Physical Activity, Fitness, Glucose Homeostasis, and Brain Morphology in Twins

MIRVA ROTTENSTEINER[1], TUIJA LESKINEN[1], EINI NISKANEN[2], SARI AALTONEN[1], SARA MUTIKAINEN[1], JAN WIKGREN[3], KAUKO HEIKKILÄ[4], VUOKKO KOVANEN[1], HEIKKI KAINULAINEN[5], JAAKKO KAPRIO[4,6,7], INA M. TARKKA[1], and URHO M. KUJALA[1]

[1]Department of Health Sciences, University of Jyväskylä, Jyväskylä, FINLAND; [2]Department of Applied Physics, University of Eastern Finland, Kuopio, FINLAND; [3]Department of Psychology, University of Jyväskylä, Jyväskylä, FINLAND; [4]Department of Public Health, Hjelt Institute, University of Helsinki, Helsinki, FINLAND; [5]Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, FINLAND; [6]Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, FINLAND; and [7]Institute for Molecular Medicine, University of Helsinki, Helsinki, FINLAND

ABSTRACT

ROTTENSTEINER, M., T. LESKINEN, E. NISKANEN, S. AALTONEN, S. MUTIKAINEN, J. WIKGREN, K. HEIKKILÄ, V. KOVANEN, H. KAINULAINEN, J. KAPRIO, I. M. TARKKA, and U. M. KUJALA. Physical Activity, Fitness, Glucose Homeostasis, and Brain Morphology in Twins. *Med. Sci. Sports Exerc.*, Vol. 47, No. 3, pp. 509–518, 2015. **Purpose**: The main aim of the present study (FITFATTWIN) was to investigate how physical activity level is associated with body composition, glucose homeostasis, and brain morphology in young adult male monozygotic twin pairs discordant for physical activity. **Methods**: From a population-based twin cohort, we systematically selected 10 young adult male monozygotic twin pairs (age range, 32–36 yr) discordant for leisure time physical activity during the past 3 yr. On the basis of interviews, we calculated a mean sum index for leisure time and commuting activity during the past 3 yr (3-yr LTMET index expressed as MET-hours per day). We conducted extensive measurements on body composition (including fat percentage measured by dual-energy x-ray absorptiometry), glucose homeostasis including homeostatic model assessment index and insulin sensitivity index (Matsuda index, calculated from glucose and insulin values from an oral glucose tolerance test), and whole brain magnetic resonance imaging for regional volumetric analyses. **Results**: According to pairwise analysis, the active twins had lower body fat percentage ($P = 0.029$) and homeostatic model assessment index ($P = 0.031$) and higher Matsuda index ($P = 0.021$) compared with their inactive co-twins. Striatal and prefrontal cortex (subgyral and inferior frontal gyrus) brain gray matter volumes were larger in the nondominant hemisphere in active twins compared with those in inactive co-twins, with a statistical threshold of $P < 0.001$. **Conclusions**: Among healthy adult male twins in their mid-30s, a greater level of physical activity is associated with improved glucose homeostasis and modulation of striatum and prefrontal cortex gray matter volume, independent of genetic background. The findings may contribute to later reduced risk of type 2 diabetes and mobility limitations. **Key Words:** EXERCISE, FITNESS, BODY COMPOSITION, GRAY MATTER VOLUME, GLUCOSE

High levels of leisure time physical activity and physical fitness are associated with reduced levels of total and visceral fat, lowered cardiometabolic risk factors, better cognitive function, reduced mortality, and reduced prevalence of metabolic syndrome, type 2 diabetes, and CHD (5, 16, 29,30). In many diseases, such as CHD, type 2 diabetes, and Alzheimer disease, a long presymptomatic phase is thought to precede clinical onset. Hence, studies assessing a low level of physical activity as a potential risk factor for such diseases among middle-age or older people require long follow-up times to avoid influence on the investigated risk factors from preclinical pathogenic processes or changes in physical activity levels arising from the prodromal phase of a disease.

In exercise science, very long-term intervention studies are challenging to accomplish because of both funding and logistical reasons. Purely observational follow-up studies, even in a longitudinal setup, also present problems in establishing cause-and-effect relations. If, because of genetic susceptibility, a person becomes ill, gains weight, or has naturally low aerobic

Address for correspondence: Urho M. Kujala, M.D., Ph.D., Department of Health Sciences, Sports and Health Laboratory, Rautpohjankatu 8, University of Jyväskylä, PO Box 35 (LL), FI-40014 Jyväskylä, Finland; E-mail: urho.m.kujala@jyu.fi.

Submitted for publication February 2014.
Accepted for publication June 2014.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.acsm-msse.org).

0195-9131/15/4703-0509/0
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DOI: 10.1249/MSS.0000000000000437

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fitness, the result can be inactivity with the consequence of selection bias in observational studies (13). Various studies have shown that physical fitness and the ability to achieve high levels of physical activity also have genetic components (6,36). Inherited biological characteristics may make it easier for individuals to exercise and therefore may favor them with lower morbidity and mortality because of this interaction (13). Childhood environment also plays a role in adult exercise behavior. A monozygotic (MZ) twin pair study design controls for somatic genetic predisposition (MZ pairs are genetically identical at the sequence level) and largely controls for childhood home environment because the pairs almost always share the same childhood environment.

According to individual-based observational studies, healthy elderly adults who have a high aerobic fitness level maintain larger specific brain volumes, especially in the hippocampus, compared with their less physically active age-matched controls (9). A larger right hippocampus is also implicated in younger exercising adults compared with those not exercising (11). However, theoretically, the observed difference reported by Killgore et al. (11) may be explained by other associated unstudied factors among unrelated individuals whereas MZ twin pairs usually show similar regional brain volumes (37).

The main aim of the present co-twin control study (the FITFATTWIN study) was to investigate how physical activity level is associated with body composition, glucose homeostasis, and brain morphology in young adult male MZ twin pairs discordant for physical activity. We studied young adult males to see whether differences arising from differing physical activity levels are observable under conditions in which chronic diseases are uncommon, and medications or possible prodromal phases thus do not interfere with interpretation of findings.

METHODS

Participants

We recruited 17 young adult male MZ twin pairs for the FITFATTWIN study, among whom 10 pairs were determined to be discordant for leisure time physical activity during the past 3 yr. The selection process is described in detail as follows.

The participants for this study were initially identified from the FinnTwin16 Cohort, which is a population-based longitudinal study on Finnish twins born between October 1974 and December 1979 (10). All twins had been sent by mail a paper questionnaire at ages 16, 17, 18.5, and 22–27 yr (mean of the last range, 24.5 yr). The latest data collection (wave 5), using a Web-based questionnaire, was conducted when the twins were age 32–37 yr (mean, 34.0 yr). All questionnaires included questions related to health, body composition, and physical activity. A total of 4183 twin individuals (1880 males) responded to the latest Web-based questionnaire, and the response rate for the overall cohort was 71.9%. The responders included 202 male MZ pairs with data on physical activity from both co-twins. The zygosity of the twins was determined using a validated questionnaire (33).

The selection of the twin pairs for the FITFATTWIN study was done on the basis of data gathered from a telephone interview, face-to-face interview, and medical examination at the laboratory, in addition to the Web-based questionnaire.

Initially, we selected all of the MZ male twin pairs from the FinnTwin16 Cohort (wave 5) and estimated their physical activity level on the basis of answers to questions about leisure time physical activity. We identified potential participants for the FITFATTWIN study by screening and including the pairs with the highest discordance in their leisure time physical activity (Fig. 1). Specifically, the difference in physical activity between the co-twins of a twin pair was assessed on the basis of frequency of leisure time physical activity, as follows: the so-called active co-twin of the twin pair was physically active ≥2 times per week, and the so-called inactive co-twin of the same pair, ≤2 times per month (inclusion criterion 1 is shown in Fig. 1). If this criterion was not met, the physically active co-twin needed to participate in leisure time physical activity ≥2 times per week at an intensity equivalent to easy or brisk running while the leisure time physical activity of the inactive co-twin needed to be less intense and less frequent or of shorter duration, and neither frequency nor duration could be more than that of his active co-twin (inclusion criterion 2 is shown in Fig. 1). Because chronic diseases can restrict the ability to be physically active, twins with specific chronic diseases were excluded. Furthermore, twins reporting heavy use of alcohol or use of medication for a chronic disease were excluded.

Among the 202 MZ male pairs of the FinnTwin16 Cohort, 26 pairs fulfilled inclusion criterion 1 and 13 pairs fulfilled inclusion criterion 2. All of these pairs (n = 39) were interviewed by telephone. The interview included questions on current health and physical activity habits during the past 3 yr, similar to those asked in our previous studies (15). Of these 39 pairs, 19 pairs were excluded from the FITFATTWIN study for the following reasons: declining to take part in the study, having specific acute diseases that affected the ability to be physically active, failure to attend the telephone interview, or recent major changes in physical activity levels (Fig. 1). Finally, 17 male MZ pairs (10 pairs meeting inclusion criterion 1 and seven pairs meeting inclusion criterion 2) accepted the invitation to participate in the study and went through our comprehensive clinical study measurements and detailed physical activity interviews (Fig. 1).

Final criteria of physical activity discordant twin pairs. After the FITFATTWIN physical activity interviews (see details in later portion), 10 of these 17 pairs were classified as discordant for leisure time physical activity (Fig. 1). These 10 pairs met the following five criteria set for maximal leisure time physical activity discordance.

1. Inclusion based on criterion 1 or 2, given previously.
2. A pairwise difference of ≥1.5 MET·h·d^{-1} between active and inactive co-twins in leisure time physical activity (including work journey activity), according to

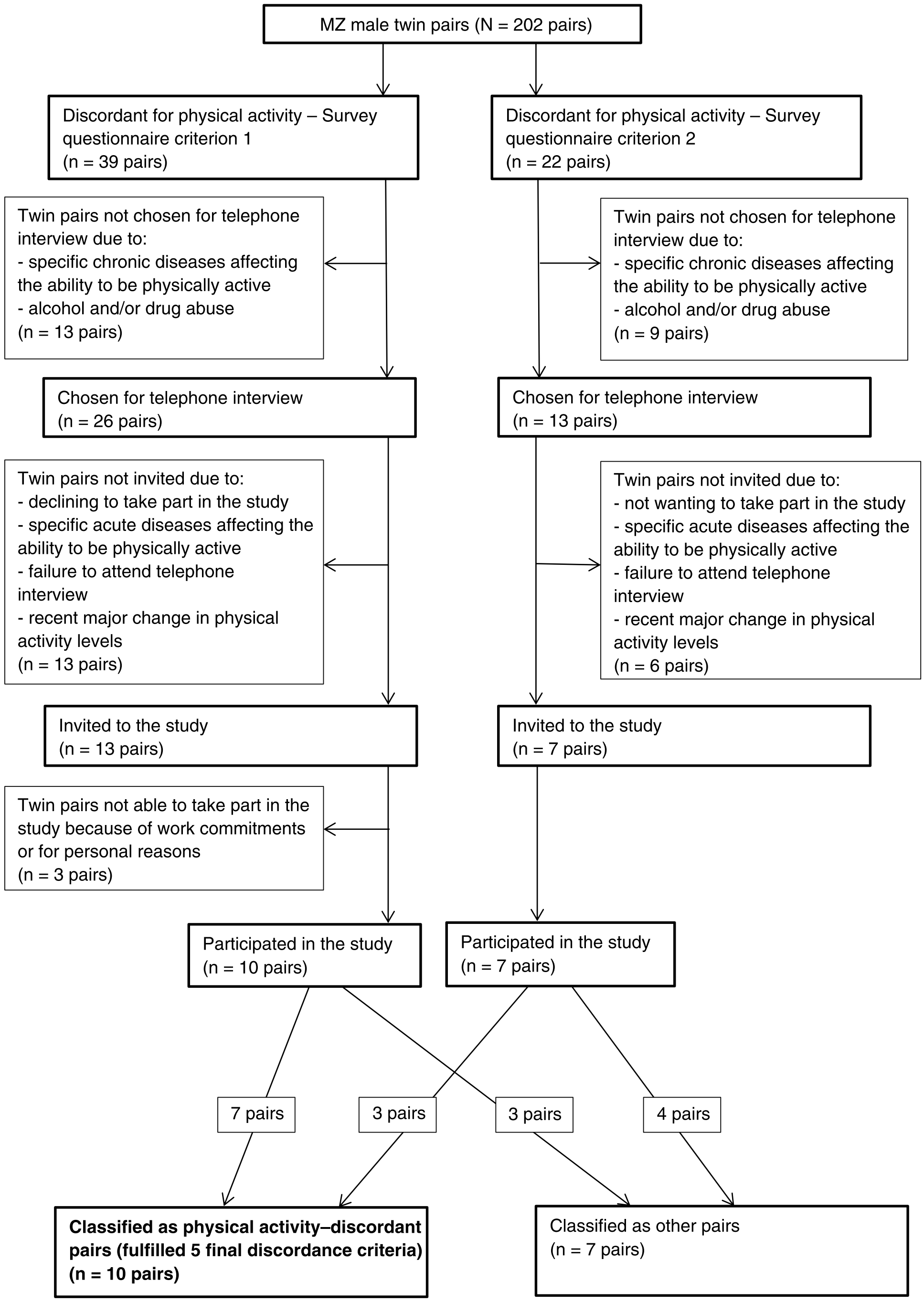

FIGURE 1—Flow chart of the participants in the FITFATTWIN study. For selection criteria 1 and 2 and the five final criteria for discordant pairs, see Methods.

the 12-month physical activity interview (12-month-LTMET index; see later portion) (17,38).

3. 12-month-LTMET index <5 MET·h·d^{-1} for the inactive co-twin.
4. ≥1 MET·h·d^{-1} pairwise difference between active and inactive co-twins in leisure time physical activity (including work journey activity) for the past 3 yr, according to the shorter physical activity interview (3-yr-LTMET index; see later portion) (15,19,38).
5. A higher Baecke sport index for the active versus the inactive co-twin (4).

Measurements

We conducted a series of comprehensive clinical measurements over two consecutive days (see Table, Supplemental Digital Content 1, List of examinations with timetable related to the FITFATTWIN study, http://links.lww.com/MSS/A423).

All of the main outcome measurements were carried out blind to physical activity status. All participants were advised not to exercise vigorously (except for walking and other daily chores) during the 2 d before the measurements because our aim was to investigate long-term adaptations to exercise. The measurements reported in this article are described in more detail, as follows.

Leisure time physical activity. The two different structured physical activity interviews were used to assess the volume of participant leisure time physical activity, including work journey activity. First, a shorter retrospective physical activity interview (15,19,38) was used to assess leisure time physical activity volume at 1-yr intervals over the past 6 yr. Leisure time physical activity volume was quantified as a leisure time MET index. Leisure time physical activities were calculated as frequency (per month) × duration (min) × intensity (MET) and work journey activity as frequency (five times per week) × duration (min) × intensity of 4 METs. The results were expressed as a sum score of MET-hours per day (MET index). The mean leisure time MET index during the past 3 yr (3-yr-LTMET index as MET-hours per day) was calculated and used as one of the criterion variables for pairwise comparison of leisure time physical activity discordance (see previously given discordance criterion 4).

The second, more detailed, structured interview that was used to determine the volume of leisure time activities, daily (nonexercise) activities, and work journey activity over the previous 12 months used a modified version of the Kuopio Ischemic Heart Disease Risk Factor Study Questionnaire (17,38). Here, "modified version" refers to the updated list of activities included in the questionnaire. This questionnaire contained a 20-item list of different types of physical activity, including leisure time (e.g., running, skiing, and swimming), daily (e.g., gardening, berry picking, do-it-yourself activities), and commuting activity (walking or cycling) along with "other" physical activities specified by the responder. Both twin brothers reported the monthly frequency of each physical activity session over the previous 12 months. They also reported the average intensity of their activity sessions on a scale from 1 to 4, as follows: 1 = recreational outdoor activities that do not cause breathlessness or sweating, 2 = conditioning exercise that induces breathlessness but not sweating, 3 = brisk conditioning exercise that induces breathlessness and sometimes sweating, and 4 = competitive strenuous exercise that induces breathlessness and extensive sweating. Each self-rated physical activity intensity was converted into MET values (2,3,17). For each activity, the average duration per exercise session was also reported to calculate the overall dose of activity (MET × average duration × frequency ($MET{\cdot}h{\cdot}d^{-1}$)). The overall dose of leisure time physical activity during the past 12 months (12-month-LTMET index as $MET{\cdot}h{\cdot}d^{-1}$) was calculated by summing the values for leisure time and work journey activity, excluding daily activities, and used in the identification of discordant pairs (see previously given criteria 2 and 3). The most common types of leisure time physical activity reported were jogging and walking.

We also used the 16-item Baecke Questionnaire to assess recent vigorous physical activity (4). We then summed the three indexes (work, sport, and leisure time excluding sports) as proposed in the original article (4). The sport index was used as a measure of vigorous physical activity.

Psychological factors. To evaluate participant motives for leisure time physical activity, the Finnish version (for details, see Aaltonen (1)) of the original 73-item version of the Recreational Exercise Motivation Measure, developed by Rogers and Morris (31), was used. The 73 Recreational Exercise Motivation Measure items comprise eight subdimensions for exercise motivation (each with 8–13 items). The subdimension "enjoyment" (i.e., "to have a good time/I enjoy exercising"), representing intrinsic motivation, was included in this initial analysis to study its associations with the other characteristics related to physical activity.

Physical fitness. Cardiorespiratory fitness was measured by a maximal exercise test with gas exchange analysis (spiroergometry) using an electrically braked bicycle ergometer. Gas exchange, including oxygen uptake, was measured breath by breath with a Vmax spiroergometer (Sensormedics, Yorba Linda, CA). The workload started at 25 W and was increased stepwise by 25 W every 2 min until exhaustion, or until maximal exercise capacity was reached, using an RPE of 19–20/20 on the Borg scale or a gas exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) of over 1.1 as the criterion. Maximal oxygen uptake was determined as the mean value of the two highest consecutive $\dot{V}O_2$ values recorded during periods of 30 s. ECG recordings were performed with the participant at rest and monitored during exercise and recovery. Blood pressure was measured at rest and during exercise and recovery at 2-min intervals.

Maximal isometric left knee extensor force was measured in a sitting position using an adjustable dynamometer chair (Good Strength; Metitur, Palokka, Finland) (35). Briefly, the left knee was set at an angle of 60° from full extension. Overall, four maximal efforts separated by a 30-s pause were performed. The best performance with the highest value was accepted as the participant's score. In our laboratory, the coefficients of variation between two consecutive measurements have been 6%.

Anthropometrics and body composition. Weight and height were measured, with the participant in bare feet and light clothing, to the nearest 100 g and 0.5 cm, respectively. Waist circumference was measured midway between the spina iliaca superior and the lower rib margin, and hip circumference, at the level of the greater trochanters. Both were measured to the nearest 0.5 cm (21). Whole body composition was determined after an overnight fast using dual-energy x-ray absorptiometry (DEXA Prodigy; GE Lunar Corp., Madison, WI).

Blood samples. Ten-hour fasting blood samples were collected by venipuncture after 10 min of supine rest. Plasma glucose was determined using a Konelab 20 XT (Thermo Fisher Scientific, Vantaa, Finland), and serum insulin, with an IMMULITE® 1000 Analyzer (Siemens Medical Solution

Diagnostics, Los Angeles, CA). The homeostatic model assessment (HOMA) index was calculated using the following formula: (fasting plasma glucose × fasting plasma insulin)/22.5 (23). After drawing the fasting blood samples, an oral glucose tolerance test was performed with a glucose load of 75 g (GlucosePro; Comed LLC, Tampere, Finland) and blood samples were taken at 30 min, 1 h, and 2 h. Plasma glucose and insulin were determined from the samples, as described previously. The Matsuda index (22) (insulin sensitivity index) was calculated according to the Web-based calculator at http://mmatsuda.diabetes-smc.jp/MIndex.html.

Brain magnetic resonance imaging and voxel-based morphometry preprocessing. Participant brain scans were acquired using a 1.5-T whole body magnetic resonance (MR) scanner (Siemens Symphony; Siemens Medical Systems, Erlangen, Germany). The three-dimensional T1-weighted MPRAGE images of whole brain were taken with the following parameters: TR, 2180 ms; TE, 3.45 ms; TI, 1100 ms; flip angle, 15°; slice thickness, 1.0 mm; in-plane resolution, 1.0 mm × 1.0 mm; and matrix size, 256 × 256. Nine pairs had complete MR images (one pair was excluded for excessive artifacts from dental work). Three participants were left-handed, and their MR images were axially flipped to create a database in which all participants had their dominant hemisphere on the left. Therefore, the voxel-based morphometry (VBM) results reported here reflect differences in gray matter (GM) volume on either the dominant or nondominant hemisphere, not on the right or left hemisphere. VBM analyses were performed with VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) for SPM8 (Wellcome Trust Center for Neuroimaging, University College London, United Kingdom) running under MATLAB R2010a (MathWorks Inc., Natick, MA). First, the MR images were segmented into GM, white matter (WM), and cerebrospinal fluid. Images were then normalized to the Montreal Neurological Institute brain template using a high-dimensional DARTEL algorithm. Nonlinearly modulated GM images were created to preserve relative differences in regional GM volume. Finally, the GM volumes were spatially smoothed with 12-mm full width at half-maximum Gaussian kernel. Total intracranial volume was calculated for each individual from the segmentation maps to be used as a covariate in statistical analysis.

Ethical Approval

This study was conducted according to good clinical and scientific practice/guidelines and the Declaration of Helsinki. The ethics committee of the Central Finland Health Care District approved the study plan on September 29, 2011, and all participants gave their written informed consent.

Statistical Analysis

Data analyses were carried out as pairwise analyses comparing inactive versus active members of twin pairs discordant for physical activity. The normality of the variables was assessed by the Shapiro–Wilk test. In the pairwise comparison, student's paired *t*-test was used for normally distributed variables and the Wilcoxon matched-pair signed rank test, for nonnormally distributed variables. Effect sizes for the motives for leisure time physical activity were calculated as Cohen *d*, which illustrates the strength of the phenomenon (means divided by the SD). The 95% confidence intervals were calculated for the absolute mean differences between the inactive versus active co-twins. The level of significance was set at $P < 0.05$. Data were analyzed using IBM SPSS Statistics 19 and StataIC 12 software.

Brain VBM analysis. The GM volume of the active twin was compared with that of the inactive co-twin using a paired *t*-test, with total intracranial volume included in the model as a covariate. A statistical threshold of $P < 0.001$ (uncorrected) with a minimum cluster size of 15 voxels was used in the analysis.

RESULTS

The characteristics of our twin participants are shown in Table 1, and the intrapair differences, in Table 2. By definition, the past 3-yr-LTMET index, the 12-month-LTMET index, and Baecke sport index, all three of which characterize leisure time physical activity level, differed between the members of the twin pairs discordant for physical activity (Table 2). According to our retrospective interviews covering year by year the time 1–6 yr before the outcome measurements, there was a pairwise difference in leisure time physical activity during past 3 yr, but no difference was seen 4–6 yr before the examinations. Among these pairs, there was no pairwise difference in leisure time physical activity according to the questionnaire data collected from the cohort at the mean age of 24.5 yr or during their late adolescence, on the

TABLE 1. Characteristics of the male MZ twin pairs discordant for physical activity (10 male MZ twin pairs, 20 individuals).

	Mean ± SD	Minimum	Maximum
Age (yr)	33.9 ± 1.3	32	36
3-yr-LTMET index ($MET \cdot h \cdot d^{-1}$)	3.4 ± 2.7	0.2	10.4
12-month-LTMET index ($MET \cdot h \cdot d^{-1}$)[a]	2.6 ± 1.8	0.1	6.7
Baecke total index	7.8 ± 1.2	5.6	9.8
Baecke sport index	2.7 ± 0.6	1.8	4.0
Exercise enjoyment (five-point Likert scale)	4.1 ± 0.5	3.1	4.9
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)[b]	40.3 ± 4.9	31.6	49.1
Leg extension strength (N)	605 ± 128	430	865
Height (cm)	179.4 ± 5.2	173.0	188.0
Weight (kg)	76.8 ± 10.5	59.2	95.9
Body mass index ($kg \cdot m^{-2}$)	23.8 ± 2.6	19.8	30.1
Waist circumference (cm)	87.0 ± 7.3	74.5	102.0
Waist-to-hip ratio	0.90 ± 0.05	0.82	0.99
Percent fat	22.4 ± 4.5	14.1	32.0
Fat mass (kg)	17.6 ± 5.8	10.3	30.8
Lean mass (kg)	56.2 ± 5.4	46.1	63.6
Systolic blood pressure (mm Hg)	115 ± 10	102	140
Diastolic blood pressure (mm Hg)	68 ± 9	50	80
Fasting glucose (mM)	5.3 ± 0.4	4.7	5.9
Fasting insulin (μU)	3.8 ± 2.2	0.2	8.5
Matsuda index (n = 44)	15.1 ± 14.2	3.7	51.5
HOMA index	0.9 ± 0.6	0.1	2.2

[a]Excludes daily activities.
[b]One active twin did not participate, and for one inactive twin, maximal oxygen uptake was extrapolated on the basis of his submaximal test.

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TABLE 2. Intrapair differences in male MZ twin pairs discordant for physical activity.

Characteristics	Inactive (n = 10)	Active (n = 10)	Mean Difference (95% CI)	P Value
Age (yr)	34 (range, 32 to 36)			
Physical activity				
3-yr-LTMET index (MET·h·d^{-1})	1.7 ± 1.3	5.0 ± 2.7	3.3 (1.9 to 4.8)	0.001
12-month-LTMET index (MET·h·d^{-1})[a]	1.2 ± 0.9	3.9 ± 1.2	2.8 (2.0 to 3.5)	<0.001
Baecke total index	7.2 ± 1.2	8.4 ± 0.9	1.2 (0.6 to 1.9)	0.002
Baecke sport index	2.2 ± 0.4	3.1 ± 0.4	0.9 (0.4 to 1.3)	0.005
Exercise enjoyment (five-point Likert scale)	3.9 ± 0.4	4.2 ± 0.6	0.3 (−0.02 to 0.6)	0.060
Physical fitness				
$\dot{V}O_{2max}$ (mL·kg^{-1}·min^{-1}) (n = 9 pairs)[b]	37.3 ± 3.5	43.6 ± 4.2	6.3 (4.1 to 8.5)	<0.001
Leg extension force (N)	591 ± 146	619 ± 114	28 (−43 to 98)	0.65
Body composition				
Body height (cm)	179.1 ± 5.2	179.8 ± 5.4	0.7 (−0.5 to 1.8)	0.21
Body weight (kg)	77.8 ± 12.7	75.8 ± 8.5	−2.0 (−6.9 to 2.9)	0.38
Body mass index (kg·m^{-2})	24.2 ± 3.3	23.4 ± 1.7	−0.8 (−2.3 to 0.8)	0.28
Waist circumference (cm)	88.6 ± 8.2	85.3 ± 6.2	−3.3 (−7.4 to 0.8)	0.099
Waist-to-hip ratio	0.91 ± 0.05	0.89 ± 0.04	−0.02 (−0.04 to −0.003)	0.027
Percent fat	24.0 ± 4.6	20.7 ± 4.0	−3.3 (−6.2 to −0.4)	0.029
Fat mass (kg)	19.2 ± 6.6	16.0 ± 4.5	−3.3 (−6.7 to 0.2)	0.059
Lean mass (kg)	55.5 ± 6.1	56.9 ± 4.8	1.4 (−0.3 to 3.0)	0.094
Glucose homeostasis				
Fasting plasma glucose (mM)	5.3 ± 0.4	5.2 ± 0.3	−0.01 (−0.2 to 0.2)	0.92
Fasting plasma insulin (μU)	4.5 ± 1.7	3.2 ± 2.6	−1.3 (−2.6 to −0.1)	0.042
Matsuda index	8.6 ± 2.2	21.7 ± 18.1	13.1 (−0.6 to 26.9)	0.021
HOMA index	1.1 ± 0.5	0.8 ± 0.7	−0.3 (−0.6 to −0.03)	0.031

[a]Excludes daily activities.
[b]One active twin did not participate, and for one inactive twin, maximal oxygen uptake was extrapolated on the basis of his submaximal test.
CI, confidence interval; MET, metabolic equivalent.

basis of questionnaire data from ages 16 to 18.5 yr (see Table, Supplemental Digital Content 2, Intrapair differences in leisure time physical activity among FITFATTWIN study participants, http://links.lww.com/MSS/A424). This means that we investigated the effects of physical activity differences during the 3-yr period before outcome measurements.

As expected, active twins had higher cardiorespiratory fitness (P < 0.001) compared with their inactive co-twins. Active twins tended to have higher exercise enjoyment (P < 0.06), with a moderate effect size (Cohen d = 0.75) compared with their inactive co-twins (Table 2). To establish more personal reasons for engaging or not engaging in leisure time physical activity, the co-twins were asked to describe in their own words their reasons for their physical activity behaviors. Six of the inactive co-twins reported that work and/or family commitments were the primary reasons for physical inactivity.

The active twins had a lower body fat percent (P = 0.029) compared with inactive co-twins, but there was no pairwise difference in lean mass (Table 2). The Matsuda index was higher (P = 0.021) and the HOMA index was lower (P = 0.031) among active twins compared with those among their inactive co-twins, indicating better insulin sensitivity/lower insulin resistance among the more active individuals (Table 2).

Segmentation of brain MR images revealed that total GM, WM, and cerebrospinal fluid volumes were similar between co-twins (P > 0.60 for all comparisons). However, the VBM analysis indicated regional GM volume differences in the nondominant striatum and prefrontal cortex between active and inactive members of the pairs. Specifically, the putamen (peak voxel coordinates 18, 6, −6; peak T = 8.8; 395 voxels in cluster) in the nondominant hemisphere showed larger GM volume in the active twins compared with their inactive co-twins (Fig. 2). In addition, nondominant prefrontal cortex (subgyral and inferior frontal gyrus (IFG), peak voxel coordinates 34.5, 33, 18; peak T = 6.6; 99 voxels in cluster) showed larger GM volume in active members than that in inactive members of the pair (Figs. 2 and 3).

DISCUSSION

Our results show that physical fitness and glucose homeostasis differed between the members of the MZ twin pairs discordant for physical activity, supporting the argument for a causal association between physical activity and risk factor profile in healthy young adult men. Interestingly, in MZ twins with a high degree of similarity in brain structure (37), we observed specific modulation in GM in the striatum and frontal cortex on the nondominant hemisphere associated with physical activity. The active member of the twin pair had larger striatal GM volume; furthermore, the nondominant prefrontal cortex in the subgyral and IFG had larger GM volume. Because in this age group, total cortical GM and WM volumes are typically stable, our finding provides evidence for the structural effects of long-term physical activity on the healthy adult brain.

Identifying MZ co-twins who have long-term discordance in their physical activity habits is challenging because participation in physical activity has a rather high heritability (14,24). In our comprehensive screening of five consecutive age cohorts of twins in Finland, we identified 10 male twin pairs who fulfilled our criteria for discordance in physical activity, which included differential participation in physical activity between the co-twins during the past 3 yr. Members of MZ twin pairs usually have rather similar health habits because they are reared together at home and differences

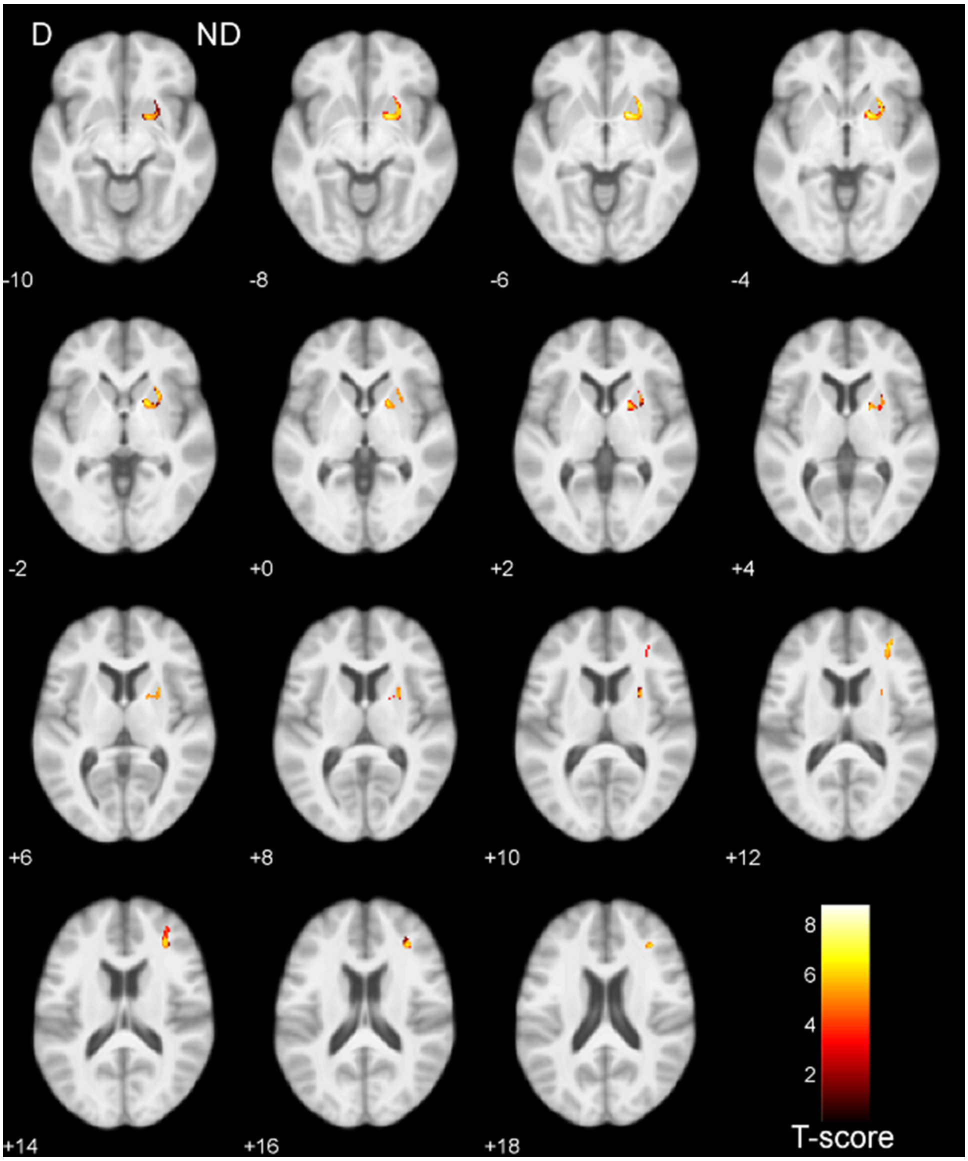

FIGURE 2—Axial MRI slices extending from the inferior tip of putamen (−10) to the superior tip of the caudate nucleus (+18), illustrating increased GM volume in *yellow* and *red* in the nondominant hemisphere of active versus inactive members of twin pairs (nine pairs). D, dominant hemisphere; ND, nondominant hemisphere.

arise mostly after they have moved out of the parental home for study or work. Among the physical activity-discordant twin pairs, the most commonly reported reason for being physically inactive given by the inactive members were work- or family-related commitments.

As expected, physical activity was associated with increased cardiorespiratory fitness in our pairwise analysis, indicating causality between physical exercise and fitness. Similar associations were not found for maximum muscular strength or power possibly because our participants usually reported participation in aerobic sports. Over the long term, the finding of increased aerobic fitness among physically active individuals has clinical significance because low cardiorespiratory fitness is a quantitative predictor of all-cause mortality (12).

For body composition, our results accord with those of our previous studies (19) on older twin pairs highly discordant for physical activity over a long period, where the inactive twins had only slightly higher body weight but markedly higher body fat percent and body fat mass than their active co-twins.

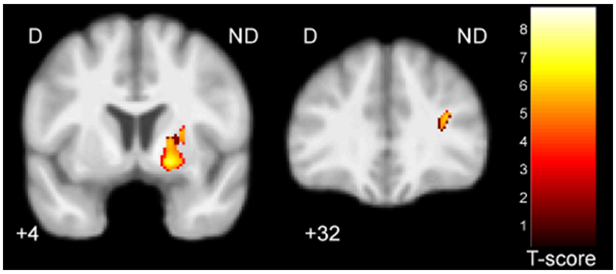

FIGURE 3—Coronal MRI slices demonstrating the extent of significantly differing GM volumes in the striatum in the nondominant hemisphere (+4) and in the subgyral prefrontal region in the nondominant hemisphere (+32). The same regions are shown in axial slices in Figure 2. D, dominant hemisphere; ND, nondominant hemisphere.

This result also is in line with the results of intervention studies showing that aerobic exercise leads to visceral fat reduction in a dose–response manner (26).

Interestingly, the young co-twins discordant for physical activity already had differences in their insulin resistance/sensitivity, as measured by both a steady-state (fasting/HOMA) index and dynamic (Matsuda) index. This finding is evidence for a reduced risk for type 2 diabetes in later life. In addition, it is in line with results of a randomized controlled trial showing that exercise can prevent the occurrence of type 2 diabetes among people with impaired glucose tolerance (28) and with our previous twin study showing lower risk for type 2 diabetes among physically active members of MZ twin pairs compared with that among their inactive co-twins (39).

A novel aim of the present study was to analyze brain morphology in young adult twin pairs discordant for physical activity. The voxel-wise whole brain analysis revealed a surprisingly extensive difference in the volume of GM between the members of the pairs in the striatum in the nondominant hemisphere and a somewhat smaller area in the IFG, also in the nondominant hemisphere, in favor of those with physically active lifestyle. Reduced basal ganglia volume was associated with metabolic syndrome in a recent study by Onyewuenyi et al. (27), who analyzed GM region of interest volumes of basal ganglia in participants of similar age to that of our group. A reduced basal ganglia volume (specifically pallidal) was associated with greater odds having metabolic syndrome (27). In addition, a large study on elderly persons showed that their walking speed decreased progressively with the decreasing volume of the basal ganglia (8). Various parts of the basal ganglia are heavily involved with motor control networks and with networks involved in frontal and prefrontal association areas and limbic networks (25). As for the healthy elderly general population, 6 months of aerobic exercise intervention increased GM volume in the anterior cingulate and supplementary motor area (SMA) as well as in the right IFG (7). Increased GM volume in the SMA and other frontal cortex regions as well as in the hippocampus has been associated with various aerobic sport activities when compared with sedentary persons or nonaerobic athletes (9, 34). Our present analysis also implicated nondominant IFG as a potential brain region to benefit from long-term physical activity. IFG is heavily connected with SMA, an important region for planning and initiating motor actions, thus being an important node in the cognitive–motor network. Recent extensive analysis of IFG functions revealed four functional clusters, three in the dominant hemisphere involved with language, memory, and emotion and one in the nondominant hemisphere involved with fine movement control. This area is known to have broad anatomical connections to visual and limbic areas, establishing its role in the cognitive–motor network (20). Effects of increased use of motor planning and execution were observed in the present GM volumes. Our young healthy twins did not show GM differences in areas connected to memory performance, such as hippocampi. It is noteworthy that the association between aerobic fitness and larger hippocampal volumes has been shown in elderly adults with diverse backgrounds (9), and it is possible that our twins' hippocampus-mediated memory functions were at a generally healthy level with absence of detectable pathology. Thus, we could not detect changes affected by exercise, or the pairwise differences were so small that they did not reach statistical power high enough to be detected in our analyses. Increased GM volumes presumably reflect the capability of the structures in question to modulate their function, e.g., to enhance local dendritic complexity. It is assumed that neuroplasticity, well known in animal and human pathological studies, is the mechanism behind the increased GM volumes. The overall interpretation is that the capacity of the brain to coordinate motor activities and the necessary associative and cognitive functions in the frontal cortex are improved.

The limitations of our study include the low number of twin pairs discordant for physical activity despite our nationwide search. Because of this low number, we had to use a relatively low statistical threshold in the VBM analysis (uncorrected $P < 0.001$). On the other hand, focusing on young healthy adult males helped us avoid bias arising from effects of sex differences, chronic diseases, degenerative changes, or medications. There was no pairwise difference in the occupational physical loading or in the daily activities among the studied pairs. There was one smoker in the inactive and the active co-twin groups, respectively, and the active compared with the inactive members of the twin pairs did not have statistically significant differences in their diet according to a food frequency questionnaire (results on diet will be reported in more detail elsewhere). Therefore, it is unlikely that smoking or dietary differences explain our findings. With respect to the comparability of our sample with the general population (generalizability), we compared the participants of this study with the other men from the FinnTwin16 Cohort, who participated in the Web-based questionnaire survey at the mean age of 34 yr (32) (see Table, Supplemental Digital Content 3, Characteristics of FITFATTWIN participants and all other men from FinnTwin16 cohort, http://links.lww.com/MSS/A425). Participants of the current study had somewhat lower BMI and mean physical activity level but otherwise rather similar subject characteristics compared with that of the other men in the cohort. The generalizability of the results to women needs further research. Our future analyses on metabolomics and properties of skeletal muscle and fat tissues will increase our understanding of the complicated underlying mechanisms, some of which have already been investigated among older twins (16,18). Our FITFATTWIN study also includes physical activity-concordant twin pairs (not included in this report), allowing us to study different associations using a larger population.

CONCLUSIONS

In healthy adult male twins, the level of leisure time physical activity is at a young age already associated with factors known to be related to reduced cardiometabolic risk. A significantly larger striatal GM volume in active twins indicates structural modulation of the brain GM as a result of long-term physical activity. When studying the effects of physical activity on health, the multidimensional influences should be considered in addition to specific single variables.

We thank Professor James A. Timmons for his comments on the manuscript.

The FITFATTWIN study was supported by the Finnish Ministry of Education and Culture (UMK), META-PREDICT (within the European Union Seventh Framework Program, HEALTH-F2-2012-277936 to U. M. K.), Juho Vainio Foundation (M. R.), and Finnish Cultural Foundation (M. R.). The data collection of the FT16 study was supported by the National Institute of Alcohol Abuse and Alcoholism (grants AA-12502, AA-00145, and AA-09203 to R. J. Rose) and the Academy of Finland (grants 100499, 205585, 118555, 141054, and 264146 to J. K.).

The authors apologize for not being able to cite all the outstanding work in this area because of constraints on space.

The authors declare that they have no competing interests.

M. R., T. L., and U. M. K. contributed equally to this article.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

1. Aaltonen S. Leisure-time physical activity in a Finnish twin study. Genetic and environmental influences as determinants and motives as correlates. Thesis. University of Jyväskylä (2013); Available from: http://urn.fi/URN:ISBN:978-951-39-5326-3.
2. Ainsworth BE, Haskell WL, Herrmann SD, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc*. 2011;43(8):1575–81.
3. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of Physical Activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc*. 2000;32(9 Suppl):S498–516.
4. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr*. 1982;36:936–42.
5. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. *Compr Physiol*. 2012;2:1143–211.
6. Bouchard C, Dionne FT, Simoneau JA, Boulay MR. Genetics of aerobic and anaerobic performances. *Exerc Sport Sci Rev*. 1992; 20:27–58.
7. Colcombe SJ, Erickson KI, Scalf PE, et al. Aerobic exercise training increases brain volume in aging humans. *J Gerontol A Biol Sci Med Sci*. 2006;61:1166–70.
8. Dumurgier J, Crivello F, Mazoyer B, et al. MRI atrophy of the caudate nucleus and slower walking speed in the elderly. *Neuroimage*. 2012;60:871–8.
9. Eriksson KI, Prakash RS, Vos MW, et al. Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus*. 2009;19:1030–9.
10. Kaprio J, Pulkkinen L, Rose RJ. Genetic and environmental factors in health-related behaviors: studies on Finnish twins and twin families. *Twin Res*. 2002;5:366–71.
11. Killgore WD, Olson EA, Weber M. Physical exercise habits correlate with gray matter volume of the hippocampus in healthy adult humans. *Sci Rep*. 2013;3:3457.
12. Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA*. 2009; 301:2024–35.
13. Kujala U. Physical activity, genes, and lifetime predisposition to chronic disease. *Eur Rev Aging Phys Act*. 2011;8:31–6.
14. Kujala UM, Kaprio J, Koskenvuo M. Modifiable risk factors as predictors of all-cause mortality: the roles of genetics and childhood environment. *Am J Epidemiol*. 2002;156:985–93.
15. Kujala UM, Kaprio J, Sarna S, Koskenvuo M. Relationship of leisure-time physical activity and mortality: the Finnish twin cohort. *JAMA*. 1998;279:440–4.
16. Kujala UM, Mäkinen V-P, Heinonen I, et al. Long-term leisure-time physical activity and serum metabolome. *Circulation*. 2013; 127:340–8.
17. Lakka TA, Salonen JT. The physical activity questionnaires of the Kuopio Ischemic Heart Disease Study (KIHD). A collection of physical activity questionnaires for health-related reseach. *Med Sci Sports Exerc*. 1997;29:S46–58.
18. Leskinen T, Rinnankoski-Tuikka R, Rintala M, et al. Differences in muscle and adipose tissue gene expression and cardio-metabolic risk factors in the members of physical activity discordant twin pairs. *PLoS One*. 2010;5:e12609. doi: 10.1371/journal.pone.0012609.
19. Leskinen T, Sipilä S, Alen M, et al. Leisure time physical activity and high-risk fat: a longitudinal population-based twin study. *Int J Obes (Lond)*. 2009;33:1211–8.
20. Liakakis G, Nickel J, Seitz RJ. Diversity of the inferior frontal gyrus—a meta-analysis of neuroimaging studies. *Behav Brain Res*. 2011;225:341–7.
21. Marti B, Tuomilehto J, Salomaa V, Kartovaara L, Korhonen HJ, Pietinen P. Body fat distribution in the Finnish population: environmental determinants and predictive power for cardiovascular risk factor levels. *J Epidemiol Community Health*. 1991;45:131–7.
22. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999;22:1462–70.
23. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab*. 2008;294:E15–26.
24. Mustelin L, Joutsi J, Latvala A, Pietiläinen KH, Rissanen A, Kaprio J. Genetic influences on physical activity in young adults: a twin study. *Med Sci Sports Exerc*. 2012;44(7):1293–301.
25. Noback CR, Strominger NL, Demarest RJ, Ruggiero DA. *The Human Nervous System. Structure and Function*. 6th ed. Totova (NJ): Humana Press; 2005. p. 477.
26. Ohkawara K, Tanaka S, Miyachi M, Ishikawa-Takata K, Tabata I. A dose–response relation between aerobic exercise and visceral fat reduction: systematic review of clinical trials. *Int J Obes (Lond)*. 2007;31:1786–97.
27. Onyewuenyi IC, Muldoon FM, Christie IC, Erickson KI, Gianaros PJ. Basal ganglia morphology links the metabolic syndrome and depressive symptoms. *Physiol Behav*. 2014;123:214–22.
28. Pan XR, Li GW, Hu YH, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care*. 1997;20: 537–44.
29. Physical Activity Guidelines Advisory Committee. *Physical Activity Guidelines Advisory Committee Report, 2008*. Washington (DC): US Department of Health and Human Services; 2008.
30. Reiner M, Niermann C, Jekauc D, Woll A. Long-term health benefits of physical activity—a systematic review of longitudinal studies. *BMC Public Health*. 2013;13:813.
31. Rogers H, Morris T. An overview of the development and validation of the Recreational Exercise Motivation Measure (REMM). In: *XI the European Congress of Sport Psychology Proceedings Book*. Copenhagen, Denmark: University of Copenhagen; 2003:144.

32. Rottensteiner M, Pietiläinen KH, Kaprio J, Kujala UM. Persistence or change in leisure-time physical activity habits and waist gain during early adulthood: a twin-study. *Obesity*. 2014;22(9):2061–70.
33. Sarna S, Kaprio J, Sistonen P, Koskenvuo M. Diagnosis of twin zygosity by mailed questionnaire. *Hum Hered*. 1978;28:241–54.
34. Schlaffke L, Lissek S, Lenz M, et al. Sports and brain morphology—a voxel-based morphometry study with endurance athletes and martial artists. *Neuroscience*. 2014;259:35–42.
35. Sipilä S, Multanen J, Kallinen M, Era P, Suominen H. Effects of strength and endurance training on isometric muscle strength and walking speed in elderly women. *Acta Physiol Scand*. 1996;156:457–64.
36. Stubbe JH, Boomsma DI, Vink JM, et al. Genetic influences on exercise participation in 37.051 twin pairs from seven countries. *PLoS One*. 2006;1(1):e22.
37. Tramo MJ, Loftus WC, Stukel TA, Green RL, Weaver JB, Gazzaniga MS. Brain size, head size, and intelligence quotient in monozygotic twins. *Neurology*. 1998;50:1246–52.
38. Waller K, Kaprio J, Kujala UM. Associations between long-term physical activity, waist circumference and weight gain: a 30-year longitudinal twin study. *Int J Obes (Lond)*. 2008;32:353–61.
39. Waller K, Kaprio J, Lehtovirta M, Silventoinen K, Koskenvuo M, Kujala UM. Leisure-time physical activity and type 2 diabetes during a 28-year follow-up in twins. *Diabetologia*. 2010;53:2531–7.