Supporting information

Supplementary methods

Identification of the MZ twin pairs discordant for leisure-time physical activity (LTPA)

TWINACTIVE study

The TWINACTIVE study included both MZ and DZ pairs discordant for LTPA. As this paper focuses on findings seen in MZ pairs this supplementary information focuses on the procedures related to the identification of the 7 MZ pairs for TWINACTIVE study. More details with flowcharts are available in Leskinen et al. (2009).

Baseline LTPA Questionnaires: The Finnish Twin Cohort includes same-sex twin pairs born in Finland before 1958 and with both co-twins alive in 1975 (Kaprio & Koskenvuo 2002; Kaprio et al. 2019). In the cohort, there were 1772 MZ, 3551 DZ and 340 twin pairs with unknown zygosity composing the cohort who were 24 to 60 years old, employed and healthy in 1981 (Kujala et al. 2002). They all had completed the LTPA questionnaire administered in 1975 and in 1981. Assessment of LTPA volume (leisure time MET index) was based on a series of structured questions on leisure activity and physical activity during journeys to and from work (Kujala et al. 1998). The leisure time MET index was then calculated by assigning a multiple of the resting metabolic rate to each form of physical activity (intensity of activity x duration of one session x monthly frequency), which was then expressed as a sum score of leisure time MET hours/day. Assessment of the intensity of activity was based on the following question: Is your physical activity during leisure time about as strenuous on average as: (1) walking, (2) alternately walking and jogging, (3) jogging, (4) running? Those who chose 2, 3 or 4 were classified as engaging in vigorous activity (Kujala et al. 1998). After calculating the leisure time MET indices, we found that 29 MZ pairs were discordant for LTPA both in participation in vigorous activity and in volume of activity both in 1975 and 1981. The criterion for the baseline discordance was that one co-twin was physically active (calculated leisure time MET index was > 2 MET h/day corresponding to about 30 min walking per day) while his/her co-twin was less active (leisure time MET index < 2 MET h/day) in both assessments (Kujala et al. 2002; Waller et al. 2008). To increase the number of LTPA discordant MZ twin pairs, we set up another selection criterion and found that 151 MZ pairs were discordant in volume of activity (2 MET criteria as described above, vigorous activity discordance ignored). Among these MZ pairs, we selected only those 19 MZ pairs whose difference in volume of activity was > 3 MET h/day between the inactive and active co-twin in both 1975 and 1981, while the average intensity of a physical activity session was the same or greater in the active vs. inactive co-twin. This resulted in 48 comprehensively selected MZ twin pairs with baseline discordance for LTPA. These twin pairs constituted a target group for our follow-up interview on LTPA habits. As our aim was to investigate the health effects of physical activity in twin pairs with long-term persistent discordance for LTPA, pairs not persistently discordant for LTPA were excluded during later stages of the study (described later).

Follow-up LTPA 1980–2005 interview: Follow-up interviews were carried out during the years 2005–2007 with 40 MZ twin pairs as only those pairs were included in which both co-twins were still alive, lived in Finland, and spoke Finnish as their mother tongue. In total 33 MZ twin pairs completed the physical activity assessment section in the follow-up interviews. The telephone interview included questions on current and past physical activity (for more details see Waller et al. 2008). In brief, physical activity was assessed by two sets of questions. The first shorter

retrospective timeline assessment of LTPA volume and intensity (with 5-year intervals) was carried out using the same physical activity questions as in 1975 and 1981. The mean MET index for all six time points from 1980 to 2005 was then calculated for both the inactive and active co-twins. To aid recall, twins were asked to describe their marital and work status for each year before the retrospective LTPA questions. The second set of questions was a detailed assessment of the volume of leisure time, daily (non-exercise activities such as gardening) and commuting activity over the previous 12 months (12-month MET index) using a modified version of the Kuopio Ischemic Heart Disease Risk Factor Study Questionnaire (Lakka & Salonen 1997). As a result of these physical activity assessments, we found 17 MZ twin pairs who, in addition to baseline discordance, were discordant for LTPA habits throughout the follow-up (i.e., 30 years).
Follow-up laboratory interview and measurements in 2007: Before issuing an invitation to participate in the laboratory study measurements, we excluded pairs whose health status/medication would severely violate our study aims. Pairs were excluded for the following reasons: one co-twin had malign cancer (2 MZ pairs), oral corticosteroid treatment for rheumatoid arthritis (1 MZ pair), severe disability/high age (1 MZ pair) and insufficient collaboration (1 MZ pair). This procedure left us with 12 MZ twin pairs. Of these pairs who were invited to the laboratory, 8 MZ twin pairs underwent our detailed health-related examinations.

Physical activity assessments at follow-up and the final number of MZ twin pairs long-term discordant for LTPA: In the follow-up measurements, LTPA volume (leisure time MET index 2007) and participation in vigorous physical activity were assessed by the same questions as used at the baseline and in the retrospective assessment. Detailed assessment of the volume of leisure time and of daily and commuting activity over the previous 12 months (total 12-month MET index 2007) was also carried out (Lakka & Salonen 1997; Ainworth et al. 2000; Ainsworth et al. 2011). After careful intrapair examination of the leisure time MET indices from 1975 to 2007, we found that 7 MZ (5 male and 2 female pairs) fulfilled our discordance criterion. In one MZ pair the leisure activity MET indices were higher among the previously inactive co-twin and lower among his previously active co-twin at the last follow-up assessment. This pair was excluded from the data analysis.

Physical activity discordance in the identified 7 MZ LTPA discordant MZ pairs: Physical activity discordance showed a decreasing trend with time during the retrospective follow-up (1980–2005). In 2007, the leisure time MET index was on average lower (–7.0 MET h/day, 95% CI –11.4 to –2.6, $p = .018$) among inactive MZ co-twins compared to their active co-twins. During the LTPA follow-up period, from 1980 to 2007, the inactive co-twins were less active than their active co-twins. When we assessed the volume of leisure time, daily and commuting activity over the previous 12 months (Lakka & Salonen 1997), significant differences between inactive and active co-twins were found in total and leisure time physical activities but not in daily and commuting activities. The two questionnaires on the volume of LTPA (the retrospective timeline vs. previous 12-month assessment) showed a good correlation ($r = 0.73$, $p < .001$).

FITFATTWIN study

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 and in more detail with flow-charts in Rottensteiner et al. (2015).

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 (Kaprio et al. 2002; Kaidesoja et al, 2019). 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 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 LTPA. We identified potential participants for the FITFATTWIN study by screening and including the pairs with the highest discordance in their leisure time physical activity. Specifically, the difference in physical activity between the co-twins of a twin pair was assessed on the basis of frequency of LTPA, 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 (preliminary inclusion criterion 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 (preliminary inclusion criterion 2). 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 preliminary inclusion criterion 1 and 13 pairs fulfilled preliminary 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 (Kujala et al. 1998). 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). Finally, 17 male MZ pairs (10 pairs meeting preliminary inclusion criterion 1 and seven pairs meeting preliminary inclusion criterion 2) accepted the invitation to participate in the study and went through our comprehensive clinical study measurements and detailed physical activity interviews.

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 LTPA. These 10 pairs met the following five criteria set for maximal LTPA discordance.

1. Inclusion based on preliminary criterion 1 or 2, given previously.
2. A pairwise difference of ≥1.5 $MET \cdot h \cdot d^{-1}$ between active and inactive co-twins in LTPA (including work journey activity), according to the 12-month physical activity interview (12-month-LTMET index; see later portion) (Lakka & Salonen 1997; Waller et al. 2008).
3. 12-month-LTMET index <5 $MET \cdot h \cdot d^{-1}$ for the inactive co-twin.
4. ≥1 $MET \cdot h \cdot d^{-1}$ pairwise difference between active and inactive co-twins in LTPA (including work journey activity) for the past 3 yr, according to the shorter physical activity interview

(3-yr-LTMET index; see later portion) (Kujala et al. 1998; Leskinen et al. 2009; Waller et al. 2008).

5. A higher Baecke sport index for the active versus the inactive co-twin (Baecke et al. 1982).

LTPA volume identification: The two different structured physical activity interviews were used to assess the volume of participant LTPA, including work journey activity. First, a shorter retrospective physical activity interview as also used in TWINACTIVE study (Kujala et al. 1998; Leskinen et al. 2009; Waller et al. 2008) was used to assess leisure time physical activity volume at 1-yr intervals over the past 6 yr. LTPA 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 LTPA 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 (non-exercise) activities, and work journey activity over the previous 12 months used a modified version of the Kuopio Ischemic Heart Disease Risk Factor Study Questionnaire (Lakka & Salonen 1997; Waller et al. 2008) which was slightly modified from what was used in TWINACTIVE study. 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. As in the TWINACTIVE study each self-rated physical activity intensity was converted into MET values (Lakka et al. 1997; Ainworth et al. 2000; Ainsworth et al. 2011). 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 LTPA 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 (Baecke et al. 1982). We then summed the three indexes (work, sport, and leisure time excluding sports) as proposed in the original article (Baecke et al. 1982). The sport index was used as a measure of vigorous physical activity.

By definition, the past 3-yr-LTMET index, the 12-month-LTMET index, and Baecke sport index, all three of which characterize LTPA level, differed between the members of the twin pairs discordant for physical activity. 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 basis of questionnaire data from ages 16 to 18.5 yr. This means that we investigated the effects of physical activity differences during the 3-yr period before outcome measurements.

There was no pairwise difference in the occupational physical loading or in the daily activities among the studied pairs.

References to supplementary methods

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Supplementary Table 1. Selected data from monozygotic twin pairs discordant for leisure time physical activity habits, TWINACTIVE and FITFATTWIN studies

Variable	FITFATTWIN (N=10 pairs 3+ -year discordant for LTPA, mean age 34, range 32-36 years)			TWINACTIVE (N=7 pairs 30+ -year discordant for LTPA; mean age 62, range 50-74 years)			Pooled data (N=17 pairs)		
	Less active co-twins	More active co-twins	Intrapair difference Mean (95% CI)	Less active co-twins	More active co-twins	Intrapair difference Mean (95% CI)	Less active co-twins	More active co-twins	Intrapair difference Mean (95% CI)
	Mean±SD			Mean±SD			Mean±SD		
VO_2peak, mlxkg^{-1}xmin^{-1}	37.3±3.5	43.6±4.2	6.3 (4.1 to 8.5)	27.4±5.3	32.2±6.0	4.8 (-0.9 to 10.5)	33.0±6.6	38.6±7.6	5.6 (3.3 to 8.0)
Leisure-time physical activity									
Past 3-year-LTMET index, MET-hours/day	1.7±1.3	5.0±2.7	3.3 (1.9 to 4.8)						
Past 30-year-LTMET index, MET-hours/day				3.0±2.6	11.2±4.9	8.2 (3.4 to 13.0)			
12-month LTMET index, MET-hours/day	1.2±0.9	3.9±1.2	2.8 (2.0 to 3.5)	3.2±2.2	9.2 ± 3.9	5.9 (2.1 to 9.7)	2.0±1.8	6.1±3.7	4.1 (2.5 to 5.6)
Body composition									
Body height, cm	179.1±5.2	179.8±5.4	0.7 (-0.5 to 1.8)	172.8±12.0	173.7±11.8	0.9 (0.2 to 2.1)	176.5±8.9	177.3±8.8	0.8 (0.1 to 1.5)
Body weight, kg	77.8±12.7	75.8±8.5	-2.0 (-6.9 to 2.6)	77.0±11.3	75.4±13.2	-1.52 (-8.9 to 5.8)	77.5±11.7	75.7±10.3	-1.8 (-5.5 to 1.8)
Body-mass index, kg/m^2	24.2±3.3	23.4±1.7	-0.8 (-2.3 to 0.8)	25.7 ± 1.5	24.8 ± 1.9	-0.9 (-3.4 to 1.7)	24.8±2.7	24.0±1.9	-0.8 (-2.0 to 0.4)
Waist circumference, cm	88.6±8.2	85.3±6.2	-3.3 (-7.4 to 0.8)	97.6±7.9	92.9±8.1	-4.7 (-10.8 to 1.4)	92.3±9.0	88.4±7.8	-3.9 (-6.9 to -0.8)
Fat percent, %	19.0±2.9	17.4±3.2	-1.6 (-3.7 to 0.6;)	27.9±5.6	22.9±4.7	-5.0 (-11.3 to 1.4)	22.6±6.0	19.7±4.7	-3.0 (-5.6 to -0.6)
Visceral adipose tissue area, cm^2	129±54	92±47	-37 (-66 to -8)	165±51	128±62	-37 (-89 to 16)	144±54	107±55	-37 (-61 to -13)
Subcutaneous adipose tissue area, cm^2	115±36	99±35	-16 (-42 to 11)	185±40	161±26	-25 (-63 to 13)	144±51	125±44	-19 (-39 to -0.2)
Visceral per subcutaneous adipose tissue ratio	1.12±0.33	0.93±0.33	-0.19 (-0.38 to -0.00)	0.92±0.33	0.81±0.38	-0.11 (-0.39 to 0.17)	1.04±0.33	0.88±0.35	-016 (-0.23 to -0.02)
Liver fat index, MRI signal intensity	7.3±3.9	7.3±4.0	-0.0 (-2.2 to 2.1)	26.1±21.6	6.0±8.2	-20.1 (-34.0 to 6.2)	15.1±16.6	6.8±5.9	-8.3 (-15.4 to -1.1)
Lipoproteins									
Apo-B, g/L	1.11±0.14	0.93±0.19	-0.18 (-0.40 to 0.04)	0.80±0.15	0.75±0.19	-0.05 (-0.12 to 0.02)	0.93±0.21	0.82±0.21	-0.11 (-0.20 to -0.02)
Apo-A1, g/L	1.81±0.28	1.82±0.27	0.02 (-0.06 to 0.09)	1.37±0.24	1.41±0.24	0.04 (-0.08 to 0.16)	1.55±0.33	1.58±0.32	0.03 (-0.04 to 0.10)
ApoB:ApoA1 ratio	0.63±0.17	0.52±0.17	-0.11 (-0.25 to 0.04)	0.59±0.14	0.53±0.13	-0.06 (-0.10 to -0.02)	0.61±0.13	0.53±0.03	-0.08 (-0.13 to -0.02)
HDL cholesterol, mol/L	1.66±0.39	1.82±0.45	0.15 (-0.03 to 0.34)	1.26±0.32	1.35±0.31	0.09 (-0.06 to 0.23)	1.43±0.10	1.54±0.11	0.11 (0.01 to 0.22)
HDL_2 cholesterol, mol/L	1.12±0.37	1.29±0.46	0.17 (-0.04 to 0.38)	0.80±0.28	0.87±0.30	0.08 (-0.05 to 0.20)	0.93±0.35	1.05±0.42	0.12 (0.02 to 0.22)
HDL diameter, nm	9.83±0.22	9.93±0.25	0.10 (-0.02 to 0.22)	9.65±0.22	9.76±0.21	0.11 (0.04 to 0.18)	9.72±0.23	9.83±0.24	0.11 (0.05 to 0.16)
Very large HDL particles, µmol/L	0.24±0.17	0.31±0.18	0.08 (-0.03 to 0.18)	0.19±0.11	0.25±0.15	0.06 (-0.01 to 0.12)	0.21±0.11	0.27±0.16	0.07 (0.02 to 0.11)
Large HDL particles, µmol/L	1.05±0.42	1.27±0.53	0.22 (-0.00 to 0.44)	0.63±0.37	0.77±0.37	0.14 (0.03 to 0.25)	0.80±0.43	0.98±0.50	0.17 (0.08 to 0.27)
Medium HDL particles, µmol/L	2.20±0.32	2.19±0.39	-0.00 (-0.03 to 0.02)	1.55±0.35	1.55±0.22	0.00 (-0.18 to 0.18)	1.82±0.47	1.82±0.43	-0.00 (-0.13 to 0.12)
Small HDL particles, µmol/L	4.96±0.23	4.88±3.20	-0.08 (-0.30 to 0.13)	4.41±0.23	4.31±0.26	-0.10 (-0.04 to 0.16)	4.64±0.36	4.55±0.39	-0.09 (-0.25 to 0.06)

Abbreviations: CI, confidence interval; LTMET-hours/day, leisure-time metabolic equivalent for physical activity during leisure-time and the commute to and from work indicating daily leisure-time physical activity volume; MRI, magnetic resonance imaging; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; HDL, high density lipoprotein.

Supplement 2. Links to PhD theses (Sports and Exercise Medicine, University of Jyväskylä) which include results from leisure-time physical activity discordant twin pairs

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