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Physical activity, sedentary time, and liver enzymes in adolescents: the HELENA study

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BACKGROUND: To examine the association between physical activity (PA) and liver enzyme levels in adolescents from nine European countries.

METHODS: The study comprised 718 adolescents (397 girls). PA was measured by accelerometry and expressed as total PA (counts/min), and time (min/d) engaged in moderate to vigorous intensity PA (MVPA). Time spent sedentary was also objectively measured. We measured serum levels of alanine aspartate aminostrasferase (AST), alanine aminostransferase (ALT), and γ-glutamyltrasnferase (GGT), and the AST/ALT ratio was computed.

RESULTS: There was an association between MVPA and AST and AST/ALT (age, sex, and center-adjusted $\beta = 0.096$, 95% confidence interval (CI): 0.016 to 0.118; and $\beta = 0.090$, 95% CI: 0.006 to 0.112, respectively). Meeting the PA recommendations (60 min/d of MVPA) was significantly associated with higher AST and AST/ALT, which persisted after further adjusting for sedentary time and waist circumference. Sedentary time was not associated with any of the studied liver enzyme levels.

CONCLUSION: Meeting the current PA recommendations of 60 min/d of MVPA is associated with higher levels of AST and AST/ALT regardless of time spent sedentary as well as total and central body fat in European adolescents.

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal liver function in young people (1), and is reaching epidemic proportions (2). Increased serum liver enzyme levels such as alanine aminostransferase (ALT), γ-glutamyltrasnferase (GGT), and even aspartate aminostrasferase (AST) are considered surrogate markers of NAFLD (2). These enzymes are associated with total and central body fat, insulin resistance, and blood lipids in children and adolescents (3–6), and are known to track from childhood to adulthood (7). Several studies in middle- and older-aged populations showed an association of liver enzyme levels with metabolic syndrome and type 2 diabetes (8–12). Hanley *et al.* (13) showed that adults with higher levels of AST-to-ALT ratio (AST/ALT) had lower risk of metabolic syndrome. Of note is that whereas an AST/ALT level <1 is indicative of nonalcoholic steatohepatitis, a ratio of ≥2 is associated with alcoholic liver disease (14). Lu *et al.* (15) showed that Han young adults with AST/ALT <1 had a higher frequency of abdominal obesity, high triglycerides, elevated blood pressure, and metabolic syndrome. More recently, a longitudinal study in young adults showed that both ALT and GGT levels predicted 16-y incidence of type 2 diabetes (16). Therefore, a greater in-depth knowledge of the factors affecting liver enzymes and NAFLD in youth will contribute to the development of effective prevention programs, counseling, and public health policy.

It is known that moderate to vigorous physical activity (MVPA) is associated with a healthier body composition (17–20), metabolic profile (21,22), and insulin sensitivity (23) in youth. Moreover, there is no doubt regarding the detrimental consequences for health of a prolonged sedentary lifestyle. Studies examining the association of objectively measured physical activity (PA) and sedentary time with liver enzymes in youth are scarce (6), yet it is biologically plausible that both lifestyle factors are associated with fatty liver enzyme levels at this age (24,25). We hypothesized that MVPA and sedentary time are associated with AST, ALT, AST/ALT, and GGT in adolescents. We tested these hypotheses in a cross-sectional study conducted on adolescents from nine European countries participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study.

RESULTS

The characteristics of the study sample are shown in **Table 1**. A total of 15% ($n = 108$) of the adolescents were overweight and

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4.5% (n = 32) were obese. Moreover, 42.5% (n = 305) of adolescents met the PA recommendations (60 min/d of MVPA). The association between the exposures, total PA, MVPA, and sedentary time, with the outcome variables, liver enzyme levels (i.e., AST, ALT, AST/ALT, and GGT) are shown in **Table 2**. There was an association of total PA and MVPA with AST (age, sex, and center-adjusted P = 0.001 and P = 0.011, respectively), which remained significant after further adjusting for sedentary time. We also observed an association of MVPA with AST/ALT (**Table 2**), which remained significant after further adjusting for sedentary time (β = 0.07, 95% confidence interval (CI): 0.014 to 0.122). The association was slightly attenuated and became borderline significant after further including fat mass index (FMI) or waist circumference in the model (β = 0.077, 95% CI: −0.002 to 0.103; and β = 0.078, 95% CI: −0.001 to 0.104, respectively). No association of MVPA with ALT or GGT was observed. There was no association between total PA with ALT (P = 0.125), AST/ALT, or GGT. The results persisted after further adjusting for FMI or waist circumference (data not shown). Sedentary time was not significantly associated with any of the study liver enzyme levels (**Table 2**). Further adjusting for MVPA (**Table 2**), FMI, or waist circumference did not change the results.

Adolescents meeting the PA recommendations had significantñy higher AST (≈7%) and AST/ALT (≈7%) (**Figure 1**). This association persisted after further adjusting for sedentary time, and FMI or waist circumference. No association was observed between meeting the PA recommendations with ALT or GGT (**Figure 1**). We repeated all the analyses adjusting for tanner stages instead of for age, and after further adjusting for accelerometer wearing time and the results did not change (data not shown).

Table 1. Descriptive characteristics of the study sample

	Males			Females		
Characteristics	*N*	Mean	SD	*N*	Mean	SD
Age (years)	321	14.7	1.2	397	14.7	1.1
Tanner stages (I/II/III/IV/V)	307	5/26/64/111/101		374	0/20/86/159/109	
BMI (kg/m^2)	321	20.7	3.3	397	21.2	3.4
FMI (kg/m^2)	308	4.1	2.9	394	5.7	2.4
WC (cm)	316	73	8	394	71	8.0
Total PA (cpm)	321	503	169	397	387	123
Sedentary time (min/d)	321	536	93	397	547	79
MVPA (min/d)	321	69	25	397	51.2	20.3
ALT (units/liter)	321	23	9	397	20	8
AST (units/liter)	321	25	7	397	20	7
AST/ALT	321	1.13	0.32	397	1.06	0.28
GGT (units/liter)	320	18	9	395	15	5

ALT, alanine aminostransferase; AST, alanine aspartate aminostrasferase; FMI, fat mass index; GGT, γ-glutamyltrasnferase; MVPA, moderate to vigorous intensity PA; PA, physical activity; WC, waist circumference.

DISCUSSION

The results of this study suggest that meeting the current PA guidelines of 60 min/d of MVPA is associated with higher levels of AST and AST/ALT regardless of time spent sedentary as well as total or central body fat in European adolescents. In contrast, sedentary time is not associated with the studied liver enzyme levels.

The observed association between MVPA and AST/ALT in adolescents might be of clinical relevance. The Insulin Resistance Atherosclerosis Study followed 633 adults free of metabolic syndrome at baseline over 5.2 y and identified that 127 had developed metabolic syndrome (13). The study showed that adults in the upper quartile of the AST/ALT had a 60% lower risk of developing metabolic syndrome (13). Whether AST/ALT levels persist from adolescence to adulthood, and whether this may have cardiovascular consequences later in life is unknown. Data from the Bogalusa Heart Study (7) showed that about 50% of the adolescents and young adults who had high levels of ALT and GGT at baseline had also high values after 12 y, indicating high tracking for these enzymes. We observed no association between MVPA with ALT and GGT. These findings do not concur with those reported by Kelishadi *et al.* (6). They showed that self-reported PA was inversely associated with ALT in adolescents of which 50% were obese. It is worth noting that this study assessed PA by a self-reported questionnaire, whereas we assessed PA by an objective methodology (i.e., accelerometry). It is known that the assessment of PA by questionnaire may have a lower accuracy, especially in young people. Moreover, the high prevalence of obesity might have

Table 2. Standardized regression coefficients (β) and 95% CI between total PA, MVPA, and sedentary time with ALT, AST, AST/ALT, and GGT in European adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescence study

	ALT (units/liter)				AST (units/liter)				AST/ALT (units/liter)				GGT (U/L)			
Exposures	β	95% CI		*P*	β	95% CI		*P*	β	95% CI		*P*	β	95% CI		*P*
Total PA (cpm)	0.062	−0.016	0.134	0.125	0.129	0.048	0.171	**0.001**	0.063	−0.014	0.115	0.126	−0.056	−0.115	0.018	0.150
MVPA (min/d)	0.010	−0.054	0.070	0.806	0.096	0.016	0.118	**0.011**	0.090	0.006	0.112	**0.029**	−0.058	−0.096	0.014	0.142
Sedentary (min/d)	−0.061	−0.268	0.026	0.106	−0.024	−0.165	0.080	0.494	0.047	−0.047	0.205	0.220	0.021	−0.092	0.168	0.564
MVPA (min/d)[a]	−0.003	−0.065	0.061	0.949	0.095	0.014	0.118	**0.013**	0.103	0.014	0.122	**0.014**	−0.056	−0.095	0.016	0.166
Sedentary (min/d)[a]	−0.062	−0.272	0.028	0.110	−0.007	−0.137	0.111	0.840	0.065	−0.018	0.237	0.093	0.011	−0.112	0.152	0.762

Data are adjusted for age, sex, and center. Bold font indicates statistically significant values.
ALT, alanine aminostransferase; AST, alanine aspartate aminostrasferase; CI, confidence interval; FMI, fat mass index; GGT, γ-glutamyltrasnferase; MVPA, moderate to vigorous intensity PA; PA, physical activity.
[a]Models mutually adjusted for MVPA and sedentary time.

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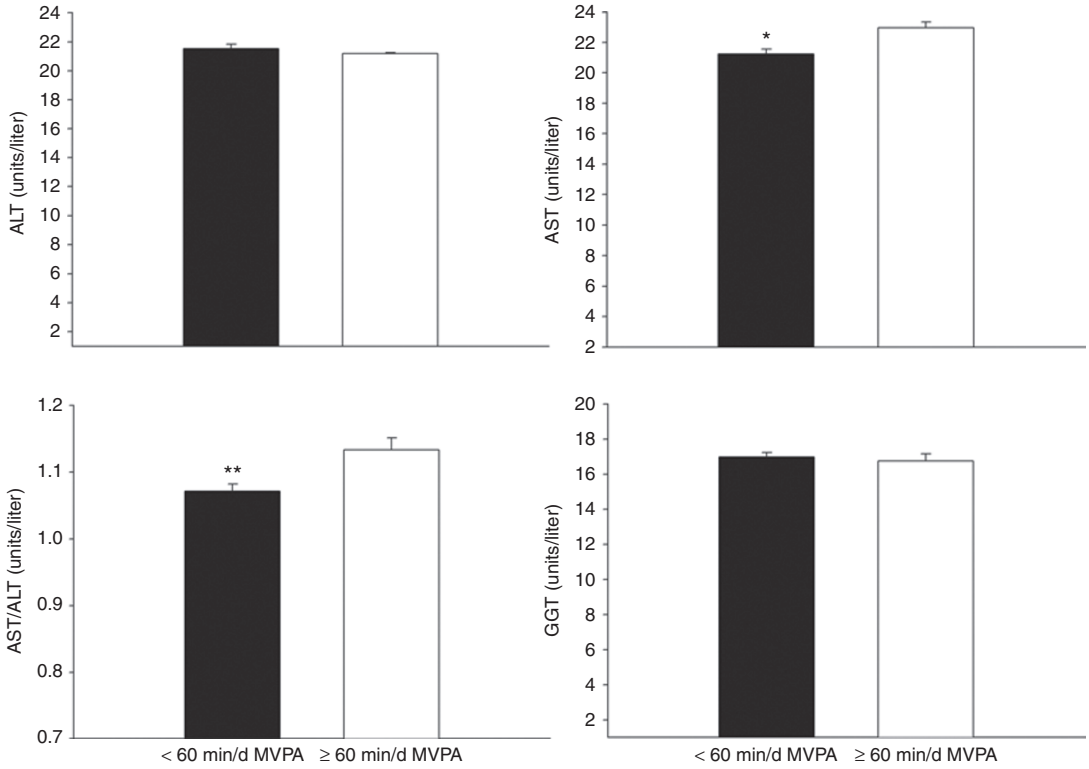

Figure 1. Levels of alanine aminotransferase (ALT; top left), aspartate aminotransferase (AST; top right) to ALT (AST/ALT; bottom left), and γ-glutamyltransferase (GGT; bottom right) according moderate to vigorous physical activity levels (MVPA, meeting and not meeting the recommendations, <60 min/d and ≥60 min/d, respectively) in European adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescence study. Data are adjusted means and standard error of the mean. Analyses were performed with Ln transformed data, but nontransformed values are shown for easier interpretation. *P* from analysis of covariance where MVPA (<60 vs. ≥60 min/d) was included as fixed factor, sex and age were entered as covariates, and center was entered as random factor. A total of 42.5% ($n = 305$) adolescents met the recommendations. $^{*}P = 0.002$; $^{**}P = 0.003$.

negatively influenced the liver enzyme levels in this group of adolescents. Indeed, the ALT levels of the obese adolescents ranged from 32.4 to 37.7 U/l, whereas in our group, mean ALT values were much lower.

Several mechanisms might partially explain the observed association of PA with AST/ALT. There is a close connection among surrogate markers of NAFLD (i.e., ALT, AST, GGT), abdominal adiposity, and insulin resistance (2). Regular PA is known to be associated with lower total and central adiposity (17,18,26) as well as with lower insulin resistance (23,27) in children and adolescents. Therefore, it is biologically plausible that regular PA preserves the hazard effects of adiposity and insulin resistance on liver function enzymes (24,25). The increased hepatic lipogenesis and triglyceride-rich lipoprotein secretion induced by excess of central adiposity (2) might also be attenuated by regular PA, as several studies have unequivocally shown that PA is associated with a healthier blood lipid profile (21,22). Likewise, results from intervention studies have also reported that regular PA improves blood lipid profile and other cardiometabolic risk factors in youth (28). Intervention studies investigating the effectiveness of regular PA on NAFLD in obese children and adolescents (29–32) support weight loss in reversing the progression of NAFLD. Recent evidence also suggests that exercise may play a key role on hepatic fat content by directly altering hepatic β-oxidation and/or lipogenesis (25).

AST is present in the liver but in considerable amounts also in other tissues including the muscles; therefore, it cannot be excluded however that the observed association of total PA and MVPA with AST and AST/ALT might be partially explained by increased AST released from muscles after PA (33), particularly when vigorous. Whether this applies to physically active healthy youth warrants further investigation. PA-based intervention studies in healthy and obese adolescents are needed to further understand the effect of regular PA on liver enzyme levels in youths.

Several cross-sectional studies showed that time spent in sedentary activities (measured by accelerometry) is associated with total and central adiposity, with insulin resistance as well as with other cardiovascular disease risk factors in youth (34,35). We observed, however, no association between sedentary time and surrogate markers of NAFLD. It is likely that the low prevalence of obesity in our study (4.5%, $n = 32$) and therefore the limited available cases of harmful effect of obesity on the study liver enzyme levels might explain the absence of association. Future studies are needed to confirm or contrast our findings.

Due to the study cross-sectional design, these findings need to be interpreted with caution. Of note is that liver enzyme levels could be affected by other liver disease or by alcohol consumption; yet results persisted after exclusion of adolescents with clinical elevations of ALT (>30 U/l, $n = 56$). Furthermore,

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in *post hoc* analysis, alcohol consumption (estimated by two no-consecutive days 24 h dietary recall) was not associated with any of the study liver enzyme levels ($n = 474$; sex and age-adjusted $r = -0.042$, $P = 0.359$; $r = -0.001$, $P = 0.980$; $r = 0.049$, $P = 0.282$; $r = -0.011$, $P = 0.807$; for ALT, AST, AST/ALT, and GGT, respectively). While we adjusted for several markers of total and central body fat, body composition was estimated through anthropometry. Note that the markers of body composition used are valid, reliable, and appropriate for epidemiological studies (36). The present study findings should be replicated in other population samples, in longitudinal studies, as well as in younger and older individuals. Randomized controlled trials examining the effects of PA on liver enzyme levels in youth are also needed.

METHODS

Study Participants

The HELENA study was conducted from 2006 to 2008 in 10 European cities (hereafter called centers) from 9 countries (37). Detailed descriptions of the HELENA sampling and recruitment approaches, standardization and harmonization processes, data collection, analysis strategies, quality control activities, and inclusion criteria were published elsewhere (37).

The current study comprised a total of 718 adolescents (397 females) aged from 12.5 to 17.49 y with complete and valid data on PA measured by accelerometry and liver enzymes (i.e., AST, ALT, AST/ALT, and GGT). There were no differences in the study key characteristics (i.e., age, sex, weight and height, and PA) between the current study sample and the original HELENA sample ($n = 3546$, all $P > 0.1$). All adolescents and their parents or guardians signed an informed written consent before being enrolled in the study. The study protocol was approved by the corresponding local Human Research Review Committees of the centers involved.

Measures

Physical examination. Body weight and height were measured following standard procedures, and body mass index was calculated by dividing body weight in kilogram by squared height in meters. The prevalence of overweight and obesity was also estimated using the International Task Force criteria (38). Waist circumference was measured in triplicate at the midpoint between the lowest rib and the iliac crest, using an anthropometric tape (SECA 200) (36). Skinfold thicknesses were measured to the nearest 0.2 mm in triplicate in the left side at triceps and subscapular using a Holtain Caliper (Crymmych, UK), and percentage body fat was estimated (39). Thereafter, FMI (fat mass in kilogram divided by squared height in meters) was calculated. FMI and waist circumference were used as surrogates of total and central adiposity, respectively. A medical doctor of the same sex as the child recorded the pubertal stage according to Tanner and Whitehouse (40).

PA. Detailed description of the assessment of PA in the HELENA study has been published elsewhere (41). Participants were instructed to wear an accelerometer (Actigraph GT1M, Pensacola, FL) on their lower back (42) during all waking hours, and to remove it while bathing or swimming. The time sampling interval (epoch) was set at 15 s. Bouts of 20 continuous minutes of 0 activity intensity counts were excluded from the analysis, and these periods were considered as nonwearing time. Monitor wearing time was calculated by subtracting nonwear time from the total registered time for the day. At least 3 d of recording with a minimum of 8 or more hours of registration per day were necessary to be included in the study (21,43). We calculated the time engaged in MVPA over the day based upon a standardized cut-off of ≥2,000 counts per minute (41). MVPA was dichotomized into <60 and ≥60 min/d according to the current PA guidelines.

Liver biomarkers. A detailed description of the blood samples analysis has been reported elsewhere (44). Venous blood was obtained by venipuncture after an overnight fast. Serum was aliquoted and sent to the central laboratory at University of Bonn. ALT (units/liter), AST (units/liter), and GGT (units/liter) levels were analyzed in fresh serum in the University Hospital, with the RxL clinical chemistry system (Dade Behring, Schwalbach, Germany). Results from stability tests for selected liver enzymes can be found elsewhere (44). We also computed the AST/ALT ratio.

Statistical Analysis

All statistical analyses were performed using the SPSS, v. 20.0 (SPSS, Chicago, Illinois) and the level of significance was set at $\alpha = 0.05$. Variables with skewed distribution (FMI, waist circumference, sedentary time, MVPA, total PA, ALT, AST, AST/ALT ratio, and GGT) were transformed in a natural logarithmic basis to obtain a more symmetrical distribution. We conducted linear regression analysis to examine the association between the exposure, total PA, MVPA, and sedentary time with the outcome variables, liver enzymes (i.e., ALT, AST, AST/ALT, and GGT). Center (entered as dummy variable) and age were included in the model as covariates. There was no significant (all $P > 0.2$) interaction effect between total PA, MVPA, or sedentary time with sex and any of the study liver enzymes; therefore, all the analyses were conducted in males and females together and sex was included in the model as covariate. We also examined the independent associations between MVPA and sedentary time with each of the outcomes by including sedentary time as a covariate when MVPA was the main exposure and by inserting MVPA as a covariate when sedentary time was the main exposure. We repeated the analysis further adjusting by FMI or waist circumference.

To study whether meeting the PA recommendations (60 min/d of MVPA) was associated with liver enzyme levels, we conducted analysis of covariance, where MVPA (<60 min/d vs. ≥60 min/d) was included as fixed factor, sex and age were entered as covariates and center was entered as random factor. We repeated the analysis after further adjusting for sedentary time and FMI or waist circumference.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

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