted for age, body mass index and dietary factors, are shown right. Error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the 4 time-points and those with skewed distributions were log(x+1)-transformed prior to analyses. HOMA-IR, homeostatic model assessment for insulin resistance.