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Citation for published version (APA):

den Hoed, M., Hesselink, M. K., van Kranenburg, G. P., & Westerterp, K. R. (2008). Habitual physical activity in daily life correlates positively with markers for mitochondrial capacity. *Journal of Applied Physiology*, 105(2), 561-568. <https://doi.org/10.1152/jappphysiol.00091.2008>

Document status and date:

Published: 01/01/2008

DOI:

[10.1152/jappphysiol.00091.2008](https://doi.org/10.1152/jappphysiol.00091.2008)

Document Version:

Publisher's PDF, also known as Version of record

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Habitual physical activity in daily life correlates positively with markers for mitochondrial capacity

Marcel den Hoed,¹ Matthijs K. C. Hesselink,² Gerrit P. J. van Kranenburg,² and Klaas R. Westerterp¹

Departments of ¹Human Biology and ²Movement Sciences, Maastricht University, Maastricht, The Netherlands

Submitted 29 January 2008; accepted in final form 23 May 2008

den Hoed M, Hesselink MK, van Kranenburg GP, Westerterp KR. Habitual physical activity in daily life correlates positively with markers for mitochondrial capacity. *J Appl Physiol* 105: 561–568, 2008. First published May 29, 2008; doi:10.1152/jappphysiol.00091.2008.—Physical exercise training is a powerful tool to maintain or improve mitochondrial density and function (mitochondrial capacity). This study aims to determine whether mitochondrial capacity is also associated with habitual physical activity in daily life (PA_{DL}). The capacity of classic markers for mitochondrial density, i.e., the capacity of citrate synthase (CS) and succinate dehydrogenase (SDH), as well as the capacity of cytochrome *c* oxidase (COX) and β -hydroxyacyl-CoA dehydrogenase (HAD), was determined in homogenized muscle biopsy samples obtained from the vastus lateralis muscle of nonexercising healthy young (age 20 ± 2 yr) subjects (31 women, 7 men). PA_{DL} was measured during two periods of 14 days using a triaxial accelerometer for movement registration. CS, SDH, and COX were positively associated with PA_{DL} [$P < 0.05$, $R = 0.36$, 95% confidence interval (CI): $1.3 \cdot 10^{-4}$ to $2.2 \cdot 10^{-3}$; $P < 0.05$, $R = 0.39$, 95% CI: $1.1 \cdot 10^{-5}$ to $9.9 \cdot 10^{-5}$; and $P < 0.05$, $R = 0.33$, 95% CI: $7.5 \cdot 10^{-6}$ to $3.6 \cdot 10^{-4}$, respectively], and HAD tended to correlate positively with PA_{DL} ($P = 0.06$, $R = 0.31$, 95% CI: $-2.2 \cdot 10^{-5}$ to $1.1 \cdot 10^{-3}$). The population was subsequently stratified based on the intensity of the activities performed. CS was only associated with PA_{DL} in subjects spending more time on high-intensity physical activity, whereas HAD was only associated with PA_{DL} in subjects spending less time on low intensity physical activity. We are the first to report that even within the range of normal daily life activities, mitochondrial capacity is positively associated with the level of habitual physical activity in daily life. Thus an active lifestyle may help to maintain or improve mitochondrial capacity.

accelerometer; intensity; citrate synthase; succinate dehydrogenase; cytochrome *c* oxidase; β -hydroxyacyl-CoA dehydrogenase

IN WESTERNIZED SOCIETIES, the prevalence of Type 2 diabetes and obesity has increased significantly over the past few decades (18, 25). A decreased physical activity has been associated with both Type 2 diabetes (13, 17) and obesity (15). Part of the pathophysiology related to these diseases has been attributed to a reduced mitochondrial capacity: the product of mitochondrial density and function (2, 16, 22).

Mitochondria are vital organelles in the oxidative degradation of macronutrients to maintain cellular ATP levels. Mitochondrial aberrations, resulting in a reduced mitochondrial capacity, may therefore seriously impair normal energy and substrate metabolism. Mitochondrial aberrations have not only been reported for metabolic disorders like Type 2 diabetes and obesity but also in diseases like chronic heart failure and chronic obstructive pulmonary disease (11, 24).

Proper mitochondrial function and maintenance of mitochondrial capacity rely on a delicate balance between mitochondrial biogenesis and degradation. While the precise mechanisms of mitochondrial biogenesis and degradation are not fully elucidated yet, it has been known for decades that one of the most potent triggers to improve or maintain mitochondrial capacity is physical exercise (9). Engagement in physical exercise programs results in increased mitochondrial biogenesis and improved mitochondrial function in healthy controls (12, 29) and is even capable of restoring mitochondrial capacity in diseased states like Type 2 diabetes and obesity (21, 30). Unfortunately, long-term adherence to strenuous exercise programs is limited (32), and life-long engagement in sports and exercise to prevent mitochondrial aberrations may be an unrealistic goal in Westernized societies. If, however, mitochondrial capacity can also be maintained by high levels of habitual physical activity in daily life (PA_{DL}), the goal of maintaining mitochondrial capacity and preventing mitochondrial aberrations by physical activity may become feasible.

Interestingly, wide ranges in PA_{DL} have been reported (23) with the most active people showing daily life activity levels approaching those reported for people actively engaged in endurance sports (5). While it is obvious that mitochondrial capacity is higher in trained athletes than in sedentary controls, it is not known to date whether differences also exist within the ranges of normal daily life activities. The aim of the present study was therefore to examine the association between markers of mitochondrial capacity with habitual physical activity in daily life in a young and healthy population. To this end, classic markers for muscle mitochondrial density, the capacity of citrate synthase (CS) and succinate dehydrogenase (SDH) (10), were measured along with the capacity of cytochrome *c* oxidase or complex IV (COX) from the electron transport chain and β -hydroxyacyl-CoA dehydrogenase (HAD) from the β -oxidation (8, 28). PA_{DL} was recorded using our validated noninvasive triaxial accelerometer approach (23). We hypothesized a higher mitochondrial enzymatic capacity in subjects with a higher PA_{DL}.

MATERIALS AND METHODS

Subjects

Thirty-eight healthy, nonsmoking subjects (31 women, 7 men), 20 ± 2 yr of age (means $\pm$ SD), gave written informed consent to participate in this study. To minimize the effect of physical exercise training, subjects spending over 2 h/wk on endurance sports or 5 h/wk on sports and exercise in general were excluded from participation. Information about the purpose and protocol of the study, as well as its

Address for reprint requests and other correspondence: M. den Hoed, P.O. Box 616, 6200 MD Maastricht, The Netherlands (e-mail: m.denhoed@hb.unimaas.nl).

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risks and discomfort, was provided both orally and in writing. The study conformed to the standards set by the Declaration of Helsinki, and the local Ethics Committee approved the study.

PA_{DL}

PA_{DL} was measured using a triaxial accelerometer for movement registration (Tracmor IV; Philips Research, Eindhoven, The Netherlands) sensitive to a wide range of body movements. The accelerometer has been validated against doubly labeled water, the gold standard for measuring energy expenditure in daily life (23). The Tracmor registers accelerations of the trunk along the anteroposterior, medio-lateral, and longitudinal axis using three uniaxial piezoelectric accelerometers [details are provided elsewhere (23)]. To ensure a valid reflection of long-term daily life activities, the accelerometer was worn for two 14-day periods under free-living conditions. Subsequently, PA_{DL} was defined as the average of both measurement periods.

Subjects were instructed to wear the Tracmor from the moment they woke up in the morning until they went back to bed at night. To verify whether subjects lived up to this instruction, waking hours and clock times of wearing the Tracmor were noted. To make sure only representative days were included, the difference between the total time the subject was awake and the time the accelerometer was worn was not allowed to exceed 75 min/day. The few days during which this difference exceeded 75 min were excluded from the analysis. This resulted in an average of 26 representative days per subject. To ensure that subjects met the inclusion criterion concerning their participation in sports and exercise, the actual sporting hours were also recorded in the diary.

PA_{DL} was acquired by summing the output of all three axes and is presented as megacounts per day (Mcnts/day). Using Tracmor data, the proportion of time subjects were physically active at a low, moderate, and high intensity (%Low, %Moderate, and %High, respectively) was determined. The cut-off points for the intensity categories were determined in a pilot study ($n = 5$). The cut-off point for low-intensity physical activity was set by Tracmor outputs associated with walking on a treadmill at 3.5 km/h, which corresponds with ~3 metabolic equivalents (METs), i.e., three times basal metabolic rate. For moderate-intensity physical activity, a Tracmor output associated with walking on a treadmill at 5 km/h was used, which corresponds with ~4.5 METs (1). The relevant Tracmor outputs were 16.0 ± 3.0 and 28.9 ± 3.0 Mcnts/min, respectively. All physical activity associated with a Tracmor output higher than the latter cut-off point was considered high-intensity physical activity. The proportion of time per intensity category was calculated as the sum of all minutes per intensity category divided by the total duration of the measurement, i.e., 28 days minus the number of excluded days.

Using linear regression analysis in a population similar to the present study with respect to PA_{DL}, body composition, and age, Plasqui et al. (23) were able to predict physical activity level (PAL), i.e., the factor by which average daily metabolic rate exceeds basal metabolic rate, with an explained variation of 70% using only megacounts per day. Whereas the MET score represents the factor by which energy expenditure exceeds basal metabolic rate for a certain predefined physical activity, PAL represents the factor by which energy expenditure exceeds basal metabolic rate during 24 h. For a proper reflection of 24-h daily life energy expenditure under free-living conditions, PAL thus provides a more valid reflection than MET scores, which in turn provide a more valid reflection of energy expenditure when considering predefined physical activities. The regression equation developed by Plasqui et al. was used in the present study to estimate PAL.

Muscle Biopsies

A muscle biopsy was obtained after an overnight fast with subjects refraining from strenuous physical activity for 24 h before the collec-

tion. The biopsy was obtained from the vastus lateralis muscle under local anesthesia (2% xylocaine) using a Bergström needle with suction (3). After muscle samples were freed from blood, visible fat, and connective tissue, they were immediately frozen in liquid nitrogen and stored at -80°C until analyzed.

Measurements of Enzyme Activities

SET buffer was prepared by dissolving 8.557 g sucrose (250 mM), 0.211 g Tris (10 mM), and 0.0744 g EDTA (2 mM) in 80 ml distilled water. pH was adjusted to 7.4, and distilled water was added to a final volume of 100 ml. Muscle samples were weighed (mean $\pm$ SD: 39.0 ± 18.8 mg) and homogenized in 1 ml of SET buffer using a Polytron homogenizer (Polytron-Aggregate; Kinematica, Littau, Lucerne, Switzerland). Homogenates were frozen and thawed two additional times using liquid nitrogen to break mitochondrial membranes and were subsequently stored at -80°C until analyzed.

On analysis, muscle homogenates were centrifuged at 13,000 g for 2 min. Supernatant was used for analysis. Absorbance changes for all enzyme assays were measured in a COBAS-FARA semiautomatic analyzer (COBAS-FARA; Roche, Basel, Switzerland). The molar extinction coefficients used were $13,600 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ for coenzyme A-DTNB at 412 nm for CS, $21,000 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ for dichlorophenol indophenol (DCPIP) at 600 nm for SDH, $15,300 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ for reduced cytochrome c at 550 nm for COX, and $63,000 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ for nicotinamide-adenine dinucleotide (NADH) at 340 nm for HAD. Enzyme capacities were expressed as micromoles per minute per gram wet weight. The compositions of the assay solutions were as follows.

1) CS. For reagent 1, 1.21 g Tris (100 mM), 4 mg DTNB (100 μM), and 4.2 mg acetyl CoA (50 μM) were dissolved in 80 ml distilled water, pH was adjusted to 8.0, and distilled water was added to a total volume of 100 ml. For reagent 2 (starting reagent), 3.3 mg oxaloacetate was dissolved in 1 ml distilled water.

2) SDH. For NaP_i 50 mM buffer, 0.890 g Na₂HPO₄·2H₂O (50 mM) (A) and 0.780 g NaH₂PO₄·2H₂O (50 mM) (B) were both dissolved in 100 ml distilled water. Subsequently, B was added to A until the pH was 7.4. For reaction reagent, 6.5 mg KCN (1 mM), 1.7 mg 2,6-DCPIP (0.06 mM), 162 mg sodium succinate (10 mM), and 50 mg albumin were dissolved in 100 ml KP_i buffer.

3) COX. For KP_i 50 mM buffer, 0.684 g KH₂PO₄ (50 mM) and 1.14 g K₂HPO₄ (50 mM) were both dissolved in 100 ml distilled water. Subsequently, both solutions were mixed, and pH was adjusted to 7.4 using KOH. For reduced cytochrome c solution, 17.6 mg ascorbic acid was added to 10 ml KP_i 50 mM buffer, and pH was adjusted to 7.4 using NaOH (1 M). One-hundred microliters of this ascorbic acid solution and 25 mg cytochrome c were mixed. This mixture was incubated at 25°C for 5 min before 900 μl KP_i 50 mM buffer was added. This solution was kept on ice until analyzed. For MgCl₂ solution, 566 mg MgCl₂ was dissolved in 10 ml distilled water (final volume). For reaction reagent, 10 ml KP_i 50 mM buffer, 200 μl MgCl₂ solution, and 400 μl reduced cytochrome c solution were mixed. Fifty microliters supernatant was mixed with 200 μl KP_i 50 mM buffer, which was kept on ice for 10 min before analysis.

4) HAD. For buffer, 3.3 g tetrasodiumpyrophosphate (100 mM) was dissolved in 80 ml distilled water, pH was adjusted to 7.3, and distilled water was added to a total volume of 100 ml. For NADH solution, 8.0 mg NADH was dissolved in 1 ml distilled water. For reagent 1, 10 ml buffer and 200 μl NADH solution were mixed. For reagent 2 (starting reagent), 5.0 mg acetoacetyl-CoA was dissolved in 2.5 ml distilled water.

All analyses for a given enzyme were performed simultaneously at 37°C . Enzyme capacities were measured twice on the same homogenate for each subject. Intra-assay coefficients of variation were 2.0% for CS, 2.1% for SDH, 5.0% for COX and 1.7% for HAD, indicating that the capacity of mitochondrial enzymes was reproducibly determined.

Body Composition

Body mass and height were measured in the morning after an overnight fast (ID 1 Plus, Mettler Toledo, Giessen, Germany; Mod. 220, SECA, Hamburg, Germany). Body volume was determined using the underwater weighing technique while correcting for residual lung volume using the helium dilution technique (Volugraph VG 2000; Mijndhardt, Bunnik, The Netherlands). Total body water was determined overnight using the deuterium dilution technique (34). Percent body fat was subsequently calculated using Siri's three-compartment model (27).

Statistics

Differences in PA_{DL} , the capacity of mitochondrial enzymes, and body composition between men and women were tested using Student's *t*-tests for unpaired samples. Simple linear regression was used to test the association between CS, SDH, COX, and HAD on the one hand and PA_{DL} on the other hand. Multiple linear regression analyses were used to test the interaction between sex and PA_{DL} for the capacity of the mitochondrial enzymes. Backward multiple linear regression analyses were used to correct the associations between the capacity of mitochondrial enzymes and PA_{DL} for sex, age, and body mass index (BMI). On log transformation, the association between PA_{DL} and the capacity of the mitochondrial enzymes with %Low, %Moderate, and %High was determined using simple linear regression analyses.

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 11 for Macintosh OS X (SPSS; Chicago, IL). Data are expressed as means $\pm$ SD. *P* values < 0.05 were considered statistically significant; 95% confidence intervals (95% CI) are provided.

RESULTS

PA_{DL} was similar between men and women (Table 1). The proportion of time subjects were physically active at a low, moderate, and high intensity was also comparable between sexes, although %High was significantly higher in men: 18

min/day vs. 9 min/day in women ($P < 0.01$, 95% CI: 2.4 to 14.3). No significant differences between men and women were found for CS, SDH, COX, or HAD, which confirms the findings of previous studies (14, 26, 29). Furthermore, no interaction was observed between sex and PA_{DL} for the capacity of the mitochondrial enzymes. Hence, both sexes were combined for further analyses.

Capacity of Mitochondrial Enzymes and Physical Activity in Daily Life

Applying the regression equation developed by Plasqui et al. (23) to the present population showed that the physical activity level, PAL, ranged from 1.62 to 2.04. Positive associations were observed between CS, SDH, and COX with PA_{DL} ($P < 0.05$, $R = 0.36$, 95% CI: $1.3 \cdot 10^{-4}$ to $2.2 \cdot 10^{-3}$; $P < 0.05$, $R = 0.39$, 95% CI: $1.1 \cdot 10^{-5}$ to $9.9 \cdot 10^{-5}$; and $P < 0.05$, $R = 0.33$, 95% CI: $7.5 \cdot 10^{-6}$ to $3.6 \cdot 10^{-4}$, respectively). HAD tended to correlate positively with PA_{DL} ($P = 0.06$, $R = 0.31$, 95% CI: $-2.2 \cdot 10^{-5}$ to $1.1 \cdot 10^{-3}$) (Fig. 1). These associations remained when sex, age, and/or BMI were taken into account.

PA_{DL} was positively associated with %Moderate and %High ($P < 0.001$, $R = 0.74$, 95% CI: $3.3 \cdot 10^{-1}$ to $6.3 \cdot 10^{-1}$; and $P < 0.001$, $R = 0.74$, 95% CI: $1.4 \cdot 10^{-1}$ to $2.6 \cdot 10^{-1}$, respectively) and negatively with %Low ($P < 0.001$, $R = 0.86$, 95% CI: -20.4 to -13.7). CS and HAD tended to correlate positively with %High ($P = 0.06$, $R = 0.31$, 95% CI: $-4.5 \cdot 10^{-3}$ to $2.6 \cdot 10^{-1}$; and $P = 0.07$, $R = 0.30$, 95% CI: $-1.0 \cdot 10^{-2}$ to $2.5 \cdot 10^{-1}$, respectively). COX and SDH on the other hand correlated positively with %Moderate ($P < 0.05$, $R = 0.37$, 95% CI: $1.1 \cdot 10^{-1}$ to 1.3 ; and $P < 0.05$, $R = 0.37$, 95% CI: $2.6 \cdot 10^{-2}$ to $3.3 \cdot 10^{-1}$, respectively). To examine if the associations between markers for mitochondrial capacity and PA_{DL} were influenced by the intensity of the activities performed, the population was subsequently stratified based on the intensity of the activities measured.

Capacity of Mitochondrial Enzymes and Physical Activity in Daily Life Stratified Based on the Intensity of Physical Activity

Stratification of the population based on the median of %High resulted in a cut-off point of 8 min/day. Within the population stratified based on %High, CS was only associated with PA_{DL} in the subgroup spending more than 8 min/day on high-intensity physical activity ($P < 0.05$, 95% CI: $7.5 \cdot 10^{-5}$ to $2.9 \cdot 10^{-3}$). SDH also correlated positively with PA_{DL} in the subgroup spending more than 8 min/day on high-intensity physical activity but tended to correlate positively with PA_{DL} in the subgroup spending less time on high-intensity physical activity as well ($P < 0.01$, 95% CI: $2.4 \cdot 10^{-5}$ to $1.2 \cdot 10^{-4}$; and $P = 0.07$, 95% CI: $-1.1 \cdot 10^{-5}$ to $2.1 \cdot 10^{-4}$, respectively). COX tended to correlate positively with PA_{DL} in both the subgroup spending more and less than 8 min/day on high-intensity physical activity ($P = 0.06$, 95% CI: $-1.3 \cdot 10^{-5}$ to $4.5 \cdot 10^{-4}$; and $P = 0.09$, 95% CI: $-7.2 \cdot 10^{-5}$ to $7.7 \cdot 10^{-4}$, respectively), and HAD was not associated with PA_{DL} in either the subgroup spending more or less time on high-intensity physical activity (Fig. 2).

Stratification of the population based on the median of %Moderate resulted in a cut-off point of 29 min/day. Within the population stratified based on %Moderate, no associations

Table 1. Results

	Men	Women
<i>n</i>	7	31
Age, yr	20 $\pm$ 2	20 $\pm$ 2
Body mass, kg	79.3 $\pm$ 14.1	63.1 $\pm$ 8.2†
Height, m	1.84 $\pm$ 0.06	1.69 $\pm$ 0.06†
BMI, kg/m ²	23.2 $\pm$ 3.0	22.0 $\pm$ 2.5
%BF	15.9 $\pm$ 5.8	26.7 $\pm$ 4.6
PA_{DL} , Mcnts/day	4,128 $\pm$ 636	3,704 $\pm$ 675
%Low, min/24 h	1,390 $\pm$ 15	1,401 $\pm$ 15
%Moderate, min/24 h	33 $\pm$ 8	30 $\pm$ 13
%High, min/24 h	18 $\pm$ 11	9 $\pm$ 6*
PAL	1.88 $\pm$ 0.10	1.80 $\pm$ 0.11
CS, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$	9.30 $\pm$ 1.75	8.05 $\pm$ 2.33
SDH, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$	0.70 $\pm$ 0.11	0.73 $\pm$ 0.10
COX, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$	0.67 $\pm$ 0.30	0.77 $\pm$ 0.39
HAD, $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$	5.10 $\pm$ 1.47	4.79 $\pm$ 1.20

Data are means $\pm$ SD. BMI, body mass index; %BF, percent body fat; PA_{DL} , habitual physical activity in daily life as measured using a triaxial accelerometer during 2 periods of 2 wk; Mcnts, megacounts; %Low, %Moderate, and %High, proportion of time subjects were physically active at a low, moderate and high intensity, respectively; PAL, physical activity level, i.e., the factor by which total energy expenditure exceeds resting energy expenditure; CS, SDH, COX and HAD, capacity of citrate synthase, succinate dehydrogenase, cytochrome *c* oxidase, and β -hydroxyacyl-CoA dehydrogenase, expressed as μmol substrate converted per min per g wet muscle weight. Significant sex difference: * $P < 0.01$, † $P < 0.001$.

Fig. 1. Mitochondrial capacity as a function of habitual physical activity in daily life. Capacity of citrate synthase (CS; A), succinate dehydrogenase (SDH; B), cytochrome *c* oxidase (COX; C), and β -hydroxyacyl-CoA dehydrogenase (HAD; D) as a function of habitual physical activity in daily life (PA_{DL}), measured using a triaxial accelerometer for movement registration, for men and women combined, is shown. A, B, and C: $P < 0.05$. D: $P = 0.06$. Mcnts, mega-counts.

were observed between markers for mitochondrial capacity and PA_{DL} in either the subgroup spending more or less time on moderate-intensity physical activity.

Stratification of the population based on the median of %Moderate and %High combined resulted in a cut-off point of 39 min/day. Within the population stratified based on the proportion of moderate- and high-intensity physical activity, CS and HAD were only positively associated with PA_{DL} in the subgroup spending more time on moderate- and high-intensity physical activity ($P < 0.05$, 95% CI: $1.9 \cdot 10^{-4}$ to $4.1 \cdot 10^{-3}$; and $P < 0.01$, 95% CI: $5.7 \cdot 10^{-4}$ to $2.6 \cdot 10^{-3}$, respectively). SDH and COX were positively associated with PA_{DL} in both the subgroup spending more and less time on moderate- and high-intensity physical activity ($P < 0.01$, 95% CI: $3.8 \cdot 10^{-5}$ to $1.7 \cdot 10^{-4}$ and $P < 0.05$, 95% CI: $1.1 \cdot 10^{-5}$ to $2.4 \cdot 10^{-4}$ for SDH; $P < 0.05$, 95% CI: $2.2 \cdot 10^{-5}$ to $6.0 \cdot 10^{-4}$ and $P < 0.05$, 95% CI: $8.4 \cdot 10^{-5}$ to $9.6 \cdot 10^{-4}$ for COX) (Fig. 3).

DISCUSSION

We are the first to show that within the range of normal daily life activities, mitochondrial capacity measured as the capacity of CS, SDH, and COX correlated positively with PA_{DL} , and HAD capacity tended ($P = 0.06$) to correlate positively with PA_{DL} . These associations were independent of sex, age, and BMI.

PAL can vary from 1.2 to 2.5 in sustainable lifestyles with 1.2 observed in bed-bound subjects that are still eating and 2.5 being defined as a highly physically active lifestyle (4). In extreme situations, such as the Tour de France cycling competition, PAL has been shown to increase up to 5 (33). The range in PAL obtained in the present study was 1.6 to 2.0, so lower than the 2.5 observed for highly physically active lifestyles. A PAL of 1.6 is regarded to reflect a sedentary lifestyle (4), and a PAL of 2.1 represents a physically active lifestyle (31). Thus our subjects covered the range of normal daily life

activity levels as reflected in PAL but were not highly physically active.

The normal range of daily life activities in the present population is confirmed by the proportion of time that subjects on average spent on moderate- and high-intensity physical activity, i.e., 30 and 11 min, respectively. These proportions are close to those recently reported in other studies that used accelerometry in healthy young adults (7, 35). McClain et al. (20), on the other hand, recruited regular endurance runners and although %Moderate was similar in their study (27 min/day), %High was more pronounced (48 min/day) compared with the present population. This suggests that although physically active subjects were evidently recruited in the present study, the proportion of high-intensity physical activity was much lower than previously observed for people actively engaged in endurance sports.

PA_{DL} correlated positively with %Moderate and with %High. Furthermore, positive associations were observed between markers for mitochondrial capacity and both %Moderate and %High. This suggests that the intensity of the activities performed may have affected the association between our markers for mitochondrial capacity and PA_{DL} . Indeed, stratification of the population based on the intensity of the activities performed made the association between markers for mitochondrial capacity and PA_{DL} more prominent. Evidently, as a classic marker for mitochondrial density, CS was only associated with PA_{DL} in the subgroup spending more than 8 min/day on high-intensity physical activity. Importantly, this suggests that although CS was associated with PA_{DL} in the entire population, there may be a lower limit for the time spent on high-intensity physical activity that should be met for the association to become apparent. Since PA_{DL} and %High were both significantly higher in the subgroup spending more time on high-intensity physical activity (data not shown), the association found could theoretically also result from an increased

Fig. 2. Capacity of CS (A and B), SDH (C and D), COX (E and F), and HAD (G and H) as a function of PA_{DL} , measured using a triaxial accelerometer for movement registration, stratified based on the proportion of time subjects were physically active at a high intensity. A, C, E, and G show the association for subjects who spent 2–8 min/day on high-intensity physical activity, and B, D, F, and H for subjects who spent 9–34 min/day on high-intensity physical activity. B: $P < 0.05$. C: $P = 0.07$. D: $P < 0.01$. E: $P = 0.09$. F: $P = 0.06$.

PA_{DL} . However, stratification of the population based on %Moderate did not reveal an association between CS or any other marker for mitochondrial capacity and PA_{DL} in either the subgroup spending more or less than 29 min/day on moderate-intensity physical activity. Therefore, we conclude that high-intensity physical activity may be a prerequisite for a positive association between CS and PA_{DL} .

Within the population stratified based on the median of the time spent on moderate- and high-intensity physical activity combined, HAD showed a positive association with PA_{DL} in

subjects spending more but not in subjects spending less than 39 min/day on moderate- and high-intensity physical activity. On first thought, this seems inconsistent since HAD was not associated with PA_{DL} within the population stratified based on %Moderate or %High separately. However, the proportion of moderate- and high-intensity physical activity combined is by definition the inverse of the proportion of low-intensity physical activity. In other words, the association observed between HAD and PA_{DL} in subjects spending more than 39 min/day on moderate- and high-intensity physical activity probably results

Fig. 3. Capacity of CS (A and B), SDH (C and D), COX (E and F), and HAD (G and H) as a function of PA_{DL}, measured using a triaxial accelerometer for movement registration, stratified based on the proportion of time subjects were physically active at a moderate and high intensity. A, C, E, and G show the association for subjects who spent 18–39 min/day on moderate- and high-intensity physical activity, and B, D, F, and H for subjects who spent 39–72 min/day on moderate- and high-intensity physical activity. B, C, E, and F: $P < 0.05$. D and H: $P < 0.01$.

from a decreased time spent on low-intensity physical activity. This implies that the capacity to oxidize fatty acids is positively associated with habitual physical activity in daily life if the latter is accompanied by a decreased time spent on low-intensity physical activity.

For SDH and COX, a positive association with PA_{DL} was found both in subjects spending more and less than 39 min on moderate- and high-intensity physical activity per day. Comparable results were obtained within the population stratified based on %High, suggesting that SDH and COX are associated

with habitual physical activity in daily life irrespective of the intensity of the activities performed.

In general, it can be concluded that a sedentary lifestyle does not result in positive associations with mitochondrial capacity, whereas an active lifestyle does. The intensity of the activities performed influences the association with PA_{DL} differently for different markers of mitochondrial capacity; it does not appear to affect the association between SDH and COX with PA_{DL}, whereas a limited time spent on low-intensity physical activity appears to be a prerequisite for a positive association between

HAD and PA_{DL} . CS on the other hand only correlated positively with PA_{DL} in subjects spending more time on high-intensity physical activity. Interestingly though, spending more than 8 min/day on high-intensity physical activity already resulted in a positive association between CS and PA_{DL} in healthy young subjects. Whether the positive association between mitochondrial capacity and habitual physical activity in daily life persists in populations at risk for developing Type 2 diabetes and obesity remains to be established. More importantly, it should be examined if spending more than 8 min/day on high-intensity physical activity accompanied by a high level of habitual physical activity in daily life suffices to maintain or improve proper mitochondrial capacity in populations at risk for developing Type 2 diabetes and obesity. It should be stressed that even if this association holds in populations at risk, no extrapolations to prevention of Type 2 diabetes and obesity can be made. In fact, based on previous studies, 8 min of high-intensity physical activity per day is unlikely to be sufficient to delay the development of Type 2 diabetes and obesity (6, 19).

In conclusion, evidence was found for a positive association between CS, SDH, and COX with PA_{DL} and a trend toward a positive association between HAD and PA_{DL} irrespective of sex, age, and BMI. With CS and SDH as classic markers for mitochondrial density, mitochondrial capacity was found to be positively associated with PA_{DL} . Thus, in our population of healthy young adults, an active lifestyle within the range of normal daily life activities is associated with a higher mitochondrial capacity than a sedentary lifestyle and may help to maintain or improve mitochondrial capacity.

ACKNOWLEDGMENTS

We thank Milou Beelen, Luc van Loon, and René Koopman for their skillful way of obtaining the muscle biopsies. We also thank Loek Wouters for analyzing the deuterium samples.

GRANTS

This study was funded by Maastricht University.

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