Objectively Measured Physical Activity and Body Fatness: Associations with Total Body Fat, Visceral Fat, and Liver Fat

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ABSTRACT

WINTERS-VAN EEKELEN, E., J. H. P. M. VAN DER VELDE, S. C. BOONE, K. WESTGATE, S. BRAGE, H. J. LAMB, F. R. ROSENDAAL, and R. DEMUTSERT . Objectively Measured Physical Activity and Body Fatness: Associations with Total Body Fat, Visceral Fat, and Liver Fat. *Med. Sci. Sports Exerc.*, Vol. 53, No. 11, pp. 2309–2317, 2021. **Purpose:** It remains unclear to what extent habitual physical activity and sedentary time (ST) are associated with visceral fat and liver fat. We studied the substitution of ST with time spent physically active and total body fat (TBF), visceral adipose tissue (VAT), and hepatic triglyceride content (HTGC) in middle-age men and women. **Design:** In this cross-sectional analysis of the Netherlands Epidemiology of Obesity study, physical activity was assessed in 228 participants using a combined accelerometer and heart rate monitor. TBF was assessed by the Tanita bioelectrical impedance, VAT by magnetic resonance imaging, and HTGC by proton-MR spectroscopy. Behavioral intensity distribution was categorized as ST, time spent in light physical activity (LPA), and moderate to vigorous physical activity (MVPA). To estimate the effect of replacing 30 min·d^{-1} of ST with 30 min·d^{-1} LPA or MVPA, we performed isotemporal substitution analyses, adjusted for sex, age, ethnicity, education, the Dutch Healthy Diet index, and smoking. **Results:** Included participants (41% men) had a mean ± SD age of 56 ± 6 yr and spent 88 ± 56 min in MVPA and 9.0 ± 2.1 h of ST. Replacing 30 min·d^{-1} of ST with 30 min of MVPA was associated with 1.3% less TBF (95% confidence interval = −2.0 to −0.7), 7.8 cm^2 less VAT (−11.6 to −4.0), and 0.89 times HTGC (0.82–0.97). Replacement with LPA was not associated with TBF (−0.03%; −0.5 to 0.4), VAT (−1.7 cm^2; −4.4 to 0.9), or HTGC (0.98 times; 0.92–1.04). **Conclusions:** Reallocation of time spent sedentary with time spent in MVPA, but not LPA, was associated with less TBF, visceral fat, and liver fat. These findings contribute to the development of more specified guidelines on ST and physical activity. **Key Words:** PHYSICAL ACTIVITY, SEDENTARY TIME, ADIPOSITY, EPIDEMIOLOGY, MIDDLE-AGE MEN AND WOMEN

Abdominal obesity is a well-established risk factor for diabetes mellitus type 2, metabolic syndrome, and cardiovascular diseases (1,2). It has been hypothesized that the accumulation of fat in the visceral area and in and around the organs (2), such as the liver, increases cardiometabolic risk. Both visceral fat and liver fat have been associated with metabolic risk factors, insulin resistance, and cardiovascular diseases (3–6), making these adipose depots important targets to prevent cardiometabolic diseases.

Previous meta-analyses of randomized controlled trials have shown that exercise can reduce both visceral fat (7) and liver fat (8). However, the included trials focused on structured exercise rather than habitual daily activities and sedentary time, and evidence on the association between habitual daily activity and different adipose depots is lacking. Most European guidelines on physical activity state to perform at least 150 min·wk^{-1} of moderate to vigorous physical activity (MVPA) and to limit sedentary time (9), as sedentary behavior has been associated with increased risks of type 2 diabetes, cardiovascular disease, and cancer, even after adjustment for physical activity (10). It must be noted that, as a day contains 24 h, less time spent sedentary means more time spent in other intensity categories. When examining the association of one particular type of activity with health outcomes, it is therefore important to take into account which behavior this is being replaced with (e.g., replacing sedentary time with the same amount of time spent with light-intensity physical activity) (11). Many studies that applied this isotemporal substitution analysis to examine the

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substitution of sedentary time by time spent with activities with higher intensity in relation to body fat used objectively assessed physical activity. However, most used surrogate outcomes for adiposity such as body mass index (BMI) or waist circumference (12) instead of direct measures of adipose tissue. Only one previous study investigating the isotemporal substitution of habitual activities used direct measurements of both visceral fat and physical activity, and it found that replacing sedentary time with moderate physical activity was associated with less visceral fat (13). However, liver fat was not assessed in this study and adjustment for total body fat (TBF) was not performed.

Knowing how different types of daily activities and their mutual substitutions are associated with different adipose depots, such as TBF, visceral fat, and liver fat, helps elucidating the underlying mechanism of how sedentary time and physical inactivity can lead to multiple adverse health outcomes. This may ultimately lead to more specific guidelines on sedentary time and physical activity. We therefore aimed to study the association between the substitution of sedentary time with time spent in other intensity of activity and TBF, visceral fat, and liver fat in a population-based cohort of middle-age participants. We hypothesized that replacing sedentary time with activity of any intensity is associated with reduced adipose depots, and this reduction will be more pronounced when replacing it with activity of higher intensity.

METHODS

Study design and study population. The Netherlands Epidemiology of Obesity (NEO) study is a population-based, prospective cohort study designed to investigate pathways that lead to obesity-related diseases. The NEO study includes 6671 individuals 45–65 yr of age who were included between September 2008 and October 2012, with an oversampling of individuals with overweight or obesity. The study design and the data collection are described in detail elsewhere (14). Men and women living in the greater area of Leiden (in the West of the Netherlands) were invited by letters sent by general practitioners and municipalities and by local advertisements. They were invited to respond if they were between 45 and 65 yr of age and had a self-reported BMI of 27 $kg \cdot m^{-2}$ or higher. In addition, all inhabitants between 45 and 65 yr of age from one municipality (Leiderdorp) were invited to participate irrespective of their BMI, allowing for a reference distribution of BMI. The Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the design of the study. All participants gave their written informed consent.

Participants were invited to a baseline visit at NEO study center of the LUMC after an overnight fast. Before this study visit, participants collected their urine over 24 h and completed a general questionnaire at home to report demographic, lifestyle, and clinical information. The participants were asked to bring all medication they were using to the study visit. At the study center, the participants completed a screening form, asking about anything that might create a health risk or interfere with magnetic resonance imaging (MRI) (most notably metallic devices, claustrophobia, or a body circumference of more than 1.70 m). A random selection of approximately 35% of all NEO study participants who were eligible for MRI underwent direct assessment of abdominal fat study participants.

The present study is a cross-sectional analysis of the baseline measurements of the NEO study. We excluded participants with missing data on objectively assessed physical activity, body fat measurements, or potential confounding factors (see Results).

Assessment of habitual physical activity. Daily levels of activity were objectively assessed in a random subsample of NEO study participants ($n = 955$) using a combined uniaxial acceleration and heart rate monitor (Actiheart, CamNtech Ltd., UK). The device weighs less than 8 g and is worn on the chest. Two standard ECG electrodes (H98SG, Tyco Healthcare, Germany) were placed at the level of the second intercostal space: one on the sternum and one 10 cm to the left of the first electrode. Participants were instructed to wear the monitor continuously for four consecutive days and nights, except when showering, bathing, or swimming, and to carry on with all normal activities during this time. The monitor was set up to record at 15-s epochs.

In a random subgroup of participants who wore a monitor, an 8-min ramped step test was performed to calibrate the individual heart rate response to activity intensity. If individual calibration was not available, a group calibration equation based on age, sex, and sleeping heart rate (SHR) was applied (15). The following group calibration equation was derived from the valid step tests ($n = 132$) in our population:

$$\begin{aligned} \text{PAEE}\ \left[\text{J}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\right] = {} & (6.38 - 0.0006 \times \text{age} + 0.52 \times \text{sex} - 0.002 \\ & \times \text{SHR} + 0.19 \times \text{betablocker}) \\ & \times \text{HRaS} - 0.07 \times \text{age} + 53.50 \times \text{sex} + 0.14 \\ & \times \text{SHR} - 0.65 \times \text{SHR} \times \text{sex} + 6.10 \\ & \times \text{betablocker} - 79.17 \end{aligned}$$

In this equation, age is displayed in years, sex coded as 1 for men and 0 for women, SHR in beats per minute, heart rate above sleep (HRaS) in beats per minute, and beta-blocker coded as 0 or 1 if the participant was taking beta-blocker medication.

A Gaussian process regression method was applied to the heart rate data to handle potential measurement noise (16). Using a branched equation algorithm, acceleration and heart rate information was summarized into calibrated estimates of physical activity energy expenditure (PAEE) and time spent at different activity intensities expressed as metabolic equivalents of task (MET) (17,18). One MET was defined as an energy expenditure of 71 $J \cdot kg^{-1} \cdot min^{-1}$. When summarizing the data, we accounted for non–wear time and any potential diurnal imbalance of wear time by weighting all hours of the day equally in the summation (19) and excluding participants who had less than 4 h of valid data in each quadrant of the day.

Sedentary time was defined as time spent in activities with an intensity ≤1.5 METs, excluding sleep time (assumed as time between 2300 and 0730 h on weekdays and time between 2330 and 0830 h on weekend days). Light physical activity

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(LPA) was defined as any activity during wear time with an intensity >1.5 and ≤3 METs. MVPA was defined as any activity >3 METs.

Participants with a valid total wear time <24 h were excluded from the analyses. No minimum bout duration was set for all activity intensity categories.

Assessment of body fat. Body weight and percent body fat were assessed by the Tanita bio impedance balance (TBF-310; Tanita International Division, UK) without shoes, and 1 kg was subtracted from the body weight to account for the weight of clothing.

Visceral adipose tissue (VAT) was quantified by a turbo spin echo imaging protocol using MRI. Imaging was performed on a 1.5 Tesla MR system (Philips Medical Systems, Best, the Netherlands). At the level of the fifth lumbar vertebra, three transverse images each with a slice thickness of 10 mm were obtained during a breath hold. Visceral fat area was converted from the number of pixels to centimeters squared using in-house–developed software (MASS, Medis, Leiden, the Netherlands), and the average of three slices was used in the analyses (14).

Hepatic triglyceride content (HTGC) was quantified by proton-MR spectroscopy (^{1}H-MRS) of the liver (20). An 8-mL voxel was positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time of 26 ms and a repetition time of 3000 ms. Data points (1024) were collected using a 1000-Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 s, and with four averages were obtained as an internal reference. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium), as described previously (21). HTGC relative to water was calculated as the sum of signal amplitudes of methyl and methylene divided by the signal amplitude of water and then multiplied by 100.

Covariates. On the questionnaire, participants reported ethnicity by self-identification in eight categories, which we grouped into White (reference) and other (Black, Turkish, Moroccan, Southeast Asian, Hindus, or other). Tobacco smoking was reported in the three categories current, former, and never smoking (reference). Highest level of education was reported in 10 categories according to the Dutch education system and grouped into high (including higher vocational school, university, and postgraduate education) versus low education (reference). Participants reported their medical history of diabetes and cardiovascular diseases. Preexisting cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. BMI was calculated by dividing the weight in kilograms by the height in meters squared. Habitual dietary intake of all participants was estimated using a semiquantitative self-administered 125-item food frequency questionnaire (22,23). Based on these variables, an adapted version of the Dutch Healthy Diet (DHD) index 2015 was calculated, which consisted of 13 components (vegetables, fruit, wholegrain products, legumes, unsalted nuts, dairy, fish, tea, liquid to solid fat ratio, red meat, processed meat, sweetened beverages, and alcohol). The score can range between 0 and 130, in which a higher score reflects better adherence to the Dutch Guidelines for a Healthy Diet of 2015 (24).

Statistical analysis. In the NEO study, there is an oversampling of persons with a BMI of 27 $kg \cdot m^{-2}$ or higher. To correctly represent associations in the general population (25), adjustments for the oversampling of individuals with a BMI ≥ 27 $kg \cdot m^{-2}$ were made. This was done by weighting individuals toward the BMI distribution of participants from the Leiderdorp municipality (26), whose BMI distribution was similar to the BMI distribution of the general Dutch population (27). All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of individuals with a BMI ≥ 27 $kg \cdot m^{-2}$. Because of the weighted analyses, percentages and proportions are given instead of numbers of participants. Other baseline characteristics are expressed as mean with standard deviation.

We performed linear regression analyses and fitted several models. First, we examined the association between 30 min of daily activities (i.e., sedentary time, LPA, and MVPA) and TBF, visceral fat, and liver fat in a crude model. Second, a multivariable model was performed that was adjusted for sex, age, ethnicity, education, DHD index, and smoking. This model describes the association between 30 $min \cdot d^{-1}$ of each of the daily activities on top of the regular activity pattern for each outcome. This model was also performed for total PAEE per 10 $kJ \cdot d^{-1} \cdot kg^{-1}$. Next, we performed an isotemporal substitution approach in our final model by adding total time awake and dropping sedentary time. Consequently, the regression coefficients of each activity represent the estimated difference in measure of body fat associated with replacing 30 min of sedentary time with this type of intensity of activity. We also performed this model with LPA as the intensity of activity to be substituted. This isotemporal substitution model on visceral fat and liver fat was additionally adjusted for TBF to study whether physical activity had an extra effect on visceral fat and liver fat beyond effects via TBF. Moreover, we performed sensitivity analyses in which we only included participants with at least 72 h of valid data instead of 24 h. Lastly, the associations between physical activity and TBF were also estimated without exclusion of participants with no MRI measurement.

Regression coefficients of TBF represent an absolute difference in TBF in percent per 30 min of a certain intensity of activity, and those of VAT an absolute difference in VAT in square centimeters per 30 min of a certain intensity of activity per day.

Because of a skewed distribution of HTGC, we used the natural logarithm of this variable in the analyses. For interpretation of the results, we back transformed the regression coefficients of HTGC toward a ratio with 95% confidence interval (CI), which is associated with 30 $min \cdot d^{-1}$ spent in a certain activity. Such ratio, for example, a ratio of 1.2, can be interpreted as each 30 $min \cdot d^{-1}$ of a certain activity being associated with a 1.2-fold increased HTGC, which would reflect an increase in liver fat content from, for example, 5% to 6%.

We tested interaction effects of age, sex, and BMI. For age and sex, we generated interaction terms of sex and age with the independent variables (e.g. sex–MVPA) and added them to the fully adjusted multivariable models (models 3). For BMI, we replaced TBF with BMI as covariate in the models for outcomes VAT and HTGC and added an interaction term of BMI in the fully adjusted models (e.g. BMI–MVPA). All *P*-values for the interaction terms were >0.10, except for PAEE–sex and PAEE–age for outcome TBF. Despite a statistically significant interaction term, stratified analyses yielded similar regression coefficients and therefore we present all analyses for total population.

Data were analyzed using STATA version 16 (StataCorp LP, College Station, TX).

RESULTS

Physical activity was objectively assessed in 955 participants, of whom 932 had a successful measurement. The group of participants with a physical activity assessment was similar in sex distribution, age, and BMI to the group without a physical activity assessment but was slightly more likely to have a history of CVD (5.6% vs 6.1%). We excluded participants with less than 24 h of measurement (n = 39) or for whom activity intensities could not be estimated (n = 32); participants without an MRI of the abdomen (n = 558) or ^{1}H-MRS measurement of the liver (n = 45); missing data on smoking status (n = 1), education (n = 7), or ethnicity (n = 1); and participants who consumed 40 g of alcohol or more (four standard glasses) per day (n = 21), leaving a total of 228 participants.

Included participants (41% men) had a mean ± SD age of 56 ± 6 yr and a BMI of 25 kg·m^{-2} (4). Participants had a mean ± SD wear time of physical activity monitoring of 85 ± 11 h, and 87% of participants had more than 72 h of valid wear time. Most time awake was spent sedentary, and least time was spent in vigorous intensity physical activity (Fig. 1). The baseline characteristics of the population stratified by sex-specific tertiles of MVPA are shown in Table 1. Participants in the highest tertile were somewhat younger, more often had a high educational background, had a lower BMI and higher PAEE, and spent less time sedentary compared with participants in the lowest tertile. To provide additional insight into the characteristics of our study population, we also provide baseline characteristics stratified by BMI and sex-specific tertiles of TBF (see Supplemental Tables 1 and 2, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C340).

Physical activity in relation to TBF. After adjustment for potential confounding factors, PAEE was inversely associated with body fat (−1.3%, 95% CI = −1.8 to −0.9). With respect to intensity, each 30 min·d^{-1} of sedentary time was associated with 0.5% more TBF (95% CI = 0.2–0.7), whereas 30 min·d^{-1} of LPA (−0.4, 95% CI = −0.8 to 0.04) and MVPA (−1.4, 95% CI = −1.8 to −0.9) was associated with less TBF (Table 2). In the isotemporal substitution model, replacing 30 min of sedentary time per day with 30 min of MVPA was associated with less TBF (−1.3%, 95% CI = −2.0 to −0.7), whereas LPA (−0.03, 95% CI = −0.5 to 0.4) was not. Replacing 30 min of LPA with sedentary time was not associated with TBF, whereas replacement with 30 min of MVPA was associated with less TBF (−1.3%, 95% CI = −2.3 to −0.3). Results were similar in participants with at least 72 h of objectively measured physical activity data (see Supplemental Table 3, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C340). Results on TBF without exclusion of participants with no MRI measurement can be found in Supplemental

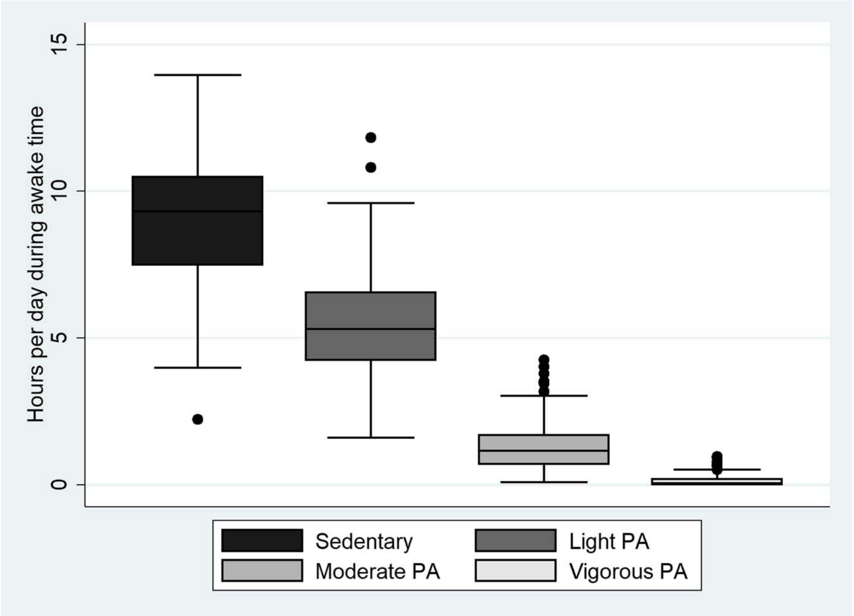

FIGURE 1—Distribution of habitual activity intensities during awake time in participants with a physical activity and TBF measurement in the NEO study (n = 228).

TABLE 1. Characteristics of participants of the NEO study: men and women between 45 and 65 yr of age with objectively measured physical activity and MRI of the abdomen, stratified by sex-specific tertiles of MVPA.

	MVPA ($min \cdot d^{-1}$)		
	T1 (men, 5.7–53.2; women, 5.5–52.2)	T2 (men, 53.8–103.5; women, 52.8–92.5)	T3 (men, 104.4–243.5; women, 93.5–284.8)
Age (yr)	57 ± 7	55 ± 6	54 ± 5
Sex (% men)	40	40	42
Ethnicity (% White)	95	99	99
Education level (% high)[a]	36	47	52
BMI ($kg \cdot m^{-2}$)	26.9 ± 4.7	25.6 ± 3.5	23.8 ± 3.3
Tobacco smoking (% current)	13	17	15
DHD index	71 ± 17	70 ± 15	72 ± 12
HDL cholesterol ($mmol \cdot L^{-1}$)	1.6 ± 0.5	1.5 ± 0.4	1.7 ± 0.5
Triglycerides ($mmol \cdot L^{-1}$)	1.1 (0.7–1.6)	1.0 (0.8–1.4)	0.8 (0.6–1.2)
CVD (%)	11	9	1
Sedentary time ($min \cdot d^{-1}$)	641 ± 92	548 ± 87	434 ± 85
LPA ($min \cdot d^{-1}$)	267 ± 87	332 ± 96	378 ± 81
MPA ($min \cdot d^{-1}$)	31 ± 13	71 ± 14	129 ± 38
VPA ($min \cdot d^{-1}$)	0 (0–4)	3 (0–7)	14 (3–31)
PAEE ($J \cdot kg^{-1} \cdot min^{-1}$)	30 ± 8	45 ± 8	69 ± 13
TBF (%)			
Men	24.7 ± 7.6	24.5 ± 5.7	21.8 ± 6.8
Women	39.4 ± 6.3	35.5 ± 5.9	33.0 ± 4.3
Visceral fat (cm^2)			
Men	110 (69–169)	98 (87–146)	63 (38–109)
Women	64 (46–116)	57 (46–80)	37 (21–57)
Liver fat (%)			
Men	3.6 (2.2–13.1)	5.1 (1.1–11.0)	3.6 (1.8–5.8)
Women	2.7 (1.2–5.0)	1.6 (0.9–1.9)	1.1 (0.8–1.5)

Results are based on analyses weighted toward the BMI distribution of the general population (n = 228). Data are shown as mean ± SD, median (interquartile range), or percentage.
[a]Low education: none, primary school, or lower vocational education as highest level of education.
CVD, cardiovascular disease; MPA, moderate physical activity; VPA, vigorous physical activity.

Table 4 (see Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C340).

Physical activity in relation to visceral fat area. After adjustment for potential confounding factors, PAEE was inversely associated with visceral fat (−7.5 cm^2, 95% CI = −11.4 to −3.7). With regard to intensity, each 30 $min \cdot d^{-1}$ of sedentary time was associated with 2.6 cm^2 more visceral fat (95% CI = 0.6–4.6), whereas 30 $min \cdot d^{-1}$ of MVPA was associated with 7.8 cm^2 less visceral fat (95% CI = −11.6 to −4.0) (Table 3). LPA was not associated with visceral fat (−1.7 cm^2, 95% CI = −4.4 to 0.9). In the isotemporal substitution model, replacing 30 $min \cdot d^{-1}$ of sedentary time with 30 $min \cdot d^{-1}$ of MVPA was associated with 8.0 cm^2 less (95% CI = −11.9 to −4.1) visceral fat, whereas replacement with LPA was not associated with visceral fat (0.0 cm^2, 95% CI = −2.8 to 2.8). The association with MVPA attenuated after additional adjustment for TBF. After additional adjustment TBF, the isotemporal substitution of LPA with sedentary time or MVPA was not associated with visceral fat. Results were similar in participants with at least 72 h of objectively measured physical activity data (Supplemental Table 1, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C340).

Physical activity in relation to liver fat content. After adjustment for potential confounding factors, PAEE was inversely associated with liver fat (0.88 times, 95% CI = 0.80–0.95). As for intensity, each 30 $min \cdot d^{-1}$ of sedentary time was associated with more liver fat (1.05 times, 95% CI = 1.01–1.10), whereas 30 $min \cdot d^{-1}$ of MVPA was associated with less liver fat (0.88 times, 95% CI = 0.81–0.96)(Table 4). LPA was not associated with liver fat (0.96, 95% CI = 0.91–1.02). In the isotemporal substitution model, replacing 30 $min \cdot d^{-1}$ of sedentary time with 30 $min \cdot d^{-1}$ of MVPA was associated with less liver fat (0.89 times, 95% CI = 0.82–0.97), whereas replacement with LPA was not associated with liver fat (0.98, 95% CI = 0.92–1.04). The association with MVPA attenuated after additional adjustment for TBF. After this additional adjustment, the isotemporal substitution of LPA with sedentary time or MVPA was not associated with liver fat. Results were

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TABLE 2. Associations of daily activities per 30 $min \cdot d^{-1}$ with TBF (%) in participants with an MRI of the abdomen and objectively measured physical activity.

	Crude	Multivariable[a]	Substitution Model[b]	Substitution Model[c]
Per 30 $min \cdot d^{-1}$	% TBF (95% CI)	% TBF (95% CI)	% TBF (95% CI)	% TBF (95% CI)
Sedentary time	0.1 (−0.3 to 0.5)	0.5 (0.2 to 0.7)	Substituted	0.03 (−0.4 to 0.5)
LPA	0.2 (−0.3 to 0.7)	−0.4 (−0.8 to 0.04)	−0.03 (−0.5 to 0.4)	Substituted
MVPA	−1.4 (−2.3 to −0.5)	−1.4 (−1.8 to −0.9)	−1.3 (−2.0 to −0.7)	−1.3 (−2.3 to −0.3)

Results reflect regression coefficients from linear regression analyses weighted toward the BMI distribution of the general population (n = 228).
[a]Adjusted for sex, age, ethnicity, education, DHD index, and smoking.
[b]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between the substitution of 30 min sedentary time with 30 min of either light or MVPA and TBF (%).
[c]Same as model 2, but coefficients represent the association between the substitution of 30 min LPA with 30 min of either sedentary time or MVPA and TBF (%).

TABLE 3. Associations of daily activities per 30 min·d^{-1} with visceral fat (cm^2) in participants with an MRI of the abdomen and objectively measured physical activity.

	Crude	Multivariable[a]	Substitution Model[b]	Substitution Model[c]	Substitution Model[d]
Per 30 min·d^{-1}	cm^2 VAT (95% CI)	cm^2 VAT (95% CI)	cm^2 VAT (95% CI)	cm^2 VAT (95% CI)	cm^2 VAT (95% CI)
Sedentary time	3.9 (2.2 to 5.7)	2.6 (0.6 to 4.6)	Substituted	Substituted	−0.2 (−2.5 to 2.1)
LPA	−3.2 (−5.3 to −1.1)	−1.7 (−4.4 to 0.9)	0.0 (−2.8 to 2.8)	0.2 (−2.1 to 2.5)	Substituted
MVPA	−8.8 (−12.2 to −5.3)	−7.8 (−11.6 to −4.0)	−8.0 (−11.9 to −4.1)	−1.4 (−5.1 to 2.3)	−1.6 (−6.7 to 3.6)

Results reflect regression coefficients from linear regression analyses weighted toward the BMI distribution of the general population (n = 228).
[a]Adjusted for sex, age, ethnicity, education, DHD index, and smoking.
[b]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between the substitution of 30 min sedentary time with 30 min of either light or MVPA and visceral fat (cm^2).
[c]Additionally adjusted for TBF.
[d]Same as model 3, but coefficients represent the association between the substitution of 30 min LPA with 30 min of either sedentary time or MVPA and visceral fat (cm^2).

similar in participants with at least 72 h of objectively measured physical activity data (Supplemental Table 3, Supplemental Digital Content, Appendix, http://links.lww.com/MSS/C340).

DISCUSSION

In this population-based cohort study of middle-age men and women, overall physical activity was associated with less TBF, visceral fat, and liver fat. Regarding the underlying intensity distribution, sedentary time was associated with more TBF, visceral fat, and liver fat, whereas MVPA was associated with less TBF, visceral fat, and liver fat. LPA only seemed associated with less TBF but not with visceral fat or liver fat. Within the range of physical activity in our study population, the replacement of 30 min of sedentary time with MVPA, but not LPA, was associated with less TBF, visceral fat, and liver fat. These associations with visceral fat and liver fat attenuated after additional adjustment for TBF. It therefore seems that the associations with visceral fat and liver fat are mainly mediated by TBF.

To our knowledge, in only one previous study using isotemporal substitution analysis, direct measures of adiposity in combination with objectively measured physical activity were investigated (13). This study observed that the isotemporal substitution of 1 h·d^{-1} of sedentary and light-intensity physical activity with other types of physical activity was associated with less visceral fat, and this association attenuated after additional adjustment for BMI (13), which is in line with our findings. However, in the previous study, liver fat was not assessed and analyses were adjusted for BMI rather than for TBF.

In another study with objectively measured levels of sedentary time and physical activity in 82 overweight or obese adults, there was no relationship between physical activity and liver fat and a weak positive association between time spent in moderate physical activity and visceral fat (28). These results could be explained by the fact that BMI was already included in the prediction model before measures of physical activity were added. These findings are consistent with our results, which show that sedentary time is positively associated with liver fat and moderate to vigorous activity negatively. However, these associations also attenuated after the additional adjustment for TBF, which suggests that they are mainly mediated by TBF. A twin study of both monozygotic and dizygotic twins who were discordant for physical activity based on questionnaires during a follow-up of more than 30 yr showed that habitual physical activity potentially prevents the accumulation of visceral and ectopic fat (29). Although several previous studies observed associations between time spent in sedentary time and the risk of nonalcoholic fatty liver (30–33), sedentary time was measured objectively in only one study (33).

Besides abdominal adiposity, increased sedentary time has also been associated with other adverse metabolic consequences. Recent studies have shown that a high amount of sedentary time is associated with multiple adverse health outcomes, including two diabetes (34). This association was also present in physically fit individuals (35). Moreover, time spent sedentary has been shown to predict higher levels of fasting insulin after adjusting for time spent in MVPA (36). This indicates that the detrimental effects of sedentary time are not merely due to a lack of sufficient physical activity. Large meta-analyses have also shown that high sedentary time was associated with all-cause mortality (37), type 2 diabetes, cardiovascular disease, and

TABLE 4. Associations of daily activities per 30 min·d^{-1} with HTGC (%) in participants with an MRI of the abdomen and objectively measured physical activity.

	Crude	Multivariable[a]	Substitution Model[b]	Substitution Model[c]	Substitution Model[d]
Per 30 min·d^{-1}	Relative Change in HTGC (95% CI)	Relative Change in HTGC (95% CI)	Relative Change in HTGC (95% CI)	Relative Change in HTGC (95% CI)	Relative Change in HTGC (95% CI)
Sedentary time	1.08 (1.03–1.12)	1.05 (1.01–1.10)	Substituted	Substituted	1.02 (0.97–1.07)
LPA	0.94 (0.89–0.99)	0.96 (0.91–1.02)	0.98 (0.92–1.04)	0.98 (0.94–1.03)	Substituted
MVPA	0.87 (0.80–0.94)	0.88 (0.81–0.96)	0.89 (0.82–0.97)	1.00 (0.92–1.09)	1.02 (0.91–1.14)

Results reflect regression coefficients from linear regression analyses weighted toward the BMI distribution of the general population (n = 228). Because of a skewed distribution of HTGC, we used the natural logarithm of this variable in the analyses. For interpretation of the results, we back transformed the regression coefficients of HTGC toward a ratio with 95% confidence interval, which is associated with 30 min·d^{-1} spent in a certain activity. Such ratio, for example, a ratio of 1.2, can be interpreted as each 30 min·d^{-1} of a certain activity being associated with a 1.2-fold increased HTGC, which would reflect an increase in liver fat content from, for example, 5% to 6%.
[a]Adjusted for sex, age, ethnicity, education, DHD index, and smoking.
[b]Additionally adjusted for total time awake and for all other daily activities, but not for sedentary time. Coefficients represent the association between the substitution of 30 min sedentary time with 30 min of either light or MVPA and HTGC.
[c]Additionally adjusted for TBF.
[d]Same as model 3, but coefficients represent the association between the substitution of 30 min LPA with 30 min of either sedentary time or MVPA and HTGC.

cancer risk, even after adjustment for physical activity (10). However, the underlying biological pathways via which sedentary time may lead to disease remain largely unknown. It has been suggested that replacing sitting with standing and LPA improves insulin sensitivity and decreases plasma triglycerides, thereby leading to a decrease in triglyceride storage in the liver (38,39). It has also been suggested that exercise results in preferential reductions in visceral fat compared with caloric restriction when the amount of exercise is sufficient to result in weight loss (40). This might be due to the fact that visceral fat is more metabolically active and more sensitive to lipolytic activation by the adrenal system (41), which occurs during vigorous exercise. We observed that the habitual replacement of sedentary time with time spent in MVPA was associated with less TBF, visceral fat, and liver fat, whereas the replacement of sedentary time with LPA was not. A recent systematic literature review of multiple observational studies on physical activity in relation to adiposity by means of isotemporal substitution analyses described that the replacement of 30 $min \cdot d^{-1}$ of sedentary time with MVPA resulted in a decreased waist circumference, BMI, and body fat percentage in healthy adult populations (12). Moreover, reallocating sedentary time to light or MVPA was associated with multiple favorable cardiometabolic biomarkers, such as insulin sensitivity (12). Although most of the included studies used BMI or waist circumference as an outcome, the results are in line with our own findings, but we did not find an association between replacing sedentary time with time spent performing LPA and measures of adiposity.

A strength of this study is the extensive phenotyping, including direct assessment of VAT and hepatic triglyceride using MRI and ^{1}H-MRS. Moreover, physical activity was objectively assessed using combined accelerometry and heart rate monitoring, which has been shown to classify physical activity more accurately than either of the two measures (42–44). Another strength is that we applied isotemporal substitution analysis to be able to investigate the replacement of sedentary time with time spent in light and MVPA.

A few limitations should also be discussed. Because of the fact that both the objective measurement of physical activity and the body fat measurements by MRI were only performed in a random subset of the participants of the NEO study, the number of participants in the analyses on visceral fat and liver fat were relatively small. It must therefore also be noted that our results apply to people that were able to undergo an MRI of the abdomen and wear a monitor during free-living. Nevertheless, this sample size seems sufficient because we were able to detect associations with visceral fat and liver fat, as were previous studies that reported on the associations between objectively measured daily activities and visceral fat and liver fat and included even smaller sample sizes ($n < 100$) (28,33). In addition, the precision of combined sensing estimates of intensity is improved by individual calibration, and not everybody in our sample performed the step test. Consequently, the misclassification of activity intensity may have occurred for whom this was estimated based on the group calibration. Because we aimed to prevent such misclassification with our population-specific calibration, if present, this may have resulted in an overestimation of our results. Furthermore, our definition of sedentary time was purely based on intensity as used in many other studies, but ideally information on sitting posture *per se* would have enhanced this classification. We also have no information in which domain the physical activity was performed. In addition, participants were not instructed to keep a log to record sleep and waking times, and therefore these were not available. Instead, we used general times during which we assumed most participants were asleep. This may have led to an over- or underestimation of sedentary time in certain participants. However, we do not expect that such misclassification would be related to the amount of body fat. Further, the amount of daily MVPA in our study population was relativity high. Whereas the Dutch population is known to be among the most active in Europe (45), a high level of MVPA has also been commonly reported in other studies where PA was assessed by Actiheart (46). Nevertheless, even if the absolute amount of MVPA was overestimated in our study, we do not expect that this would have affected our results in relation to the different measures of adiposity. Moreover, our population consisted mainly of White participants, so results need to be confirmed in other ethnic groups. Lastly, the observational cross-sectional study design precludes from any causal inference, and therefore larger prospective studies are needed to confirm these associations. In the present cross-sectional analyses, reverse causation, e.g., people engaging in less MVPA due to their high TBF, cannot be ruled out. Nevertheless, the results on visceral fat and liver fat are less likely affected by reverse causation because people are not aware of the amount of visceral fat or liver fat in their body.

To conclude, in this population-based study of middle-age men and women, sedentary time was associated with more TBF, visceral fat, and liver fat. The replacement of 30 min of sedentary time per day with MVPA was associated with less TBF, visceral fat, and liver fat. In our analyses, the replacement of sedentary time with light activities was not associated with TBF, visceral fat, or liver fat. The associations for visceral fat and liver fat attenuated after additional adjustment for TBF, which suggests that the associations with visceral fat and liver fat mainly flow via TBF.

This study provides knowledge on how reducing sedentary time by replacing it with MVPA is negatively associated with multiple TBF and abdominal fat, which is important for the prevention of abdominal obesity and ultimately cardiometabolic diseases.

The authors' responsibilities were as follows: R. dM. and F. R. R. designed the study; R. dM. and H. J. L. conducted the study; E. W.-vE., S. C. B., J. H. P. M. vdV., K. W., and S. B. performed data collection and Actiheart data processing; E. W.-vE. analyzed data;E. W.-vE. drafted the manuscript; E. W.-vE. and RdM had primary responsibility for final content. All authors read and approved the final manuscript.

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