Supplementary Material

Patients and Methods

Study Sample

The Framingham Heart Study is a multi-generational cohort study. Beginning in 1971, the offspring and spouses of the offspring were enrolled in the Offspring Cohort. Between 2002 and 2005, the grandchildren of the original cohort and children of the Offspring cohort were enrolled in the Third Generation Cohort.(1) Starting in 1994, in order to reflect the growing ethnic diversity of the community, the Omni Cohort, which consists of minority residents of Framingham, MA and surrounding towns, was recruited. A second Omni Cohort was recruited between 2003 and 2005, with examinations paralleling that of the Third Generation Cohort. Between September 2008 and December 2011, Offspring, Third Generation and Omni 2 Cohort participants were enrolled in the Multi-Detector Computed Tomography 2 substudy and underwent chest and abdominal computed tomographic (CT) scanning. The median length of time between the CT scans and measurement of PA was 0.86 months with an interquartile range of 0.03 months to 4.21 months.

Measurement of physical activity by accelerometer

Participants were asked to remove the accelerometer only when bathing. At the end of the 8 day period, participants mailed the monitor to the FHS clinic. Data was visually screened for spurious data by FHS clinical staff. Standard quality control metrics were applied to the data and only data from participants with a minimum of 5 of 7 days of “valid” wear time were included in analyses as noted above. A “valid” wear day was defined as a minimum of 10 hours of wear time.(2) We used algorithms

developed to process raw activity data from the Actical accelerometer to define sedentary, light, and MVPA minutes using analytical software as previously described (Kinesoft, version 3.3.63; KineSoft, Loughborough, UK).(3, 4) Briefly, time spent in each PA category is based on the accelerometer measured “counts” per minute which are calibrated to correspond to metabolic equivalents (METs) based on prior studies.(24) Light-intensity activity is defined as >200 counts/minute and <3 METs, moderate-intensity activity as between 3-6 METs and vigorous-intensity activity as >6 METs.(24, 25) Sedentary time is defined as ≤200 counts/minute. The device was considered not worn if there were 60 minutes of consecutive zero counts allowing for 1-2 minute interruptions of non-zero counts.(25) Since the accelerometer was worn for 24 hours/day in order to maintain a high level of adherence with wearing the device, sleep time was defined empirically as between the hours of 2200 hours and 0600 hours and sedentary activity was not considered during this time period. All other count data contributed to wear time. Because the total time spent at vigorous-intensity activity was so small, moderate and vigorous-intensity minutes were summed to obtain the total MVPA time. Based on the national PA guidelines for Americans which recommends that all adults participate in at least 150 minutes of MVPA per week with activity accumulated in bouts of ≥ 10 minutes (24), we additionally defined a bout of MVPA as at least 10 minutes of continuous MVPA, allowing for 2-minute interruptions to simulate conditions such as slowing a jog for a traffic light as has been done in prior research.(25) For this investigation, we defined compliance with the national PA guidelines as participating in ≥ 150 minutes of MVPA over 5 to 7 days in bouts or in total MVPA, regardless of bout duration.

Multi-detector CT scan protocol and measurement of VAT

For the CT scans, participants were placed in the supine position and twenty-five contiguous 5-mm-thick slices (120 kVp, 400mA, gantry rotation time 500 ms, and table feed 3:1) covering 125 mm above S1 were obtained using an 8-slice multi-detector abdominal CT scanner (LightSpeed Ultra, General Electric, Milwaukee, WI). A calibration phantom (Image Analysis, Lexington, KY) with a water equivalent compound (CT-Water, Light Speed Ultra; General Electric, Milwaukee, WI) and calcium hydroxyapatite at 0, 75, and 150 mg/cm^3 was placed under each participant and was visualized on each image obtained. Three areas from the liver, two areas from the spleen and one from the external phantom were measured and averaged to create liver spleen ratios and liver phantom ratios (LPR). The intra-class correlation coefficient was 0.99 on a subset of 50 randomly selected participants. Because the spleen was not visualized on all scans, the liver phantom ratio was used as the indexed standard.

VAT and subcutaneous adipose tissue (SAT) were measured using an image display window of -195 to -45 Hounsfield Units (HU) and a window center of -120 HU to identify pixels containing fat. The VAT compartment was separated from the SAT compartment by a single reader who manually traced the muscular abdominal wall separating these depots. VAT and SAT volumes were subsequently quantified using a semiautomatic segmentation technique at a dedicated offline workstation (Aquarius 3D Workstation; TeraRecon, San Mateo, CA) as described.(5) The interclass correlation coefficients between two independent readers on a subset of 100 randomly selected participants were 0.992 and 0.997 for VAT and SAT, respectively.

Covariate and baseline measurements

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were obtained from fasting morning samples using an automated Roche method (Roche cobas 501). Data on ethnicity was obtained from self-administered questionnaires, while alcohol use and smoking status were assessed on the basis of physician-administrated questionnaires. Alcohol use was recorded as drinks per week or drinks per month. Participants were considered current smokers if they had smoked at least one cigarette per day in the year preceding the FHS examination. Anthropometric measurements were obtained by trained technicians using standard protocols for measuring height, weight and waist circumference. BMI was defined as weight (kg)/height^2 (m^2).

References:

1. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ*, et al.* The Third Generation Cohort of the National Heart, Lung, and Blood Institute's Framingham Heart Study: design, recruitment, and initial examination. *American journal of epidemiology* 2007;**165:** 1328-1335.

2. Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. *Medicine and science in sports and exercise* 2008;**40:** 181-188.

3. Glazer NL, Lyass A, Esliger DW, Blease SJ, Freedson PS, Massaro JM*, et al.* Sustained and shorter bouts of physical activity are related to cardiovascular health. *Medicine and science in sports and exercise* 2013;**45:** 109-115.

4. Heil DP. Predicting activity energy expenditure using the Actical activity monitor. *Research Quarterly for Exercise and Sport* 2006;**77:** 17.

5. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY*, et al.* Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation* 2007;**116:** 39-48.

Supplementary Table 1: Multivariable-adjusted logistic regressions examining the association between 30 minutes/day increment of sedentary time and hepatic steatosis (Liver Phantom Ratio ≤ 0.33).

	Women (n=501)		Men (n=559)		Overall (n=1060)		P Value for Sex Interaction
	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	
Sedentary (min/day)							0.70
Multivariable (MV) Model*	0.98 (0.88-1.08)	0.64	1.0 (0.92-1.09)	0.99	0.99 (0.93-1.06)	0.76	
MV + BMI	1.0 (0.88-1.13)	0.98	1.03 (0.93-1.13)	0.62	1.02 (0.94-1.10)	0.67	
MV + VAT	1.0 (0.88-1.14)	0.99	1.03 (0.93-1.13)	0.61	1.02 (0.94-1.10)	0.64	

BMI indicates body mass index; VAT, visceral adipose tissue

*Multivariable (MV) Model includes age, sex (in overall model only), cohort (Third Generation or Omni), accelerometer wear time (valid days of use), light PA minutes/day, MVPA minutes/day, current smoking status and drinks per week.

Supplementary Table 2: Multivariable-adjusted linear regressions examining the associations between 30 minutes/day increment of physical activity and log-adjusted aminotransferase levels.*

	Log-AST		Log-ALT	
	Beta Coefficient (95% CI)	P Value	Beta Coefficient (95% CI)	P Value
MVPA (min/day)				
Multivariable (MV) Model**	0.17 (0.08-0.26)	0.0002	-0.04 (-0.12-0.05)	0.41
MV + BMI	0.17 (0.08-0.26)	0.0002	0.02 (-0.06-0.10)	0.62
MV + VAT	0.18 (0.09-0.27)	0.0001	+0.04 (-0.04-0.13)	0.30
MVPA Bouts (min/day)***				
Multivariable (MV) Model**	0.29 (0.15-0.42)	<0.0001	-0.09 (-0.21-0.04)	0.18
MV + BMI	0.30 (0.16-0.43)	<0.0001	-0.01 (-0.13-0.12)	0.91
MV + VAT	0.30 (0.16-0.43)	<0.0001	0.00 (-0.12-0.13)	0.95
Light (min/day)				
Multivariable (MV) Model**	0.03 (-0.01-0.06)	0.16	0.01 (-0.03-0.04)	0.71
MV + BMI	0.03 (-0.01-0.06)	0.16	0.01 (-0.03-0.04)	0.61
MV + VAT	0.03 (-0.01-0.06)	0.15	0.02 (-0.02-0.05)	0.33
Met MVPA Guidelines****				
Multivariable (MV) Model**	0.17 (0.04-0.29)	0.008	-0.08 (-0.20-0.03)	0.17
MV + BMI	0.17 (0.05-0.30)	0.007	-0.00 (-0.12-0.11)	0.96
MV + VAT	0.17 (0.05-0.30)	0.007	0.02 (-0.09-0.13)	0.70
Met MVPA Guidelines*****				
Multivariable (MV) Model**	0.31 (0.13-0.49)	0.0007	-0.05 (-0.22-0.12)	0.54
MV + BMI	0.32 (0.14-0.50)	0.0006	0.03 (-0.14-0.20)	0.72
MV + VAT	0.32 (0.14-0.50)	0.0006	0.04 (-0.13-0.20)	0.66

AST indicates aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; VAT, visceral adipose tissue

*There were no significant sex interactions.

**Multivariable (MV) Model includes age, sex, cohort (Third Generation or Omni), accelerometer wear time (valid days of use), current smoking status and drinks per week.

***A bout of MVPA is defined as at least 10 minutes of continuous MVPA allowing for a 2 minute interruption.

****Met MVPA Guidelines is defined as participating in ≥ 150 minutes of moderate-to-vigorous physical activity per week.

*****Met MVPA Bout Guidelines is defined as participating in ≥ 150 minutes of moderate-to-vigorous physical activity per week accrued in bouts of at least 10 minutes.

Beta estimates provided above give standard deviation increase in log AST/ALT.