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ORIGINAL ARTICLE

Moderate physical activity reduces 10-year diabetes incidence: the mediating role of oxidative stress biomarkers

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Abstract
Objectives To evaluate the effect of physical activity levels on 10-year diabetes incidence and investigate the potential mechanism.
Methods In 2001–2002, a random sample of apparently healthy 3042 men and women (18–89 years) was selected to participate in the ATTICA study. Several socio-demographic, clinical and lifestyle characteristics were recorded. Physical activity level was recorded through a translated, validated, version of International Physical Activity Questionnaire (IPAQ); MET min/week was calculated and quartiles constructed. Diabetes diagnosis was defined according to the ADA criteria. During 2011–2012, a 10-year follow-up was performed.
Results $n = 191$ cases were recorded, yielding an incidence of 12.9%. In multivariable analysis, moderate physical activity level (331–1484 MET min/week) was found to decrease 10-year diabetes incidence by 53% compared to very low physical activity (< 150 MET min/week) (OR = 0.47; 95% CI 0.24, 0.93). For high physical activity level (> 1484 MET min/week), the results were not significant. The antidiabetic effect was found to be mediated by oxidized LDL and total antioxidant capacity.
Conclusions The current work revealed the significant beneficial role of moderate physical activity against diabetes development, potentially through attenuating oxidative stress.

Keywords Physical activity · Diabetes incidence · Oxidative stress

Abbreviations

ADA	American Diabetes Association
CI	Confidence interval
BMI	Body mass index
HDL	High-density cholesterol lipoprotein
IPAQ	International Physical Activity Questionnaire
LDL	Low-density cholesterol lipoprotein
MET	Metabolic equivalent of task
OR	Odds ratio
SPSS	Statistical Package for Social Sciences
T2DM	Type 2 diabetes mellitus

Introduction

In the last few decades, the prevalence of type 2 diabetes mellitus (T2DM) is increasing rapidly worldwide, mainly because of changes in factors, particularly obesity and physical inactivity, which interact with individual genetic susceptibility (Gill and Cooper 2008). It has been estimated that the world prevalence of diabetes in 2030 will be 7.7%, affecting 439 million adults (aged 20–79 years) (Shaw et al. 2010). In Greece, the 10-year diabetes incidence was recently recorded, indicating an increase in diabetes prevalence, in line with global trends (Koloverou et al. 2014). Lack of treatment makes primary prevention a cornerstone of the global response to the disease.

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Physical activity seems to be beneficial for individuals with T2DM, along with diet and medication (Wasserman and Zinman 1994), as it can prevent, delay or even treat various diabetes-related complications, such as cardiovascular disease, hyperlipidemia, hypertension, fibrinolysis and obesity, and help achieve better glycemic control (American Diabetes Association 2004). Physical activity may also reduce the incidence of stroke and ischemic heart disease, and decrease all-cause mortality (Wei et al. 2000). However, only almost two out of three elder patients poorly adhere to current physical activity recommendations (Panagiotakos et al. 2008); i.e., 150 min per week of moderate physical activity, "spread out during at least 3 days during the week, with no more than 2 consecutive days between bouts of aerobic activity" (Colberg et al. 2010).

Physical activity may also be a promising strategy in diabetes primary prevention. It has been earlier demonstrated that physical activity was associated with lower diabetes risk in a dose–response way (Hu et al. 1999). Modest levels of physical activity are associated with reduced diabetes risk (Fretts et al. 2012), both from leisure-time exercise or daily activity (Villegas et al. 2006), whereas a deleterious association of increasing sedentary behavior and diabetes development has been proposed (Joseph et al. 2016), which was confirmed by a recent metanalysis (Cloostermans et al. 2015). Physical activity has been suggested to ameliorate the detrimental effects of obesity on insulin resistance (Kavouras et al. 2007); nevertheless, a recent meta-analysis proposed that the benefit is not entirely attributed to reduced adiposity (Aune et al. 2015).

Despite the well-stated effect of physical activity on diabetes, debate still exists regarding (i) the level of physical activity that confers the greatest antidiabetic effect, i.e., whether there is a dose–response relationship and (ii) the exact mechanism through which exercise exerts its antidiabetic effect (i.e., whether it is adiposity related or not). Thus, the aim of this work was to investigate the relationship between physical activity levels and 10-year diabetes development taking into consideration several socio-demographic, lifestyle and clinical factors, and evaluate the potential mediating effect of obesity, as well as several inflammatory and oxidative stress biomarkers, under the context of a large-scale prospective cohort study of the Greek adult population (i.e., the ATTICA study).

Methods

Baseline sampling procedure (2001–2002)

The ATTICA is a large-scale prospective study, which was carried out during 2001–2002, in Athens metropolitan area (including 78% urban and 22% rural areas). People with cardiovascular disease or those living in institutions or having chronic viral infections were not eligible for participation. Exclusion of cardiovascular disease at baseline was ensured with a detailed clinical evaluation, following standard criteria. From the 4056 inhabitants, randomly invited to participate, 3042 were finally enrolled in the study (75% participation rate); 1514 were men, 46 $\pm$ 13 years, and 1528 were women, 45 $\pm$ 13 years.

All participants were interviewed by trained personnel (i.e., cardiologists, general practitioners, dietitians and nurses) who used a standard questionnaire. Protocols were submitted to and approved by our institutional ethics committee. Informed consent was obtained from all individual participants included in the study. Further details about the aims, design and methods used in the ATTICA study can be found elsewhere (Pitsavos et al. 2003).

Baseline measurements

The baseline evaluation included information about: socio-demographic characteristics (age, sex and years of school), personal history of hypertension, hypercholesterolemia and diabetes, family history of cardiovascular disease, dietary and other lifestyle habits (i.e., smoking and physical activity status). Smokers were defined as those who smoked at least one cigarette per day or had quit within the previous year. Dietary habits were evaluated using a validated semi-quantitative food-frequency questionnaire, from the Unit of Nutritional Epidemiology of Athens Medical School (Katsouyanni et al. 1997). Based on the Mediterranean-diet pyramid, the diet score ranged from 0 to 55. *MedDietScore* was also electronically calculated. *MedDietScore* reflects the person's adherence to the Mediterranean diet, and was used as a marker of the food habits of the study sample. More information on *MedDietScore* calculation can be found elsewhere (Panagiotakos et al. 2006, 2007).

Body mass index (BMI) was calculated as weight (in kg) divided by standing height (in meters squared). Waist (in cm) was also measured; Regarding other clinical characteristics, arterial blood pressure (3 recordings) was measured at the end of the physical examination with the subject in sitting position and being at least 30 min at rest. Participants whose average blood pressure levels were $\geq$ 140/90 mmHg or were under antihypertensive medication were classified as being hypertensive.

Physical activity was assessed through a translated version of the validated "International Physical Activity Questionnaire" (IPAQ), suitable for assessing population levels of self-reported physical activities (Papathanasiou et al. 2009). The short version of IPAQ (7 items) that we used provided information on weekly time spent walking,

in vigorous intensity, moderate intensity and sedentary activity. Participants were instructed to refer to all domains of physical activity and report only episodes of activities of at least 10 min, since this is the minimum required to achieve health benefit. Afterward, the sum of MET (metabolic equivalent of task) min/week was calculated for each participant, and the quartiles of MET min/week were constructed, yielding four categories, i.e., very low, low, moderate and high physical activity level. This type of analysis was preferred to quantify the total physical activity across all domains and thus provide an overall picture of physical activity's effect on diabetes development.

Biochemical measurements were carried out in the same laboratory that followed the criteria of the World Health Organization Lipid Reference Laboratories. Blood samples were collected from the antecubital vein between 8 and 10 am, in a sitting position after 12 h of fasting and alcohol abstinence. Serum total cholesterol, HDL cholesterol, triglycerides and glucose concentrations were measured using chromatographic enzymic method in a Technicon automatic analyzer RA-1000. LDL cholesterol was calculated using the Friedewald formula. Diagnosis of diabetes was based on the criteria of the American Diabetes Association (American Diabetes Association 1997); i.e., participants who had fasting blood glucose > 125 mg/dL during the examination or reported use of antidiabetic medication were defined as having diabetes. Blood glucose levels (mg/dL) were measured with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA, USA). Hypercholesterolemia was defined as total cholesterol levels > 200 mg/dL or the use of lipids lowering agents. The intra- and inter-assay coefficients of variation of cholesterol levels did not exceed 9%. Serum insulin concentrations were assayed by radioimmunoassay. Inflammatory markers were assayed using the following techniques: C-reactive protein (CRP) and serum amyloid-A (SAA) by particle-enhanced immunonephelometry, interleukin 6 (IL-6) by a high-sensitivity enzyme-linked immunoassay, human tumor necrosis factor-α (TNF-α) by the enzyme-linked immunosorbent assay method for the quantitative determination, homocysteine levels by an automatic analyzer based on the technology of fluorescence polarization immunoassay and fibrinogen by automatic nephelometry. Finally, serum TAC was measured with a colorimetric test and plasma-oxidized LDL cholesterol with an enzyme-linked immunosorbent assay kit.

10-year follow-up evaluation (2011–2012)

During 2011–2012, the 10-year follow-up was performed. Of the $n = 3042$ participants, $n = 2583$ were found during the follow-up (85% participation rate). Specifically, 224 individuals were lost due to false contact details (telephone numbers or addresses) provided at baseline and $n = 235$ participants refused to participate. No statistically significant differences existed between those who participated and those who did not. $n = 210$ participants were further excluded due to baseline diabetes, and for $n = 888$ participants no information about diabetes status was available at the 10-year follow-up, since some participants refused clinical evaluation and did not know if they had diabetes or not (diagnosed by a physician). In total, for 1347 diabetes-free participants at baseline, the 10-year diabetes status could not be recorded. Thus, the final working sample consisted of $n = 1485$ participants without diabetes at baseline. Statistically, but not clinically significant, differences between our working sample ($n = 1485$ individuals) and participants who were not included in these analyses ($n = 1347$ participants), because there was no available information about the 10-year diabetes status, existed for (43 ± 13 vs. 45 ± 13 years, $p < 0.001$) hypertension status (30 vs. 26%, $p = 0.036$), smoking status (58 vs. 54%, $p = 0.028$), fasting glucose (88 ± 12 vs. 80 ± 13, $p = 0.005$) and fasting insulin (12 ± 3.0, 13 ± 3.4 μU/ml, $p = 0.014$). No statistically significant differences existed for gender, years of education, hypercholesterolemia, family history of diabetes, metabolic syndrome occurence, BMI and energy intake ($ps > 0.05$).

Diabetes diagnosis was based on American Diabetes Association criteria, as performed in the baseline examination. This sample was adequate to achieve 92% statistical power to evaluate a relative risk of 0.70 between the null hypothesis and the alternative two-sided hypothesis, when the exposure variable was increased by 1 unit (by level increase of physical activity) and with a significance level (alpha) of 0.05.

Statistical analysis

Incidence of T2DM was calculated as the ratio of new cases to the $n = 1485$ participants, free of T2DM at baseline, who participated in the follow-up. For the description of participant's characteristics by level of physical activity, continuous variables are presented as mean values and standard deviation, while categorical variables are presented as relative frequencies. In the case of categorical variables, the tested hypotheses were performed using contingency tables and the calculation of Chi-squared test (e.g., incidence of T2DM by physical activity level). Comparisons between differences of mean values of normally distributed variables between groups of exercise were tested using the analysis of variance (ANOVA), after ensuring normality (assessed through Shapiro–Wilk test and Q–Q plots) and homogeneity of variances. Post hoc analyses using the Bonferroni rule

were performed to account for the inflation of the probability of type I error. For non-normally distributed variables or if homogeneity of variances was not fulfilled, Kruskal–Wallis test was performed. To assess the potential effect of physical activity levels on diabetes incidence, binary logistic regression analysis was performed for all participants and odds ratios (OR) with the corresponding 95% confidence intervals (CI) were calculated, since the exact time to event (i.e., development T2DM) was not known. All known confounders were included in the models after testing for colinearity. Interactions of all variables with physical activity status were checked in all steps, and if significant they would remain in the model. The Hosmer and Lemeshow's goodness-of-fit test was calculated to evaluate the model's goodness of fit and residual analysis was implicated using the dbeta, the leverage and Cook's distance D statistics to identify outliers and influential observations. All reported p values are based on two-sided hypotheses and compared to a significance level of 5%. For all the statistical calculations, the SPSS version 18 statistical software was used (SPSS Inc., Chicago, IL, USA).

Results

10-year diabetes incidence

During the 10-year follow-up period, 191 subjects were classified as having diabetes, yielding a crude incidence of 129 per 1000 participants (or 12.9%), with 97 men (13.4%) and 94 women (12.4%) ($p = 0.79$ for gender difference) (Koloverou et al. 2014).

Participants' baseline characteristics by physical activity level

The demographic and clinical characteristics of participants, by physical activity level at baseline, are presented in Table 1. Unadjusted analysis revealed slight differences in age and gender distribution along the four quartiles. Similar differences were noted for education status, fasting plasma glucose, BMI and *MedDietScore*. Participants who engaged in moderate physical activity at baseline had significantly lower mean values in waist circumference and triglycerides, compared to participants in the other quartiles. Small significant differences were also observed for some inflammatory markers, i.e., IL-6, homocysteine and TNF-α as well as for the two oxidative stress biomarkers, i.e., ox-LDL and TAC. Smoking status decreased accordingly with physical activity level. No significant differences existed for family history of diabetes, hypertension, hypercholesterolemia, fasting insulin levels, CRP, SAA and fibrinogen ($ps > 0.05$).

The 10-year diabetes incidence and physical activity level

The 10-year incidence of diabetes was $n = 68$ cases (13%) among participants reporting very low physical activity level (Quartile 1), $n = 48$ (14%) in the low physical activity group (Quartile 2), $n = 25$ (9.0%) in the moderate physical activity group (Quartile 3) and $n = 50$ (14%) in the high physical activity group (Quartile 4) ($p = 0.25$) (Table 1). The distribution of participants who developed diabetes at the 10-year follow-up among the four physical activity groups (34, 22, 19 and 25% for Quartiles 1–4) was similar to the distribution of participants who did not develop diabetes (36, 25, 13 and 26%, respectively).

To control for known confounders (e.g., family history of diabetes, smoking, diet) as well as variables that were found to differ significantly at baseline (i.e., triglycerides), multi-adjusted analysis was performed through nested models. In age–sex adjusted model (Table 2, Model 1), a significant inverse association was observed for participants with moderate physical activity level (MET min/week 331–1484), compared to participants with very low physical activity level (MET min/week < 150) (OR = 0.56, 95% CI 0.34, 0.92). For participants in the low and high physical activity groups, an inverse trend was observed, though not statistically significant. The protective effect of moderate physical activity remained significant even after controlling for family history of diabetes and cardiovascular risk factors (i.e., hypertension, hypercholesterolemia and smoking status) (Table 2, model 2) and educational status, *MedDietScore*, triglycerides and fasting glucose (Table 2, model 3). To test for the potential mediating effect of adiposity in the observed association, waist circumference was added to the model. However, the results remained unchanged, indicating that the observed effect was not attributed to body weight (Table 2, model 4). Thus, in the fully adjusted models individuals reporting moderate level of physical activity experienced 53% lower risk for developing diabetes within a decade, compared to completely inactive individuals (OR = 0.47; 95% CI 0.24, 0.93).

Finally, it was further evaluated whether achieving 600 MET min/week (the minimum level recommended by WHO, achieved by 37% of the study sample) confers any benefit against diabetes mellitus; however, results were not significant in age–sex adjusted model (OR = 0.91, 95% CI 0.66, 1.26), indicating that not all participants with > 600 MET min/week were benefited, further strengthening the

Table 1 Distribution of baseline lifestyle and clinical characteristics of the ATTICA study's participants, according to physical activity level, assessed through MET min/week ($n = 1485$), at baseline (2000–2002)

	Physical activity level (MET min/week)				
	Very low ($n = 509$)	Low ($n = 334$)	Moderate ($n = 271$)	High ($n = 371$)	p
MET min/week, range	0–150	150–330	331–1484	1484–8500	< 0.001
Diabetes cases (n, %)	68 (13)	48 (14)	25 (9)	50 (14)	0.25
Age	44 ± 12	49 ± 13	46 ± 15	45 ± 14	< 0.001
Male sex, n (%)	244 (48)	162 (48)	118 (44)	202 (54)	0.048
Education, years of school	12 ± 3.4	11 ± 3.6	12 ± 3.7	13 ± 3.6	< 0.001
Body mass index, kg/m^2	26 ± 4.6	27 ± 4.0	26 ± 4.1	26 ± 4.3	< 0.001
Waist circumference, cm	90 ± 15	91 ± 16	87 ± 13	88 ± 14	0.013
MedDietScore (range 0–55)	26 ± 7.8	25 ± 5.0	27 ± 7.7	25 ± 5.9	< 0.001
Hypertensive, n (%)	144 (29)	96 (32)	72 (28)	103 (30)	0.65
Hypercholesterolemic, n (%)	197 (39)	134 (40)	110 (41)	140 (38)	0.87
Total cholesterol, mg/dL	191 ± 37	201 ± 47	194 ± 44	191 ± 40	0.11
LDL cholesterol, mg/dL	121 ± 34	127 ± 40	126 ± 40	120 ± 37	0.28
HDL cholesterol, mg/dL	48 ± 13	49 ± 19	50 ± 12	50 ± 15	0.058
Triglycerides, mg/dL	114 ± 70	126 ± 84	103 ± 53	110 ± 68	0.007
Current smokers, n (%)	289 (57)	195 (58)	138 (51)	180 (49)	0.02
Family history of diabetes	107 (22)	65 (23)	55 (23)	64 (19)	0.54
Fasting glucose, mg/dL	89 ± 13	88 ± 12	92 ± 12	89 ± 13	0.007
Fasting insulin, μU/mL	13 ± 3.4	14 ± 5.4	13 ± 1.5	13 ± 2.1	0.89
TAC, μmol/L	233 ± 36	232 ± 46	234 ± 46	235 ± 42	0.017
ox-LDL, mg/dL	57 ± 25	67 ± 30	59 ± 27	61 ± 30	0.001
IL-6, pg/mL	1.44 ± 0.40	1.44 ± 0.52	1.46 ± 0.48	1.39 ± 0.56	0.024
TNF-a, pg/mL	6.4 ± 4.0	5.7 ± 4.8	6.0 ± 4.4	6.3 ± 4.5	0.011
CRP, mg/L	1.98 ± 2.48	1.96 ± 2.55	1.84 ± 2.42	1.54 ± 2.02	0.13
Homocysteine, μmol/L	11.7 ± 6.8	12.2 ± 4.2	12.5 ± 6.7	12.1 ± 6.8	0.049
SAA, mg/dL	4.28 ± 4.1	4.52 ± 4.1	4.88 ± 5.3	4.15 ± 4.6	0.19
Fibrinogen, mg/dL	312 ± 64	310 ± 69	303 ± 74	298 ± 70	0.11

Data are presented as mean values and standard deviation or absolute and relative frequencies. p values are derived from ANOVA for the normally distributed variables for which homogeneity of variances existed in all groups (glucose) and Kruskal–Wallis test for the non-normally distributed variables (MET minutes, fasting insulin, years of school, triglyceride, HDL, TAC, ox-LDL, TNF-a, IL-6, CRP, homocysteine, SAA, fibrinogen) or variables for which homogeneity of variances test did not exist (age, waist circumference, BMI, MedDietScore, LDL, total cholesterol). For categorical variables, Chi-square test was performed

TAC total antioxidant capacity, *ox-LDL* oxidized LDL cholesterol, *IL-6* interleukin-6, *TNF-α* tumor necrosis factor-α, *CRP* C-reactive protein, *SAA* serum amyloid-A

aforementioned results about the importance of moderate physical activity.

To investigate an alternative potential mechanism underlying the protective effect of moderate physical activity, baseline biomarkers of oxidative stress (i.e., ox-LDL and TAC) and inflammation (i.e., IL-6, SAA, TNF-a, CRP, fibrinogen and homocysteine) were sequentially, and separately, entered in the fully adjusted model (model 4). Statistical analysis revealed the mediating effect of TAC and ox-LDL in the examined relationship (p values > 0.05) (Table 3), suggesting that physical activity may ameliorate oxidative stress, decreasing through this mechanism the risk for developing diabetes. No change in statistical significance was observed after the inclusion of any of the inflammatory markers.

Discussion

Physical activity is an established "heart-healthy" strategy, with a potential to extend its benefit beyond cardiovascular disease. In this work, the effect of physical activity levels on diabetes incidence was evaluated, in a prospective cohort of Greek adult population. After adjusting for several known confounders, participants engaged in moderate physical activity activities (331–1484 MET min/week)

Table 2 Results from multiple logistic regression models (ORs and 95% CIs) that evaluated participants' ($n = 1485$) physical activity status, assessed through MET min/week, as well as other characteristics, in relation to 10-year incidence of diabetes (2002–2012)

	Physical activity level			
	Very low ($n = 509$)	Low ($n = 334$)	Moderate ($n = 271$)	High ($n = 371$)
Model 1	Ref	0.83; 0.55, 1.26	0.56; 034, 0.92	0.91; 0.61, 1.37
Model 2	Ref	0.81; 0.51, 1.31	0.51; 0.29, 0.89	0.98; 0.62, 1.54
Model 3	Ref	0.71; 0.39, 1.30	0.47; 0.25, 0.90	0.95; 0.56, 1.62
Model 4	Ref	0.77; 0.41, 1.49	0.47; 0.24, 0.93	1.04; 0.59, 1.82

Model 1 was adjusted for age and sex; model 2 = model 1, plus family history of diabetes, smoking status, hypercholesterolemia and hypertension; model 3 = model 2, plus years of school, adherence to the Mediterranean diet, blood glucose and triglycerides; model 4 = model 3, plus waist circumference

Table 3 Results (OR and 95% CI) from multiple logistic regression models that evaluated ATTICA study participants' ($n = 1485$) physical activity status, assessed through MET min/week, in relation to 10-year incidence of diabetes (2002–2012), after one-by-one inclusion of various biomarkers in the fully adjusted model (model 4)

	Physical activity level			
	Very low ($n = 509$)	Low ($n = 334$)	Moderate ($n = 271$)	High ($n = 371$)
Oxidative stress				
TAC, μmol/L	Ref	2.53; 0.44, 14.6	0.70; 0.10, 4.82	1.04; 0.24, 4.5
ox-LDL, mg/dL	Ref	0.85; 0.38, 1.86	0.12; 0.001, 1.31	1.31; 0.65, 2.67
Inflammatory markers				
IL-6, pg/mL	Ref	0.73; 0.36, 1.45	0418; 0.20, 0.84	1.02; 0.57, 1.81
TNF-α, pg/mL	Ref	0.68; 0.31, 1.48	0.37; 0.17, 0.80	0.54; 0.28, 1.06
CRP, mg/L	Ref	0.76; 0.38, 1.52	0.40; 0.20, 0.83	1.05; 0.59, 1.87
Homocysteine, μmol/L	Ref	0.91; 0.41, 2.02	0.38; 0.17, 0.84	0.45; 0.22, 0.91
SAA, mg/dL	Ref	0.92; 0.45, 1.89	0.46; 0.21, 1.01	1.33; 0.71, 2.47
Fibrinogen, mg/dL	Ref	0.92; 0.47, 1.78	0.53; 0.27, 1.05	1.001; 0.57, 1.79

TAC total antioxidant capacity, *ox-LDL* oxidized LDL cholesterol, *IL-6* interleukin-6, *TNF-a* tumor necrosis factor-a, *CRP* C-reactive protein, *SAA* serum amyloid-A

faced 53% lower 10-year diabetes risk, compared to completely inactive individuals (MET min/week < 150). It was interesting that no benefit was observed for high physical activity group, i.e., MET min/week > 1484. Finally, an effort to identify potential mediators of physical activity–diabetes inverse association was attempted. It was revealed that changes in TAC and ox-LDL may underlie diabetes pathogenesis mechanism, suggesting that moderate physical activity may offer its antidiabetic effect through a decrease in oxidative stress. Despite the limitations of the present observational study, the large representative sample, prospective design and follow-up of 10 years, as well as the detailed assessment of lifestyle information and, therefore, the ability to adjust for several known confounders, may guarantee that the reported findings have important public health implications and shed light on physical activity's antidiabetic effect, as well as the level of activity that exerts the greatest effect, thus confirming the importance of moderation even in this aspect of diabetes primary prevention.

In line with the present findings, other studies have also reported that a physically active lifestyle can protect against diabetes development. In a review of 20 prospective studies, high physical activity level was associated with 20–30% decrease in diabetes risk (Gill and Cooper 2008), while in another review of 10 prospective studies, moderate physical activity was also connected to a 30% lower risk, compared to being sedentary (Jeon et al. 2007). In this work, moderate physical activity was found to be beneficial; however, more intense activity did not confer any benefit. This finding was recently proposed by a meta-analysis of 55 studies, which documented that the major gains for diabetes incidence occurred at lower levels of activity and there were diminishing returns at levels higher than 3000–4000 MET min/week (Kyu et al. 2016). On the other hand, two other meta-analyses point toward a dose–response relationship. Specifically, Huai et al. concluded that moderate and high levels of leisure-time physical activities were associated with 21 and 31% lower diabetes risk (Huai et al. 2016), while Aune et al. (2015) reported 32

and 39% risk reductions, respectively, from moderate and vigorous activity.

The effectiveness of physical activity against the development of diabetes lies with its ability to alter several mechanistic factors involved in diabetes pathogenesis. Firstly, exercise can normalize liver and skeletal muscle insulin resistance, not only by itself but also through the weight loss process (Yeo and Coker 2008). When exercising large changes in energy utilization occur, which require the mobilization of fatty acids and glucose in the blood. In muscles, exercise enhances insulin-stimulated glycogen synthesis via an increased expression of glucose transporter type four (GLUT4), a transporter that plays a significant role in glucose uptake from insulin-sensitive tissues, and increased activity of glycogen synthase. Moreover, exercise increases muscle mass, resulting in an elevated proportion of insulin-sensitive types of muscle fibers which further improves insulin sensitivity (Goodyear and Kahn 1998). It is important to note that the aforementioned beneficial effects last a few days; thus, the consistency in a physical activity module throughout the year is a key concept for improving insulin resistance and delaying diabetes development (Morrato et al. 2007).

In this article, two oxidative stress biomarkers were identified as mediators of the studied association: ox-LDL and TAC, which seems plausible since insulin production and secretion gets defective when the pancreas is chronically exposed to high oxidative stress levels (Evans et al. 2003). Regular exercise attenuates oxidative damage in the brain, liver, kidney, skeletal muscles, blood and heart (Sallam and Laher 2016), as well as oxidative stress derived from white adipose tissue under a state of obesity (Sakurai et al. 2017). Our results for a moderate-specific effect are backed up by two studies with regard to exercise intensity. Moderate exercise has been proven more effective in reducing the susceptibility of oxidative damage following a high fat meal, compared to rest or high-intensity exercise (Lopes Kruger et al. 2016), while high-intensity exercise for 12 weeks has been shown to increase the indices of oxidative stress in young men (Goto et al. 2003).

Regardless of the aforementioned findings shown in the present study, we cannot rule out the limitations of an observational study. These include the baseline evaluation which was performed once, which may be prone to measurement error. The diagnosis of diabetes in this study was based on self-report and/or physician diagnosis, but this is common for prospective studies. Moreover, the accurate date of diabetes development was not accessible (only the date of diagnosis was identified); as a result, hazard ratios were estimated through ORs that may have overestimated the true effect. However, it has been reported that for low-frequency diseases, OR is an accurate estimate (converges) of the relative risk. Furthermore, physical activity was quantified by a questionnaire-based method and not an accelerometer; which may have underestimated the strength of the reported relationships.(Celis-Morales et al. 2012) Another concern in prospective studies is that associations with disease incidence are based on baseline information; however, many lifestyle factors (i.e., physical activity, energy intake) may have changed during the 10-year time period without timely information updates. However, this study has several strengths including a prospective design for a time period of 10 years, a large, representative sample, the detailed assessment of physical activity levels using a previously validated questionnaire and the assessment of numerous lifestyle factors which gave us the ability to control for potential confounders.

Conclusions

In conclusion, the current study has provided additional evidence in the literature concerning the benefits of physical activity levels on 10-year incidence of type 2 diabetes mellitus. The presented results concerning physical activity levels are discouraging, as two out of three participants were physically inactive, not meeting WHO recommendations for 600 MET min/week, in line with global trends toward an increasingly sedentary lifestyle. However, our results carry a hopeful public health message for individuals, suggesting that especially moderate, not high, physical activity independently of body weight and other lifestyle and clinical factors decreases diabetes risk by more than 50%. This suggests that targeting very sedentary individuals might be particularly important from a public health perspective. It is consequently imperative to promote strategies to increase physical activity levels especially among inactive individuals, while it is also important to recognize facilitators and barriers that patients with established diabetes deal with, in terms of physical activity compliance. For example, long-term monitoring and support have been proven essential for these individuals, not only to participate in a supervised exercise program, but also to adhere to it after the program's completion (Casey et al. 2010). More studies are deemed necessary to define the best "physical activity prevention module", in terms of duration, frequency and intensity, against type 2 diabetes mellitus.

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