Abating abdominal adiposity: Modifiable lifestyle risk factors for visceral and liver fat deposition

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6

Reallocating sedentary time to moderate to vigorous physical activity is associated with reduced total body fat, visceral fat and liver fat

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ABSTRACT

Background

It is unclear how habitual physical activity and sedentary time are associated with the amount of visceral fat and liver fat. We therefore aimed to study substitution of sedentary time with other daily activities and total body fat (TBF), visceral adipose tissue (VAT) and hepatic triglyceride content (HTGC) in middle-aged men and women.

Methods

In this cross-sectional analysis of the NEO study, we objectively assessed physical activity in 932 participants using a combined heart rate monitor and accelerometer (Actiheart). Of those with a valid physical activity measurement, total body fat was assessed by bio impedance balance, VAT by magnetic resonance imaging (MRI) in 317 participants, and HTGC by proton-MR spectroscopy in 265 participants. Activities were categorized as sedentary time, light physical activity (LPA) or moderate to vigorous physical activity (MVPA) and expressed in blocks of 30 minutes. We performed isotemporal substitution analyses adjusted for sex, age, ethnicity, education, Dutch Healthy Diet index, and smoking to estimate associations of replacing 30 minutes/day of sedentary time with 30 minutes/day of other activities.

Results

Participants (41% men) had a mean (SD) age of 56 (6) years and performed 82 (55) minutes of MVPA and spent 9.1 hours (2.1) sedentary per day. Replacing 30 minutes/day of sedentary time with 30 minutes of MVPA was associated with 0.5% less TBF (95% CI: -0.9, -0.1), 7.2 cm2 less VAT (-10.7, -3.6) and with less HTGC (0.89 times; 0.82, 0.97). Replacement with LPA was not associated with TBF (-0.17 %; -0.45, 0.11), VAT (0.4 cm2; -2.1, 2.9) or HTGC (0.98; 0.92, 1.04).

Conclusions

Replacing sedentary time with MVPA, but not with LPA, was negatively associated with total body fat, and visceral and liver fat. These findings contribute to the development of more specified guidelines on sedentary time and physical activity.

INTRODUCTION

Abdominal obesity is a well-established risk factor for diabetes mellitus, the metabolic syndrome and cardiovascular diseases [1,2]. In particular accumulation of fat in the visceral area and in and around the organs [2], such as the liver, carries extra 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 cardio metabolic diseases.

Meta-analyses have shown that exercise can reduce both visceral fat [7] and liver fat [8]. However, the included studies 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 advise to perform at least 150 minutes per week of moderate to vigorous physical activity and to limit sedentary time [9], as sedentary behaviour has been associated with increased risk of type 2 diabetes, cardiovascular disease, and cancer, even after adjustment for physical activity [10]. Obviously, less time spent sedentary implies more time spent in other activities. For assessment of the association of one particular type of activity with a certain outcome, it is therefore important to take into account with which activity this is replaced, e.g., whether sedentary time is replaced with the same time spent while physically active. Although most studies that applied such isotemporal substitution analysis to examine replacement of sedentary time by time spent on other activities in relation to body fat used objectively assessed physical activity, most used surrogate outcomes for adiposity such as body mass index or waist circumference [11] instead of direct measures of adipose tissue. Only one study on isotemporal substitution of habitual activities used direct measurements of both physical activity and visceral fat, and it was reported that replacing sedentary time with time spent on moderate physical activity was associated with a reduction in visceral fat [12]. However, liver fat was not assessed in this study and adjustment for total body fat was not performed, which may attenuate associations.

Knowledge on how different types of daily activities and their mutual substitutions are associated with different adipose depots, such as total body fat, visceral and liver fat, helps elucidating the underlying mechanism of how sedentary time or physical inactivity can lead to multiple adverse health outcomes. This may lead to more specified guidelines on sedentary time and physical activity. We therefore aimed to study the association between substitution of sedentary time with other daily activities and total body fat, visceral fat and liver fat in a population-based cohort of middle-aged participants.

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 started in 2008 and includes 6,671 individuals aged 45–65 years, with an oversampling of individuals with overweight or obesity. The study design and data collection are described in detail elsewhere [13]. Men and women living in the greater area of Leiden (in the West of the Netherlands) were invited to participate if they were aged between 45 and 65 years and had a self-reported BMI of 27 kg/m^2 or higher. In addition, all inhabitants aged between 45 and 65 years 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. Prior to this study visit, participants collected their urine over 24 hours 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). Of the participants who were eligible for MRI, approximately 35% were randomly selected to undergo direct assessment of abdominal fat. Another random subsample (n=955) received the Actiheart device (Actiheart, CamNtech Ltd, UK) to carry for four days to objectively assess daily levels of physical activity.

6

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.

Objective assessment of daily activities

Daily levels of physical activity were objectively assessed the NEO study participants (n=955) who carried the Actiheart device, a uniaxial activity monitor capable of measuring acceleration and heart rate (Actiheart, CamNtech Ltd, UK). The device weighs less than 8 g and is worn directly onto the skin. 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.

A Gaussian process regression method was applied to the heart rate data to handle potential measurement noise [14]. Using a branched equation algorithm the acceleration and heart rate information was summarised into calibrated estimates of physical activity energy expenditure (PAEE) and time spent at different activity intensities expressed as metabolic equivalents of task (MET) [15, 16]. When summarising 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 [17]. In a subgroup of 132 participants who were equipped with an Actiheart monitor, an 8-min ramped step test was performed to calibrate the individual heart rate response to activity intensity. In addition, a group calibration equation was applied to the results of the other participants, which was derived from the valid step tests in this population [18].

Sedentary time was defined as time spent in activities with an intensity ≤1.5 MET, excluding sleep time. Sleep time was assumed as the time between 23:00h and 07:30h on weekdays and between 23:30h and 07:30h on weekend days unless Actiheart detected activity took place. Light intensity physical activity (LPA) was defined as any activity during wear time with an intensity >1.5 and ≤3 MET. Moderate-to-vigorous physical activity (MVPA) was defined as any activity >3 MET [19]. Participants with a valid wear time <24 hours were excluded from the analyses. No minimum bout duration was set for the activity intensity categories.

Assessment of body fat

6

Body weight and percent body fat were assessed by a Tanita bio impedance balance (TBF-310, Tanita International Division, UK) without the participant wearing shoes and one kilogram was subtracted from the body weight. BMI was calculated by dividing the weight in kilograms by the height in meters squared. In a random subgroup without contraindications, imaging was performed on a 1.5 Tesla MR system (Philips Medical Systems, Best, the Netherlands). Visceral adipose tissue (VAT) was quantified by a turbo spin echo imaging protocol using MRI. 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 [13]. 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 3,000 ms. Data points (1,024) were collected using a 1,000 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]. Hepatic triglyceride content 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.

Confounding factors

On the questionnaire, participants reported ethnicity by self-identification in eight categories which we grouped into white (reference) and 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 post-graduate education) versus low education (reference). Participants reported their medical history of diabetes and cardiovascular diseases. Pre-existing cardiovascular disease was defined as myocardial infarction, angina, congestive heart failure, stroke, or peripheral vascular disease. Habitual dietary intake of all participants was estimated using a semi-quantitative self-administered 125-item food frequency questionnaire (FFQ)[22, 23]. Based on these variables, an adapted version of the Dutch Healthy Diet Index (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 DHD-index ranges 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/m² or higher. To correctly represent associations in the general population [25], adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m² were made. This was done by weighting individuals towards 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/m². 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 minutes of daily activities (i.e. sedentary time, light, and moderate to vigorous physical activity) and total body fat, visceral fat and liver fat in a crude model. Second, a multivariable model was applied that was adjusted for sex, age, ethnicity, education, DHD-index, and smoking. This model describes the association between 30 minutes/day of each of the daily activities on top of the regular activity pattern for each outcome. Third, we performed an isotemporal substitution approach in our final model including waking time and excluding sedentary time. Consequently, the regression coefficients of each activity represent the estimated difference in measure of body fat associated with replacing 30 minutes of sedentary time with 30 minutes spent on this type of activity. This isotemporal substitution model on visceral fat and liver fat was additionally adjusted for total body fat to study whether physical activity is associated with visceral fat and liver fat beyond effects via total body fat. Regression coefficients of total body fat represent an absolute difference in TBF in % per 30 minutes of a certain activity, and those of VAT an absolute difference in VAT in cm² per 30 minutes of a certain activity per day.

Due to 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 towards a ratio with 95% confidence interval, which indicates the relative excess liver fat content for each 30 minutes per day spent additionally on a certain activity. This ratio, for example a ratio of 1.2, can be interpreted as each 30 minutes per day of sedentary time replaced with similar time spent performing moderate to vigorous physical activity being associated with a 1.2-fold HTGC. This would reflect an increase in liver fat from, for example, 5% to 6% .

Data was analyzed using STATA v.14 (StataCorp LP, College Station, TX, USA).

RESULTS

Physical activity was objectively assessed in 955 participants, of whom 932 had a valid measurement. The group of participants with a physical activity assessment was similar in sex distribution, age, and BMI to the group without an objective assessment of physical activity, but were slightly less likely to have a history of CVD (5.6% versus

6.1%). We excluded participants with fewer than 24 hours of measurement (n=39) or for whom daily activities could not be estimated (n=32). For the analyses on total body fat, we excluded participants with missing data on smoking status (n=1), education (n=7) and ethnicity (n=1), leaving a total of 852 participants.

These participants (41% men) had a mean (SD) age of 56 (6) years and BMI of 26 kg/m^2 (4). Participants had a mean (SD) valid Actiheart wear time of 83 (12) hours and 80% of participants had more than 72 hours of valid wear time. Most waking time was spent sedentary, and least time performing vigorous physical activity (**Figure 1**). The baseline characteristics of the total population for the analyses on total body fat stratified by tertiles of moderate to vigorous physical activity are shown in **Table 1**. Participants in the highest tertile of activity were slightly younger, more often men, more often non-smokers, had a lower BMI and spent less time in sedentary time than participants in the lowest tertile of MVPA.

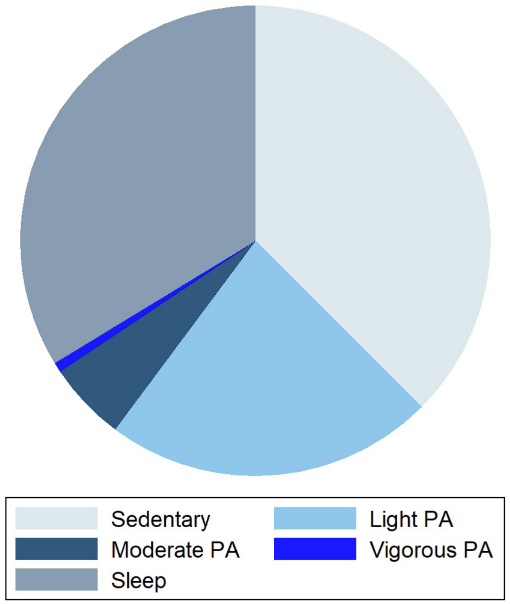

Figure 1. Distribution of daily activities during an average 24-hour period in participants with a physical activity and total body fat measurement in the Netherlands Epidemiology of Obesity study (N=852)

Table 1. Characteristics of participants of the Netherlands Epidemiology of Obesity study , men and women between 45 and 65 years of age with objectively assessed physical activity stratified by tertiles of moderate to vigorous physical activity

	Tertiles of moderate to vigorous physical activity (min/d)		
	T1 (0.2-44.8)	T2 (45.4-85.5)	T3 (85.6-394.8)
Age (y)	57 (6)	56 (6)	55 (6)
Sex (% men)	37	32	53
Ethnicity (% white)	95	95	97
Education level (% high)[1]	33	45	42
BMI (kg/m^2)	27.4	26.2	25.4
Tobacco smoking (% current)	22	15	12
Dutch Healthy Diet Index	69.0 (16.1)	72.0 (13.6)	71.7 (13.7)
HDL-Cholesterol (mmol/l)	1.5 (0.5)	1.6 (0.5)	1.6 (0.4)
Triglycerides (mmol/l)	1.4 (0.9)	1.2 (0.7)	1.1 (0.7)
CVD (%)	12	2	6
Sedentary time while awake (min/day)	523 (76)	445 (70)	362 (80)
LPA (min/day)	256 (93)	325 (92)	369 (98)
MPA (min/day)	27 (13)	61 (12)	120 (47)
VPA (min/day)	1 (3)	4 (6)	14 (16)
Total body fat (%)			
Men	25.6 (7.2)	26.0 (5.9)	23.7 (5.5)
Women	38.3 (7.8)	37.2 (5.8)	34.7 (5.5)
Visceral fat (cm^2)[2]			
Men	118.9 (73.8)	122.4 (62.2)	103.6 (53.1)
Women	88.6 (52.6)	73.3 (35.0)	46.2 (22.7)
Liver fat (%)[3]			
Men	7.8 (7.7)	10.3 (15.1)	6.7 (8.2)
Women	5.9 (9.6)	5.2 (8.3)	1.9 (2.5)

From this population, 294 participants had a measurement of visceral fat by MRI. These 294 participants were similar in age (mean 56, SD 6) to those without an MRI of the abdomen (mean 56, SD 6) and CVD history (both 6%), but were more often men (45% versus 39%) and had a slightly lower BMI (25.7 kg/m^2 versus 26.5 kg/m^2).

Lastly, for the analyses on liver fat, we additionally excluded participants without ^{1}H-MRS measurement (n=45) and those who consumed 40 grams of alcohol or more (4 standard glasses) per day (n=21), leaving a total of 228 participants.

Sedentary time and physical activity in relation to total body fat

After adjustment for confounding, 30 minutes per day of sedentary time was associated with 0.27% more total body fat (95% CI 0.11, 0.43), whereas 30 minutes per day of light physical activity (-0.25%, 95% CI -0.49, -0.02) and moderate to vigorous physical activity (-0.61%, 95% CI -0.97, -0.25) were associated with less total body fat. In the isotemporal substitution model, replacing 30 minutes of sedentary time per day with 30 minutes of moderate to vigorous physical activity was associated with less total body fat (-0.51%, 95% CI -0.94, -0.07), whereas light physical activity (-0.17%, 95% CI -0.45, 0.11) was not **(Table 2)**.

Table 2. Associations of daily activities per 30 minutes with total body fat (%) in participants with a measurement of total body fat by bioimpedance analysis and physical activity by Actiheart

	Crude	**Multivariable[1]**	**Substitution model[2]**
Per 30 min/day	**% TBF (95% CI)**	**% TBF (95% CI)**	**% TBF (95% CI)**
Sedentary time	0.15 (-0.06; 0.36)	0.27 (0.11; 0.43)	*substituted*
Light physical activity	0.04 (-0.22; 0.30)	-0.25 (-0.49; -0.02)	-0.17 (-0.45; 0.11)
Moderate to vigorous physical activity	-1.15 (-1.58; -0.72)	-0.61 (-0.98; -0.25)	-0.51 (-0.94; -0.07)

Results are based on analyses weighted toward the BMI distribution of the general population (n=852).
[1]Adjusted for sex, age, ethnicity, education, DHD-index and smoking
[2]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between substitution of 30 minutes sedentary time with 30 minutes of either light or moderate to vigorous physical activity and total body fat (%).

Sedentary time and physical activity in relation to visceral fat

In adjustment analyses, 30 minutes per day of sedentary time was associated with 2.0 cm^2 more visceral fat (95% CI 0.1, 4.9), whereas 30 minutes per day of moderate to vigorous physical activity was associated with 6.7 cm^2 less (95% CI -10.3, -3.1) visceral fat. Light physical activity was not associated with visceral fat (-1.0 cm^2, 95% CI -3.4, 1.3). In the isotemporal substitution model, replacing 30 minutes per day of sedentary time with 30 minutes per day of moderate to vigorous physical activity was associated with 7.2 cm^2 less (95% CI -10.7, -3.6) visceral fat, whereas replacement with light physical activity was not associated with visceral fat (0.4 cm^2, 95% CI -2.1, 2.9). This association attenuated after additional adjustment for total body fat (**Table 3**).

Sedentary time and physical activity in relation to liver fat

In adjusted analyses, 30 minutes per day of sedentary time was associated with more liver fat in adjusted analyses (1.05-fold, 95% CI 1.01, 1.10) whereas 30 minutes per day of moderate to vigorous physical activity was associated with less liver fat (0.88-folds, 95% CI 0.81, 0.96). Light physical activity seemed not or only marginally associated with liver fat (0.96-fold, 95% CI 0.91, 1.02). In the isotemporal substitution model, replacing 30 minutes per day of sedentary time with 30 minutes per day of moderate to vigorous physical activity was associated with less liver fat (0.89-fold, 95% CI 0.82, 0.97), whereas replacement with light physical activity showed little association with liver fat (0.98-fold, 95% CI 0.92, 1.04). This association attenuated after additional adjustment for total body fat (**Table 4**).

Table 3. Associations of daily activities per 30 minutes with visceral fat (cm^2) in participants with a direct measurement of visceral fat by MRI and physical activity by Actiheart

	Crude	**Multivariable[1]**	**Substitution model[2]**	**Substitution model[3]**
Per 30 min/day	**cm^2 VAT (95% CI)**	**cm^2 VAT (95% CI)**	**cm^2 VAT (95% CI)**	**cm^2 VAT (95% CI)**
Sedentary time	3.7 (2.0; 5.4)	2.0 (0.1; 3.9)	*substituted*	*substituted*
Light physical activity	-3.2 (-5.1; -1.2)	-1.0 (-3.4; 1.3)	0.4 (-2.1; 2.9)	0.4 (-1.6; 2.4)
Moderate to vigorous physical activity	-8.2 (-11.8; -4.6)	-6.7 (-10.3; -3.1)	-7.2 (-10.7; -3.6)	-0.6 (-3.9; 2.7)

Results are based on analyses weighted toward the BMI distribution of the general population (n=294).
[1]Adjusted for sex, age, ethnicity, education, DHD-index and smoking
[2]Additionally adjusted for all other daily activities
[2]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between substitution of 30 minutes sedentary time with 30 minutes of either light or moderate to vigorous physical activity and visceral fat (cm^2).
[3]Additionally adjusted for total body fat

Table 4. Associations of daily activities per 30 minutes with hepatic triglyceride content (%) in participants with a direct assessment of hepatic triglyceride content by ^{1}H-MRS and physical activity by Actiheart

	Crude	**Multivariable[1]**	**Substitution model[2]**	**Substitution model[3]**
Per 30 min/day	**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*
Light physical activity	0.94 (0.89; 0.99)	0.96 (0.91; 1.02)	0.98 (0.92; 1.04)	0.98 (0.94; 1.03)
Moderate to vigorous physical activity	0.87 (0.80; 0.94)	0.88 (0.81; 0.96)	0.89 (0.82; 0.97)	1.00 (0.92; 1.09)

Results are based on analyses weighted toward the BMI distribution of the general population (n=228).
[1]Adjusted for sex, age, ethnicity, education, DHD-index and smoking
[2]Additionally adjusted for all other daily activities
[2]Additionally adjusted for total time awake, and for all other daily activities but not for sedentary time. Coefficients represent the association between substitution of 30 minutes sedentary time with 30 minutes of either light or moderate to vigorous physical activity and hepatic triglyceride content.
[3]Additionally adjusted for total body fat

DISCUSSION

In this population-based cohort study of middle-aged men and women, more sedentary time was associated with more total body fat, visceral fat and liver fat, whereas moderate to vigorous physical activity was associated with less total body fat, visceral fat and liver fat. Replacing 30 minutes of sedentary time with moderate to vigorous physical activity was associated with reduced total body fat, visceral fat and liver fat. Replacing 30 minutes of sedentary time with light physical activity was not associated with total body fat, visceral fat or liver fat. These associations with visceral fat and liver fat attenuated after additional adjustment for total body fat.

To our knowledge, there is only one previous study that reported isotemporal substitution analysis with objectively measured physical activity in combination with direct measures of adiposity [12]. There, isotemporal substitution of 1 hour per day of sedentary and light intensity physical activity with other types of physical activity was associated with less visceral fat [12], which is in line with our findings. However, in this study liver fat was not assessed and analyses were adjusted for BMI rather than for objectively measured total body fat. Our study adds to this that we also assessed associations with liver fat, and were able to additionally adjust for total body fat. Our results show that replacing sedentary time with moderate to vigorous physical activity is associated with less total body fat, visceral fat and liver fat, whereas replacing sedentary time with light physical activity has no or minimal effect. The intensity of light physical activity appears to be too low . A recent systematic literature review has shown that multiple studies have investigated physical activity in relation to adiposity by means of isotemporal substitution analyses. However, most use body mass index or waist circumference as an outcome [11]. This review described that replacing 30 min/day of sedentary time with moderate to vigorous physical activity resulted in a decreased waist circumference, body mass index and body fat percentage in healthy adult populations [11]. Moreover, reallocating sedentary time to light or moderate to vigorous physical activity was associated with multiple favourable cardiometabolic biomarkers, such as insulin sensitivity [11], which may possibly be due to the associated lower body fat percentage we observed in our study.

In our study, we observed that replacing sedentary time with moderate to vigorous physical activity was associated with less total body fat, visceral fat and liver fat. However, the associations with visceral fat and liver fat attenuated when total body fat was included in the model. This attenuation for associations between physical activity and visceral fat and liver fat was also observed in other studies that included BMI into the model [12, 28]. It therefore seems that there is no extra effect on visceral fat beyond effects via total body fat.

Several previous studies reported associations between time spent in sedentary time and the risk of non-alcoholic fatty liver [29-32], although in only few studies sedentary time was measured objectively [32]. In one study researchers aimed to assess whether objectively measured levels of sedentary time and physical activity correlated with levels of directly assessed visceral fat and liver fat in 82 overweight or obese adults, but found no relationship between physical activity and liver fat and a weak positive association between time spent in moderate physical activity and visceral fat [28]. Contrastively, 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 years showed that habitual physical activity potentially prevents accumulation of visceral and ectopic fat [33]. These findings are consistent with our results, which show that sedentary time was positively associated with visceral fat and liver fat, and moderate to vigorous activity negatively. Prospective studies with objective assessment of physical activity and sedentary time are needed to confirm these associations.

Besides abdominal adiposity, sedentary time has also been associated with other adverse metabolic consequences. Recent studies have shown that a high amount of time spent in sedentary time is associated with multiple adverse health outcomes, among which type 2 diabetes [34]. This association was also present in fit individuals [35]. Furthermore, time spent sedentary predicts higher levels of fasting insulin independent of time spent performing moderate to vigorous physical activity [36], indicating 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 incidence, and cardiovascular disease and 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, although it has been suggested that replacing sitting with standing and LPA improves insulin sensitivity and decreases plasma triglycerides, thereby leading to a decrease in intrahepatic triglyceride storage [38, 39]. In our study, we observed that replacing standing with light or moderate to vigorous physical activity was not associated with liver fat after adjustment for total body fat, suggesting that the association is mainly driven by overall adiposity.

Important strengths of this study are the objective assessment of physical activity and sedentary time using an Actiheart monitor that combines a heart rate monitor and an accelerometer into a single device, which has been shown to classify physical activity more accurately than individual measures [40-42], and the direct assessment of visceral adipose tissue and hepatic triglyceride using MRI and ^{1}H-MRS. Another strength is that we applied isotemporal substitution analysis to be able to investigate replacement of sedentary time with light and moderate to vigorous physical activity in relation to measures of body fat. Finally, the extensive phenotyping allowed detailed adjustment for confounding factors.

A few limitations should also be discussed. Because both the physical activity measurements and the body fat measurements were performed in a random subset of the participants of the Netherlands Epidemiology of Obesity study, the number of participants in the analyses on visceral fat and liver fat were relatively small. Our results apply only to people without contraindications for MRI and with a valid Actiheart measurement. However, previous studies that reported on the associations between objectively measured daily activities and visceral fat and liver fat as measured by MRI and MRS included smaller sample sizes (N<100)[28, 32]. Additionally, even though the Actiheart combines accelerometry with heart rate monitoring, which provides valid estimates of physical activity intensity, defining sedentary time may be less valid as information on posture is lacking. In addition, sleep and wake times were not available and therefore we used general times during which we assumed participants were asleep. This may have led to over- or underestimation of sedentary time. Furthermore, our population consisted mainly of Caucasian participants, and results need to be confirmed in other ethnic groups. Lastly, inherent to the observational cross-sectional study design we cannot exclude residual confounding and reverse causation, as people with more body fat may be less physically active because of their higher weight. Reallocations of sedentary time to light or moderate to vigorous physical activity are model-based and actual changes might not represent causal relationships. Clinical trials should therefore confirm whether actual reallocation of sedentary time with other activities leads to decreases in visceral fat and liver fat content.

To conclude, in this population-based study of middle-aged men and women, sedentary time was associated with more total body fat, visceral fat, and liver fat. Replacing 30 minutes per day with moderate to vigorous physical activity, but not light physical activity, was associated with less total body fat, visceral fat and liver fat. The associations for visceral fat and liver fat attenuated after additional adjustment for total body fat, suggesting that there is no extra effect on visceral fat and liver fat beyond effects via total body fat. This study provides knowledge on how a reduction of sedentary time by replacing it with moderate to vigorous physical activity is negatively associated with multiple adipose tissue depots, which is important for the prevention of overall and abdominal obesity, and ultimately cardiometabolic diseases.

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6

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