Association of physical activity with high-density lipoprotein functionality in a population-based cohort: the REGICOR study

Asociación de la actividad física con la funcionalidad de las lipoproteínas de alta densidad en una cohorte de base poblacional: el estudio REGICOR

Raúl VIADAS,[a,b] Andrea TOLOBA,[a] Isabel FERNÁNDEZ,[a] Sergi SAYOLS-BAIXERAS,[a,c,d] Álvaro HERNÁEZ,[a,e,f,g] Helmut SCHROEDER,[a,h] Irene R. DÉGANO,[a,d,i] Camille LASSALE,[a,d] Jaume MARRUGAT,[a,d] Roberto ELOSUA[a,d,i,*]

[a] *Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain*

[b] *Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain*

[c] *Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden*

[d] *Centro de Investigación en Red de Enfermedades Cardiovasculares (CIBERCV), Spain*

[e] *Centre for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway*

[f] *Centro de Investigación en Red de Obesidad y Nutrición (CIBEROBN), Spain*

[g] *Facultat de Ciències de la Salut Blanquerna, Universitat Ramon Llull, Barcelona, Spain*

[h] *Centro de Investigación en Red de Epidemiología y Salud Pública (CIBERESP), Spain*

[i] *Facultat de Medicina, Universitat de Vic-Universitat Central de Catalunya, Vic, Barcelona, Spain*

***Corresponding author:**

Hospital del Mar Medical Research Institute, Dr Aiguader 88, 08003 Barcelona, Spain

Email address: relosua@imim.es (R. Elosua).

RESUMEN

Introducción y objetivos: Determinar la relación dosis-respuesta entre la actividad física en el tiempo libre (AFTL) actual y pasada, total y según su intensidad, y la funcionalidad de las lipoproteínas de alta densidad (HDL).

Métodos: Se seleccionaron a 642 participantes de un estudio poblacional: edad media 63,2 años y 51,1% mujeres. Se incluyeron datos de la visita inicial y de un seguimiento a 4 años. La AFTL se evaluó mediante cuestionarios validados. Se determinó la capacidad de eflujo de colesterol y antioxidante en el seguimiento. Se utilizaron modelos de regresión lineal y aditivos para evaluar la relación dosis-respuesta.

Resultados: Se observó una relación inversa y lineal entre la AFTL total actual (entre 0-400 MET x min/día) y la capacidad antioxidante de HDL (coeficiente de regresión [beta]: -0,022; IC95%: -0,030; -0,013), con una meseta por encima de este umbral. Se observaron resultados similares para la AFTL de intensidad moderada (beta: -0,028; IC95%: -0,049; -0,007) y vigorosa (beta: -0,025; IC95%: -0,043; -0,007), pero no para AFTL de intensidad ligera. La AFTL en el seguimiento no se asoció con la capacidad de eflujo de colesterol. La AFTL basal no se asoció con la funcionalidad de HDL.

Conclusiones: La AFTL de intensidad moderada-vigorosa actual se asocia de forma no lineal con una mayor capacidad antioxidante de las partículas de HDL. Se observa un beneficio máximo con dosis intermedias-bajas de AFTL (0-400 MET x min/día). Nuestros resultados concuerdan con las recomendaciones de práctica de AFTL y sugieren una asociación con la funcionalidad de HDL.

Palabras clave: actividad física, funcionalidad HDL, dosis-respuesta

ABREVIATURAS

CEC: Capacidad de eflujo de colesterol

HDL: Lipoproteína de alta densidad

HAC: Capacidad antioxidante del HDL

LTPA: Actividad física en el tiempo libre

MET: Equivalente Metabólico de Actividad

PA: Actividad física

ABSTRACT

Introduction and objectives: To determine the dose-response association between current and past leisure time physical activity (LTPA), total and at different intensities, and high-density lipoprotein (HDL) functionality parameters.

Methods: Study participants (n = 642) were randomly drawn from a large population-based survey. Mean age of the participants was 63.2 years and 51.1% were women. The analysis included data from a baseline and a follow-up visit (median follow-up, 4 years). LTPA was assessed using validated questionnaires at both visits. Two main HDL functions were assessed, cholesterol efflux capacity and the HDL antioxidant capacity, at the follow-up visit. Linear regression and linear additive models were used to assess the linear and non-linear association between LTPA and HDL functionality.

Results: Total LTPA at follow-up showed an inverse and linear relationship between 0 and 400 METs x min/day with HDL antioxidant capacity (regression coefficient [beta]: -0.022; 95%CI, -0.030, -0.013), with a plateau above this threshold. Similar results were observed for moderate (beta: -0.028; 95%CI, -0.049, -0.007) and vigorous (beta: -0.025; 95%CI, -0.043, -0.007), but not for light intensity LTPA. LTPA at follow-up was not associated with cholesterol efflux capacity. Baseline LTPA was not associated with any of the HDL functionality parameters analyzed.

Conclusions: Current moderate and vigorous LTPA showed a nonlinear association with higher HDL antioxidant capacity. Maximal benefit was observed with low-intermediate doses of total LTPA (up to 400 METs x min/day). Our results agree with current recommendations for moderate-vigorous LTPA practice and suggest an association between PA and HDL functionality in general population.

Key words: physical activity, HDL functionality, dose-response

ABBREVIATIONS

C: Cholesterol Efflux Capacity

HDL-C: High Density Lipoprotein Cholesterol

HAC: HDL antioxidant capacity

LTPA: Leisure Time Physical Activity

MET: Metabolic Equivalent of Task

PA: Physical Activity

INTRODUCTION

Regular leisure time physical activity (LTPA) is related to a lower risk of cardiovascular diseases and all-cause mortality.[1-4] It has been estimated that physical inactivity causes 6% of the burden of coronary heart disease and 9% of the premature mortality burden.[5] The World Health Organization recommends that all adults undertake 150-300 min of moderate-intensity or 75-150 min of vigorous-intensity physical activity (PA), or some equivalent combination of moderate-intensity and vigorous-intensity aerobic PA, per week.[6] Moreover, PA should be incorporated and performed regularly across the lifespan.[6]

PA improves cardiometabolic clinical phenotypes such as lipid profile, blood pressure, carbohydrates metabolism, hemostasis, and inflammation.[7] However, the mechanisms by which PA induces cardiovascular health benefits are still not fully understood.[7,8] One of the best-known effects of PA is to increase the levels of high-density lipoprotein-cholesterol (HDL-C).[9] HDL-C levels have been consistently and inversely related to cardiovascular risk in observational studies, but Mendelian randomization and experimental studies question the causality of this association.[10,11] Therefore, the anti-atherogenic properties of HDL particles could be related to qualitative and functional characteristics of the lipoprotein rather than the quantity of HDL-C.[12] Among these functional characteristics, cholesterol efflux capacity (CEC)[13] and HDL antioxidant capacity (HAC)[14,15] have been related to cardiovascular risk.

The relationship between LTPA and HDL functionality parameters has been analyzed in several studies.[16-20] However, the dose-response pattern of the association considering current and past LTPA, and PA intensities in a population-based study has not been previously addressed. The aim of this study was to determine the dose-response association between current and past LTPA and HDL functionality in a population-based sample. We also considered the relevance of PA intensity (light, moderate or vigorous) in this association.

METHODS

Study design and population

The Registre Gironí del Cor (REGICOR) study, begun in 1978, aims to contribute to the understanding of the epidemiology of cardiovascular diseases.[21] One of the components of the REGICOR study is a population-based cohort including 6,352 individuals recruited between 2003 and 2006 and reexamined between 2008 and 2013 (4,280 attended). Participants were 35 to 79 years old, and residents in the referral area.

In this analysis, we included a random subsample of 642 individuals who participated in both exams. In this subset of participants HDL functionality traits were measured. The study was approved by the local ethics committee and all participants provided their written informed consent.

Measurement of leisure time physical activity

The Minnesota Leisure Time Physical Activity Questionnaire was used to measure PA practice in the baseline visit. This questionnaire has been validated for the Spanish adult population[22,23] and assesses leisure and active commuting domains and frequency, duration, and intensity dimensions. Briefly, from a list of 64 activities, participants marked those they had practiced during the year prior to the visit, and a trained interviewer collected information related to the frequency of practice and the duration of each session. Each PA is assigned an intensity based on metabolic equivalents of task (MET).[24] The Minnesota Leisure Time Physical Activity Questionnaire allows estimating the daily average energy expenditure in the previous year (METs x min/day) and further classification as light-intensity LTPA if the activity required < 4 METs (e.g., slow-paced walking), moderate-intensity LTPA if it required 4 to 5.9 METs (e.g., brisk walking), and vigorous-intensity LTPA if it required ≥ 6 METs (e.g., jogging).[25] Thus, for each participant we estimated:

Total LTPA = light-intensity LTPA + moderate-intensity LTPA + vigorous-intensity LTPA

At the follow-up visit, a short version of the Minnesota Leisure Time Physical Activity Questionnaire was administered. The short version collects data about the monthly frequency, and average daily duration of practice of six types of PA: walking, brisk walking, gardening, walking trails, climbing stairs, and sport activities. This short version provides the same information as the original questionnaire, and has been validated in the Spanish population.[26] In the validation study, the Spearman correlation coefficients between the extended and the short questionnaires were 0.82 for total LTPA, 0.89 for light LTPA, 0.79 for moderate LTPA and 0.68 for vigorous LTPA. The short questionnaire also includes 2 questions related to sedentary behavior and one about occupational physical that were not considered in this analysis.

HDL functionality traits

We measured CEC and HAC in apolipoprotein-B depleted plasma at the 2008-2013 follow-up visit as previously described.[27]

Preparation of apolipoprotein-B depleted plasma (ABDP).

All HDL functionality experiments were performed in apolipoprotein-B depleted plasma (ABDP) in which only high-density lipoproteins are present. Plasma from the participants was incubated with a suspension of 20% polyethylene glycol 8000 (Sigma, United States) in a 200 mM glycine buffer pH 7.4 (Sigma), at 4 °C for 20 minutes. The mixture was then centrifuged (10,000 rpm, 15 minutes, 4 °C); supernatants were collected and finally stored at -80 °C upon use.

Cholesterol efflux capacity.

THP-1 monocytes were grown in RPMI 1640 medium, supplemented with 10% heat inactivated FBS, 1% sodium pyruvate, 1% L-glutamine, and 1% penicillin-streptomycin. Cells were refreshed every 72h. Monocytes were differentiated into macrophages through their incubation with phorbol-myristate-

acetate (Sigma) 200 nM, for 96h. THP-1 monocyte-derived macrophages were then incubated with 0.2 µCi/mL of [1,2-3 H(N)]-cholesterol (Perkin-Elmer, United States) for 24h, washed, incubated in fresh RPMI 1640 medium supplemented with 1% bovine serum albumin (BSA, Sigma) for 24h, washed again, and finally cultured in fresh RPMI 1640 medium + 1% BSA in the presence of 5% ABDP from the participants, or without (control), for 16 h. The culture supernatants were obtained, and the cell culture lipids were extracted with ice-cold isopropanol for 60 minutes. Radioactivity in both supernatant medium and cell lipids was measured in a beta scintillation Tri-Card 2800TR counter (Perkin-Elmer). Finally, CEC for each well was calculated according to this equation:

$$\text{Cholesterol efflux capacity} = \frac{\frac{radioactivity\ in\ supernatant}{(radioactivity\ in\ supernatant+cells)} \times 100}{efflux\ value\ of\ the\ pool}$$

We ran samples in duplicate and the mean value was considered for the analyses. We also corrected inter-assay variation by a pooled ABDP normalization as follows: a pool of ABDP obtained from 20 healthy volunteers was included in each experiment as inter-assay control, and we divided the values of all cholesterol efflux results of the volunteers by the efflux value of this pool. The interassay coefficient of variation of this pooled normalization method was 9.6%.

HDL antioxidant capacity measurement

HDL antioxidant capacity (HAC) was measured following the "HDL inflammatory index" technique. In brief, we measured the capacity of participants' HDL to avoid the oxidative modification of 2'-7'-dichlorodihydrofluorescein diacetate (H2DCF-DA, Life Technologies, Thermo Fisher Scientific, United States) in the presence of oxidized low-density lipoproteins (LDL). H2DCF-DA was diluted in methanol (final concentration: 2 mg/mL) for 30 minutes, to obtain its deacetylated form (H2DCF). Oxidized LDL was prepared from a pool from plasma of 20 healthy participants by density gradient ultracentrifugation. LDL were oxidized, diluted to 100 mg/L and stored at -80ºC upon use. Finally, 5 µL of ABDP from the volunteers was incubated with H2DCF (final concentration: 3 µg/mL) and oxidized

LDL (final concentration: 1.5 µg/mL) in 96-well, black polystyrene plates, at 37ºC. Fluorescence was measured every 3 minutes for 75 minutes in an Infinite M200 reader (Tecan Ltd, Switzerland). The greater the oxidation of H2DCF, the greater the fluorescent signal and the lower the HDL antioxidant capacity. To calculate the antioxidant capacity of HDLs, the slope between 15 and 75 minutes was calculated (the relationship between fluorescence and time was lineal between these times). We analyzed samples in duplicate and the mean value was considered for the analyses. We also corrected inter-assay variation by a pooled ABDP normalization. The inter-assay coefficient of variation of this pooled normalization method was 4.6%.

Covariates

Trained personnel administered a series of validated questionnaires and carried out measurements following a standardized methodology to collect information on sociodemographic (age, residence, sex, educational level), lifestyle (smoking status, diet) and anthropometric variables (weight, height, body mass index), blood pressure, and drug treatments. In addition, a series of complementary laboratory tests on serum were carried out, including total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and glucose. These assessments were performed at both visits (Figure 1).

Statistical analysis

Quantitative variables are presented as mean and standard deviation or median and interquartile range, categorical variables as counts and percentages. In the bivariate analyses, Spearman correlation was used to determine the association between two quantitative variables and to analyze the linear trend of the association between LTPA quartiles and the variables of interest and covariates, and Chi-squared tests to compare proportions between groups. To assess the dose-response pattern of the association of physical activity with HDL functionality, linear regression and additive regression models

were fitted. Additive regression models allow to explore non-linear relations between an independent continuous trait and a dependent outcome (binary or continuous) based on a number of knots, points in which the association deviates from linearity. We defined a maximum of three knots to avoid overfitting and enhance the interpretability of the model. The non-linear dose-response pattern of the association was assessed visually and also considering the p-value. When the pattern of the association was non-linear the analysis was split using the visual value that best defined the knot, and two conventional linear regression models were used: one to explore the association when LTPA was in the range of values from 0 METs x min/day to the knot, and another model to explore the association when LTPA was in the range of values from the knot to higher values.

The dependent variables were those of HDL functionality: CEC, HAC. The independent variables of interest were on one hand past LTPA, and on the other hand current LTPA. Moreover, physical activity was considered as total LTPA, independently of its intensity, in one model; and considering the intensity of PA in another model, which included LTPA in light intensity PA, in moderate intensity PA, and in vigorous intensity PA. Classic cardiovascular risk factors (age, sex, smoking, diabetes, LDL cholesterol) as well as HDL-cholesterol were included as covariates in the multivariable models. Moreover, we designed two models, differentiated by the exclusion (Model 1) or inclusion (Model 2) of body mass index to explore the potential mediating effect of this variable.

R software (Version 4.0.3) and Rstudio were used for statistical analyses. For the linear component a p-value <0.008 was considered as statistically significant after considering multiple comparisons (3 independent PA variables –light, moderate and vigorous LTPA– * 2 independent parameters of HDL functionality = 6; 0.05/6=0.008).

RESULTS

Study population

Characteristics of the 642 participants at the follow-up visit across total LTPA quartile groups are shown in table 1. The proportion of men and the HDL-C concentration increased across the quartiles of total PA practice, whereas body mass index and HAC decreased as LTPA increased. CEC was similar across total LTPA quartiles.

Bivariate associations between variables

Table 2 shows the Spearman correlation coefficients of the association between all the variables of interest, including the covariates, at the follow-up visit. CEC was directly associated with total cholesterol and HDL-C concentrations, and inversely associated with glycaemia and triglyceride levels. HAC was directly associated with body mass index, blood pressure, glycaemia, and triglyceride levels, and inversely associated with HDL-C levels and with total, light, and vigorous LTPA.

Physical activity and cholesterol efflux capacity

The linear and non-linear dose-response relationship between LTPA and CEC is shown in figure 1 of the supplementary data. In the multivariable linear regression analyses, no significant associations between past or current LTPA and CEC were observed (table 3).

1.1 Physical activity and HDL antioxidant capacity (HAC)

Past LTPA was not associated with HAC (table 3). The relationship between current total LTPA and the HAC showed both linear and nonlinear components (figure 1A), with a knot (cut-point) around 400 METs x min/day. Below this threshold, total LTPA was inversely associated with HAC: each unit of 100 METs x min/day was associated with a 0.022-unit decrease in HAC; above this threshold, no association was observed (table 3).

Consistent with total LTPA results, moderate LTPA and vigorous LTPA showed a nonlinear association with HAC (figure 1C, D), with a knot (cut-off point) around 200 METs x min/day. Below this threshold, moderate and vigorous LTPA showed an inverse and similar magnitude of association with HAC: each

100 METs x min/day was associated with a decrease in HAC of -0.028 and -0.025 units, respectively; above this threshold, no association was observed (table 3). Further adjustment by body mass index did not modify the magnitude of the association (table 1 of the supplementary data). Current light LTPA was not associated with HAC (figure 1B and table 3).

DISCUSSION

In this study, we observed that current total, moderate, and vigorous intensity LTPA were nonlinearly associated with HAC (Figure 2). Current total LTPA presents an inverse and linear relationship between 0 and 400 METs x min/day, with a plateau above this threshold. Similar results were observed for current moderate and vigorous intensity LTPA, but not for light intensity LTPA, with a cut-point around 200 METs x min/day. Current LTPA was not associated with CEC and past LTPA was not associated with any of the HDL functionality parameters analyzed.

CEC is the capacity of HDLc to promote reverse cholesterol transport from peripheral cells to the liver.[28] Experimentally, CEC quantifies the movement of labeled cholesterol from the inside of the cell to the extracellular medium.[17] The effect of LTPA on this process has been previously assessed, with heterogeneous results.[17] Our results, indicate a lack of association between LTPA and CEC at any intensity level. Hernáez et al. reached similar conclusions after analyzing 296 individuals at high cardiovascular risk.[16] However, Khan et al. studied the effect of weight loss and exercise in metabolic syndrome patients, and concluded that CEC improves after these interventions.[18] Consistent with this finding, other groups have pointed to a beneficial effect of PA on CEC[19,29-32] but with some differences in the level of CEC increase achieved. Most of these studies indicate that moderate and vigorous intensity LTPA has the strongest effect on CEC.[4,19,32] These inconsistencies could be related to the lack of a standardized method to measure CEC in humans. Most of the studies used murine J774 macrophages for laboratory tests, while the present study and Hernáez et al. used human THP1 monocytes. The design of the studies (experimental vs observational), the type of PA intervention or

the method used to measure PA practice, the characteristics of the population included in the studies, the use of concomitant drugs, or dietary differences in diets could also partially explain this heterogeneity.

The HAC measures the ability of HDL to prevent LDL oxidation. Therefore, an elevated antioxidant capacity of HDL reduces the oxidation of LDL. HDL antioxidant capacity is inversely associated with cardiovascular death, ischemic heart disease, and hospitalization for myocardial infarction, among others.[15] The dose-response effect of LTPA on HAC had not been previously studied in the general population. In our data, we observed LTPA was associated with decreased HAC values up to 400 METs x min/day. This pattern concurs with the association of LTPA to cardiovascular events and all-cause mortality in the same population[4]: increasing levels of total LTPA were inversely related to the incidence of cardiovascular events and all-cause mortality until a cut-point of 400 METs x min/day, beyond which no further benefits were observed. Two studies have also reported a shift from pro-oxidant/inflammatory to anti-oxidant/inflammatory in the HDL profile after a short training program, in metabolic syndrome patients (10-week intervention)[33] and overweight individuals (3-week intervention).[34] Changes observed in the HDL lipidome, proteome, or its structure could mediate the anti-inflammatory and antioxidant capacities of the lipoprotein and consequently modulate cardiovascular risk.[18] With respect to the effects of PA intensity, moderate and vigorous LTPA, also related to lower cardiovascular risk in the same REGICOR cohort,[4] had a similar magnitude of association with HAC. The association of moderate LTPA with HAC was not statistically significant, likely as a consequence of the low amount and low variability of the practice of this type of PA, hampering the statistical power of our analysis.

Our study has several strengths. Our analysis included a population-based sample, assessed the dose-response pattern of the association between LTPA and HDL functionality parameters, and considered different types of LTPA according to their intensity at baseline and at 4-years follow-up.

The study also has some limitations. This was an observational study and PA was assessed using questionnaires. Although they were validated, some misclassification of the exposure of interest

cannot be excluded. Second, the causal relationship between PA and HDL functionality could be difficult to ascertain using observational approach. The dose-response association, temporal trend (current but not past LTPA), plausibility, consistency with experimental studies support the causal relationship between PA and HDL functionality; however, we cannot exclude the presence of residual confounding in the estimated effect of this association. Third, HDL functionality was assessed with in vitro techniques, and was limited to cholesterol efflux capacity and HDL antioxidant capacity measured with the HII method, and no other functionality parameters or methods were used (Apo A1, paraoxonase…). Finally, the distribution and low variability of moderate intensity LTPA practice in our sample limits the statistical power of our study.

CONCLUSIONS

In summary, this population-based study evaluated the dose-response relationship between LTPA and HDL functionality parameters. Current moderate and vigorous intensity LTPA showed a nonlinear association with higher HDL antioxidant capacity (Figure 2). Maximal benefit was observed with low-intermediate doses of PA, with a plateau above 400 METs x min/day for total LTPA. Our results agree with current recommendations of low-intermediate doses of moderate-vigorous intensity LTPA practice, and suggest an association between PA and HDL functionality in general population.

ACKNOWLEDGEMENTS

We thank Elaine M. Lilly, PhD, for her critical reading and revision of the English text.

FUNDING

This work was supported by the Carlos III Health Institute–European Regional Development Fund [CIBERCV, CIBEROBN, CIBERESP]; PERIS from Agència de Gestió d'Ajuts Universitaris i de Recerca [SLT002/16/00088]; and the Government of Catalonia through the Agency for Management of University and Research Grants [2017SGR946].

CONFLICTS OF INTEREST

The authors declare they have no conflict of interest, and that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

AUTHOR CONTRIBUTION

• Contribute substantially to the study conception: Raúl Viadas, Roberto Elosua, Camille Lassale, Álvaro Hernáez, Sergi Sayols-Baixeras

• Contribute substantially to the study design: Jaume Marrugat, Helmut Schroeder, Roberto Elosua

• Contribute substantially to the acquisition of data: Álvaro Hernáez, Sergi Sayols-Baixeras, Jaume Marrugat, Roberto Elosua

• Contribute substantially to data analysis: Raúl Viadas, Andrea Toloba, Roberto Elosua

• Contribute substantially to the interpretation of the results: Raúl Viadas, Andrea Toloba, Isabel Fernández, Sergi Sayols-Baixeras, Álvaro Hernáez, Helmut Schroeder, Irene R. Dégano, Camille Lassale, Jaume Marrugat, Roberto Elosua.

• Write the article: Raúl Viadas, Roberto Elosua

• Critical review of the intellectual content: Andrea Toloba, Isabel Fernández, Sergi Sayols-Baixeras, Álvaro Hernáez, Helmut Schroeder, Irene R. Dégano, Camille Lassale, Jaume Marrugat.

• Give final approval to the version to be published: Raúl Viadas, Andrea Toloba, Isabel Fernández, Sergi Sayols-Baixeras, Álvaro Hernáez, Helmut Schroeder, Irene R. Dégano, Camille Lassale, Jaume Marrugat, Roberto Elosua.

• Agree to assume responsibility for all aspects of the article and investigate and resolve any issues related to the accuracy and veracity of any part of the work: Raúl Viadas, Andrea Toloba, Isabel Fernández, Sergi Sayols-Baixeras, Álvaro Hernáez, Helmut Schroeder, Irene R. Dégano, Camille Lassale, Jaume Marrugat, Roberto Elosua.

WHAT IS KNOWN?

- Physical activity reduces the risk of coronary artery disease.
- Physical activity improves lipid profile and increases HDL cholesterol.
- HDL cholesterol levels are not causally related to the risk of coronary artery disease.
- The mechanisms explaining the benefits of physical activity are not fully understood.

WHAT DOES THIS STUDY ADD?

- Current physical activity between 0 and 400 METs x min/day is linearly related to HDL antioxidant capacity with a plateau above this threshold.
- Current moderate and vigorous intensity physical activity showed a similar pattern of association, whereas light intensity physical activity was not associated with HDL antioxidant capacity.
- Current physical activity was not associated with cholesterol efflux capacity.
- Past physical activity was not associated with any of the HDL functionality parameters analyzed.

REFERENCES

1. Ekelund U, Tarp J, Steene-Johannessen J, et al. Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: Systematic review and harmonised meta-analysis. BMJ. 2019;366.
2. Liu Y, Shu XO, Wen W, et al. Association of leisure-time physical activity with total and cause-specific mortality: A pooled analysis of nearly a half million adults in the Asia Cohort Consortium. Int J Epidemiol. 2018;47:771-9.
3. Lear SA, Hu W, Rangarajan S, et al. The effect of physical activity on mortality and cardiovascular disease in 130 000 people from 17 high-income, middle-income, and low-income countries: the PURE study. Lancet. 2017;390:2643-2654.
4. Clará A, Berenguer G, Pérez-Fernández S, et al. Analysis of the dose-response relationship of leisure-time physical activity to cardiovascular disease and all-cause mortality: the REGICOR study. Rev Esp Cardiol. 2021;74:414-420.
5. Lee IM, Shiroma EJ, Lobelo F, et al. Effect of physical inactivity on major non-communicable diseases worldwide: An analysis of burden of disease and life expectancy. Lancet. 2012;380:219-229.
6. Bull FC, Al-Ansari SS, Biddle S, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. Br J Sports Med. 2020;54:1451-1462.
7. Neufer PD, Bamman MM, Muoio DM, et al. Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits. Cell Metab. 2015;22:4-11.
8. Gabriel BM, Zierath JR. The Limits of Exercise Physiology: From Performance to Health. Cell Metab. 2017;25:1000-1011.
9. Lin X, Zhang X, Guo J, et al. Effects of exercise training on cardiorespiratory fitness and biomarkers of cardiometabolic health: A systematic review and meta-analysis of randomized controlled trials. J Am Heart Assoc. 2015;4:e002014.
10. Landmesser U, Hazen S. HDL-cholesterol, genetics, and coronary artery disease: The myth of the “good cholesterol”? Eur Heart J. 2018;39:2179-2182.
11. Prats-Uribe A, Sayols-Baixeras S, Fernández-Sanlés A, et al. High-density lipoprotein characteristics and coronary artery disease: a Mendelian randomization study. Metabolism. 2020;112:154351.
12. Sacks FM, Jensen MK. From high-density lipoprotein cholesterol to measurements of function: Prospects for the development of tests for high-density lipoprotein functionality in cardiovascular disease. Arterioscler Thromb Vasc Biol. 2018;38:487-499.
13. Ebtehaj S, Gruppen EG, Bakker SJL, Dullaart RPF, Tietge UJF. HDL (High-Density Lipoprotein) Cholesterol Efflux Capacity Is Associated With Incident Cardiovascular Disease in the General Population. Arterioscler Thromb Vasc Biol. 2019;39:1874-1883.
14. Ajala ON, Demler O V., Liu Y, et al. Anti-inflammatory hdl function, incident cardiovascular events, and mortality: A secondary analysis of the jupiter randomized clinical trial. J Am Heart Assoc. 2020;9:16507.

15. Jia C, Anderson JLC, Gruppen EG, et al. High-Density Lipoprotein Anti-Inflammatory Capacity and Incident Cardiovascular Events. Circulation. 2021;143:1935-1945.

16. Hernáez Á, Soria-Florido MT, Castañer O, et al. Leisure time physical activity is associated with improved HDL functionality in high cardiovascular risk individuals: a cohort study. Eur J Prev Cardiol. 2020;2047487320925625.

17. Ruiz-Ramie JJ, Barber JL, Sarzynski MA. Effects of exercise on HDL functionality. Curr Opin Lipidol. 2019;30:16-23.

18. Khan AA, Mundra PA, Straznicky NE, et al. Weight Loss and Exercise Alter the High-Density Lipoprotein Lipidome and Improve High-Density Lipoprotein Functionality in Metabolic Syndrome. Arterioscler Thromb Vasc Biol. 2018;38:438-447.

19. Sarzynski MA, Ruiz-Ramie JJ, Barber JL, et al. Effects of Increasing Exercise Intensity and Dose on Multiple Measures of HDL (High-Density Lipoprotein) Function. Arterioscler Thromb Vasc Biol. 2018;38:943-952.

20. Pagonas N, Vlatsas S, Bauer F, et al. The impact of aerobic and isometric exercise on different measures of dysfunctional high-density lipoprotein in patients with hypertension. Eur J Prev Cardiol. 2019;26:1301-1309.

21. REGICOR. Available at: https://www.regicor.cat/. Accessed 23 Dec 2021.

22. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Med Sci Sports Exerc. 2000;32:1431-1437.

23. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol. 1994;139:1197-1209.

24. Ainsworth BE, Haskell WL, Herrmann SD, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. Med Sci Sports Exerc. 2011;43:1575-1581.

25. Kaminsky LA, Montoye AHK. Physical activity and health: What is the best dose? J Am Heart Assoc. 2014;3:e001430.

26. Molina L, Sarmiento M, Peñafiel J, et al. Validation of the regicor short physical activity questionnaire for the adult population. PLoS One. 2017;12:e0168148.

27. Sayols-Baixeras S, Hernáez A, Subirana I, et al. DNA Methylation and High-Density Lipoprotein Functionality-Brief Report: The REGICOR Study (Registre Gironi del Cor). Arterioscler Thromb Vasc Biol. 2017;37:567-569.

28. Patel PJ, Khera AV, Jafri K, Wilensky RL, Rader DJ. The anti-oxidative capacity of high-density lipoprotein is reduced in acute coronary syndrome but not in stable coronary artery disease. J Am Coll Cardiol. 2011;58:2068-2075.

29. Koba S, Ayaori M, Uto-Kondo H, et al. Beneficial effects of exercise-based cardiac rehabilitation on high- density lipoprotein-mediated cholesterol efflux capacity in patients with acute coronary syndrome. J Atheroscler Thromb. 2016;23:865-877.

30. Furuyama F, Koba S, Yokota Y, Tsunoda F, Shoji M, Kobayashi Y. Effects of cardiac rehabilitation on high-density lipoprotein-mediated cholesterol efflux capacity and paraoxonase-1 activity in patients with acute coronary syndrome. J Atheroscler Thromb. 2018;25:153-169.

31. Boyer M, Mitchell PL, Poirier P, et al. Impact of a one-year lifestyle modification program on cholesterol efflux capacities in men with abdominal obesity and dyslipidemia. Am J Physiol - Endocrinol Metab. 2018;315:E460-E468.

32. Wesnigk J, Bruyndonckx L, Hoymans VY, et al. Impact of Lifestyle Intervention on HDL-Induced eNOS Activation and Cholesterol Efflux Capacity in Obese Adolescent. Cardiol Res Pract. 2016;2016:2820432.

33. Sang H, Yao S, Zhang L, et al. Walk-run training improves the anti-inflammation properties of high-density lipoprotein in patients with metabolic syndrome. J Clin Endocrinol Metab. 2015;100:870-879.

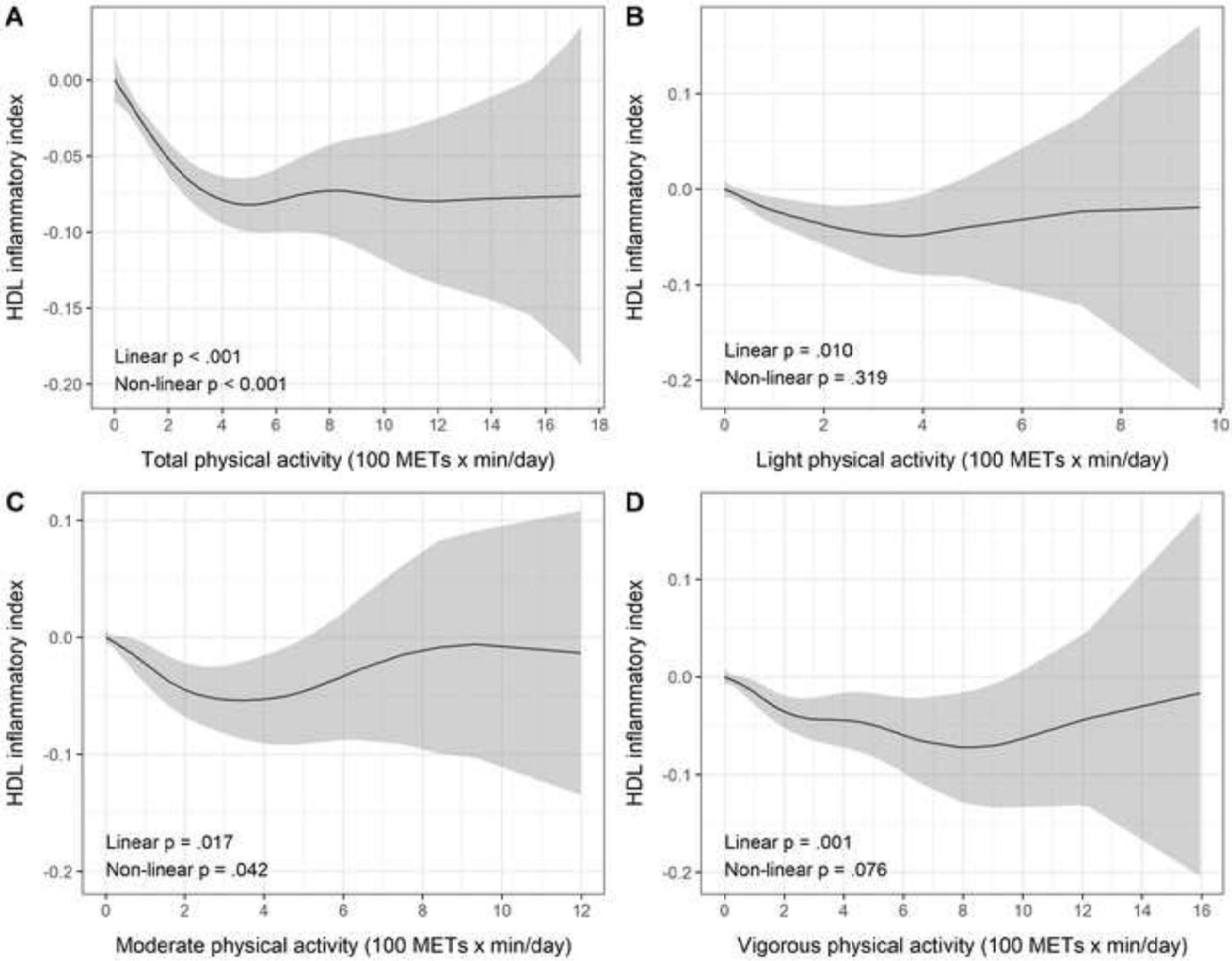
34. Roberts CK, Ng C, Hama S, Eliseo AJ, Barnard RJ. Effect of a short-term diet and exercise intervention on inflammatory/antiinflammatory properties of HDL in overweight/obese men with cardiovascular risk factors. J Appl Physiol. 2006;101:1727-1732.

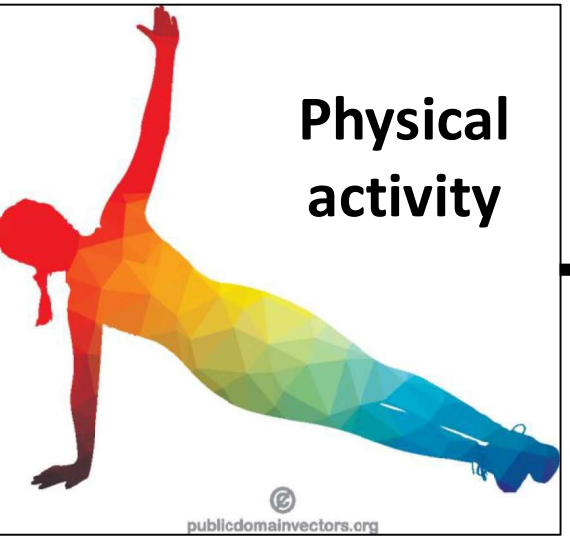
FIGURE LEGENDS

Figure 1. Dose-response association of different intensities of current leisure time physical activity (total, and light, moderate and vigorous intensity – 100 METs x min/day –) and the HDL antioxidant capacity, assessed by restricted cubic splines. The black line represents the estimated effect size of the association and the grey area the 95% confidence interval. HDL, high density lipoproteins; MET, metabolic equivalent of task.

Figure 2. Central illustration showing the dose-response association between current total leisure time physical activity and HDL antioxidant capacity. The dose-response association between light, moderate and vigorous intensity physical activity and HDL antioxidant capacity is also shown.

PA: Physical activity; HAC: HDL antioxidant capacity; MET: Metabolic Equivalent of Task





High density lipoprotein –HDL–
functionality:
1.-Cholesterol efflux capacity-CE
2.-HDL antioxidant capacity-HAC

rrent Total PA-HAC

Δ 100 MET x min/day
→ - 0.22 units

0 MET x min/day

Current Light PA-HAC

Current Moderate PA-HAC

Δ 100 MET x min/day
→ - 0.28 units

200 MET x min/day

Current Vigorous P

Δ 100 MET x m
→ - 0.25 units

200 MET x min/d

Tablas (Tables)

Table 1. Characteristics of the participants at the follow-up visit across total leisure time physical activity practice quartiles (METs x min/day)

		Total leisure-time physical activity (METs x min/day)					
	All (n = 642)	Q1 (n = 161)	Q2 (n = 160)	Q3 (n = 161)	Q4 (n = 160)	*P* for trends	*N*
		(0-44.56][a]	(44.56-154.51] [a]	(154.51-359.64] [a]	(359.64-1733.27) [a]		
Age, years [b]	63.2 (11.7)	62.6 (12.3)	62.8 (12.5)	62.5 (11.9)	64.9 (10.1)	.114	642
Sex [d]						< .001	642
Male	314 (48.9%)	58 (36.0%)	76 (47.5%)	82 (50.9%)	98 (61.3%)		
Female	328 (51.1%)	103 (64.0%)	84 (52.5%)	79 (49.1%)	62 (38.8%)		
Smoking status [d]						.682	642
Never	340 (53.0%)	83 (51.6%)	81 (50.6%)	92 (57.1%)	84 (52.5%)		
Current or ex-smoker (< 1 y)	107 (16.7%)	34 (21.1%)	33 (20.6%)	18 (11.2%)	22 (13.8%)		
Exsmoker (> 1 y)	195 (30.4%)	44 (27.3%)	46 (28.7%)	51 (31.7%)	54 (33.8%)		
Diabetes [d]	86 (13.4%)	25 (15.5%)	24 (15.0%)	22 (13.7%)	15 (9.38%)	.100	642
BMI, kg/m² [b]	26.9 (4.05)	27.7 (4.62)	26.8 (4.07)	26.6 (3.55)	26.5 (3.83)	.007	640
Total cholesterol, mg/dL [b]	209 (36.4)	212 (37.3)	206 (35.2)	209 (35.5)	208 (37.5)	.524	642
HDL cholesterol, mg/dL [b]	53.0 (12.3)	51.5 (12.2)	52.5 (12.4)	54.3 (12.3)	53.8 (12.4)	.045	642
LDL cholesterol, mg/dL [b]	135 (32.2)	140 (32.9)	133 (31.5)	135 (32.2)	135 (32.1)	.264	638
Triglycerides, mg/dL [c]	89.0 [67.0;121]	94.0 [68.0;125]	89.0 [69.0;121]	89.0 [66.0;122]	82.5 [62.5;115]	.039	642
Glycaemia, mg/dL [b]	97.7 (20.5)	97.2 (19.4)	98.8 (23.7)	98.7 (22.1)	96.1 (15.8)	.635	642
SBP, mmHg [b]	131 (18.5)	131 (20.8)	128 (17.7)	130 (18.0)	133 (17.0)	.183	642
DBP, mmHg [b]	76.0 (9.92)	76.1 (11.2)	75.2 (9.83)	75.5 (9.34)	77.3 (9.20)	.281	642
LTPA [c]							
Total, METs x min/d	155 [44.6;360]	17.5 [3.50;30.9]	94.4 [65.0;120]	240 [195;288]	555 [443;714]	< .001	642
Light, METs x min/d	30.4 [0.00;95.9]	4.00 [0.00;12.0]	36.0 [6.88;71.9]	63.9 [16.0;160]	95.9 [0.00;240]	< .001	642
Moderate, METs x min/d	1.93 [0.00;61.8]	0.00 [0.00;5.79]	8.14 [0.00;39.1]	0.00 [0.00;79.9]	79.9 [0.00;282]	< .001	642
Vigorous, METs x min/d	21.0 [1.79;149]	2.31 [0.00;7.96]	14.0 [1.36;42.2]	70.1 [6.99;160]	242 [90.4;429]	< .001	642
Cholesterol efflux capacity [b]	0.92 (0.12)	0.93 (0.12)	0.92 (0.11)	0.92 (0.12)	0.93 (0.12)	.765	642
HDL antioxidant capacity [b]	1.08 (0.12)	1.11 (0.12)	1.10 (0.12)	1.06 (0.12)	1.05 (0.11)	< .001	642

BMI, body mass indx; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; LTPA, leisure-time physical activity.

a Minimum and maximum value of LTPA in each quantile;

b Values are means (standard deviation);

c Values are medians (interquartile range);

d Values are counts (proportions).

Table 2. Spearman correlation (rho coefficient, above the diagonal; p-value, below the diagonal) between variables of interest at the follow-up visit

									LTPA (METs x min/day)				
Age	BMI	SBP	DBP	Glycaemia	Total-C	HDL-C	LDL-C	TG	Total PA	Light PA	Mod. PA	Vig. PA	CEC	HAC
1	0.126	0.420	-0.149	0.179	-0.051	-0.022	-0.077	0.107	0.058	0.224	-0.100	-0.157	-0.013	-0.033
0.001	1	0.274	0.192	0.319	-0.030	-0.259	-0.010	0.308	-0.117	-0.006	-0.076	-0.152	-0.089	0.121
0.000	0.000	1	0.514	0.314	0.017	-0.111	0.010	0.191	0.062	0.103	-0.036	-0.063	0.043	0.130
0.000	0.000	0.000	1	0.114	0.110	-0.086	0.104	0.146	0.057	-0.027	0.063	0.067	0.013	0.119
0.000	0.000	0.000	0.004	1	-0.016	-0.210	-0.023	0.262	-0.003	0.079	-0.023	-0.101	-0.118	0.177
0.195	0.446	0.672	0.005	0.687	1	0.315	0.942	0.245	-0.042	-0.075	0.035	0.019	0.167	-0.023
0.578	0.000	0.005	0.029	0.000	0.000	1	0.114	-0.418	0.062	-0.014	0.063	0.064	0.492	-0.173
0.053	0.792	0.808	0.008	0.569	0.000	0.004	1	0.177	-0.052	-0.093	0.027	0.031	0.036	-0.017
0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1	-0.096	0.034	-0.054	-0.108	-0.138	0.182
0.142	0.003	0.118	0.152	0.948	0.287	0.119	0.193	0.015	1	0.448	0.366	0.635	-0.007	-0.192
0.000	0.888	0.009	0.487	0.046	0.059	0.722	0.019	0.387	0.000	1	-0.113	0.059	-0.065	-0.089
0.011	0.054	0.361	0.113	0.557	0.381	0.109	0.495	0.169	0.000	0.004	1	0.129	0.009	-0.015
0.000	0.000	0.110	0.092	0.011	0.627	0.108	0.440	0.006	0.000	0.138	0.001	1	0.022	-0.090
0.735	0.025	0.274	0.745	0.003	0.000	0.000	0.361	0.000	0.851	0.101	0.813	0.585	1	-0.048
0.399	0.002	0.001	0.003	0.000	0.559	0.000	0.668	0.000	0.000	0.024	0.699	0.022	0.225	1

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Total-C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; Total PA, total physical activity; Light PA, light physical activity; Mod. PA, moderate physical activity; Vig. PA, vigorous physical activity; CEC, cholesterol efflux capacity; HAC, HDL antioxidant capacity.

Table 3. Relationship between past and current physical activity (total and by intensity), and cholesterol efflux capacity and HDL antioxidant capacity, adjusted for confounding variables

	CHOLESTEROL EFFLUX CAPACITY			**HDL ANTIOXIDANT CAPACITY**		
	β	**95%CI**	***P***	**β**	**95%CI**	***P***
TOTAL PHYSICAL ACTIVITY						
Past total LTPA (100 METs x min/day)	0.001	-0.002, 0.004	.404	-0.001	-0.004, 0.003	.691
Current total LTPA (100 METs x min/day)	0.000	-0.003, 0.003	.885	Non-linear *P* < .001		
< 400 METs x min/day current total LTPA	---	---	-	-0.022	-0.030, -0.013	< .001
≥ 400 METs x min/day current total LTPA	---	---	-	0.002	-0.005, 0.008	.632
PHYSICAL ACTIVITY ACCORDING TO INTENSITY						
Past physical activity practice						
Past light LTPA (100 METs x min/day)	0.000	-0.008, 0.007	.909	-0.008	-0.016, 0.000	.039
Past moderate LTPA (100 METs x min/day)	0.001	-0.005, 0.006	.791	0.001	-0.005, 0.007	.677
Past vigorous LTPA (100 METs x min/day)	0.003	-0.003, 0.008	.310	0.001	-0.004, 0.007	.638
Current physical activity practice						
Current light LTPA (100 METs x min/day)	-0.005	-0.013, 0.002	.166	-0.011	-0.019, -0.003	.010
Current moderate LTPA (100 METs x min/day)	-0.001	-0.007, 0.004	.630	Non-linear *P* = .042		
< 200 METs x min/day current moderate LTPA	---	---	-	-0.028	-0.049, -0.007	.010
≥ 200 METs x min/day current moderate LTPA	---	---	-	0.007	-0.005, 0.019	.265
Current vigorous LTPA (100 METs x min/day)	0.003	-0.001, 0.007	.174	Non-linear *P* = .076		
< 200 METs x min/day current vigorous LTPA	---	---	-	-0.025	-0.043, -0.007	.007
≥ 200 METs x min/day current vigorous LTPA	---	---	-	-0.004	-0.012, 0.005	.363

LTPA = leisure time physical activity; β = linear regression coefficient; CI, confidence interval.
Adjusted for age, sex, smoking status, diabetes, HDL-Cholesterol, and LDL-Cholesterol