How voluntary exercise behavior runs in families

Twin studies and beyond

Charlotte Huppertz

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TABLE OF CONTENTS

Chapter **1**

GENERAL INTRODUCTION

Our forefathers were hunter-gatherers for more than 2 million years. Physical activity was an essential aspect of their daily lives, crucial for securing food supply and escaping from predators. Our bodies are well-adapted to this lifestyle. However, in the last 150 years or so, a - in evolutionary terms - sudden shift has occurred in that people worldwide are becoming less and less physically active despite joint efforts of researchers, practitioners and policy makers to counteract this development. The average human energy expenditure has decreased dramatically in modern civilizations where engines have replaced the majority of physically exertive labor, where walking is largely unnecessary due to the invention of motorized transportation, and where food can be accessed easily in many parts of the world. At the same time, our genetic architecture has barely changed over the past 50,000 years, and it appears not to be suited to deal with sedentary lifestyles (Cordain, Gotshall, Eaton & Eaton, 1998). In the past, communicable diseases were the major threat to the global population. Nowadays, diabetes, cancer and heart disease have clearly outpaced these. More than 36 million people die a year because of non-communicable diseases (http://www.who.int/mediacentre/factsheets/fs355/en/, accessed July 2015) and physical inactivity has been frequently cited to be a major reason for this alarming situation (Lee et al., 2012).

As a consequence, counteracting the spread of non-communicable diseases has become a global public health priority and leading health organizations and state departments have set up guidelines outlining physical activity levels that are beneficial for health (e.g., the *Global Recommendations on Physical Activity for Health* by the World Health Organization, 2010; the *EU Physical Activity Guidelines* by the EU Working Group "Sport and Health", 2008; the *Physical Activity Guidelines for Americans* by the U.S. Department of Health and Human Services, 2008). It is generally recommended that adults accumulate approximately 150 minutes of physical activity a week and children even more than that. Although knowledge on the benefits of regular physical activity has grown rapidly (Bortz, 1982; Janssen & Leblanc, 2010; Lee et al., 2012) and numerous interventions promoting physical activity have been implemented across various settings (Heath et al., 2012; Metcalf, Henley & Wilkin, 2012), a large proportion of the global population is not sufficiently active (Hallal et al., 2012).

In order to change physical activity patterns on a population level in the long run, a better understanding of the determinants of physical (non-)activity is of utmost importance. Much research has focused on environmental determinants such as socioeconomic status or the built environment. However,

even under identical circumstances, some individuals are more predisposed towards a physically active lifestyle than others. The aims of this thesis were 1) to better understand why some individuals choose to exercise on a regular basis whilst others do not and 2) to investigate the relationship between exercise behavior and two commonly studied covariates, attitudes and body mass index (BMI), using genetically informative designs.

REGULAR VOLUNTARY EXERCISE BEHAVIOR

This thesis focuses entirely on regular voluntary exercise behavior during leisure time, which is different from general physical activity. General physical activity is defined as "any bodily movement produced by skeletal muscles that requires energy expenditure", whereas exercise behavior is "a subcategory of physical activity that is planned, structured, repetitive, and purposeful in the sense that the improvement or maintenance of one or more components of physical fitness is the objective" (http://www.who.int/dietphysicalactivity/pa/en/, accessed July 2015). The reason for focusing on exercise behavior during leisure time is two-fold. First, in order to reliably study the causes of individual differences in exercise behavior with genetically informative designs, large amounts of population-based data are needed whose collection is currently only feasible using surveys. Since general physical activity behavior (e.g., the time spent walking, taking stairs etc.) cannot be reliably measured by self-report (Adamo, Prince, Tricco, Connor-Gorber & Tremblay, 2009; Prince et al., 2008), we preferred exercise behavior. As exercise activities that are done during leisure time are deliberately initiated and often clearly defined in time, self-reports on this behavior are much more accurate. Excellent test-retest reliability has been established in our own data (de Moor, Boomsma, Stubbe, Willemsen & de Geus, 2008; Stubbe, de Moor, Boomsma & de Geus, 2007). A detailed description of how we quantified exercise behavior can be found in Chapter 2. The second reason for focusing on regular exercise behavior in leisure time is that it represents a well-defined and efficient target for interventions. Moderate-to-vigorous activities have been shown to have the largest protective effect on mortality (Samitz, Egger & Zwahlen, 2011) and exercise activities are - for most individuals - the major source of bouts that have a sufficient intensity and duration to induce these effects. Cordain et al. (1998) even suggested that simply increasing walking might not be sufficient for optimizing health benefits as we are lacking too far behind the energy expenditure of our forefathers.

TWIN RESEARCH AND GENE FINDING STUDIES

Heritability of regular voluntary exercise behavior

To disentangle causes of individual differences in a complex trait like exercise behavior, genetically informative designs, such as twin and twin-sibling studies, are the solution. Twin (-sibling) studies aim to clarify the etiology of (behavioral) traits by decomposing phenotypic differences between individuals into 1) differences that are due to genetic factors and 2) differences that are due to environmental factors (Falconer & Mackay, 1996). To this end, the resemblance of monozygotic (MZ) twin pairs is compared to the resemblance of dizygotic (DZ) twin pairs. MZ twins originate from the same fertilized egg and are therefore (nearly) genetically identical, whereas DZ twins only share, on average, 50% of their segregating genes. A larger phenotypic resemblance (correlation) in MZ twin pairs compared to DZ twin pairs must be due to **A**dditive or **D**ominant genetic influences (called "**A**" and "**D**", respectively), under the assumption of equal environments for MZ and DZ twins as related to the studied phenotype. The remaining variance must be due to environmental influences - either environmental influences that the two twins of a pair share ("**C**ommon environment" or "**C**", e.g., growing up in the same family and neighborhood) or those environmental influences that they do not share ("unique **E**nvironment" or "**E**", e.g., twin-specific diseases or different peers). Shared environmental influences are reflected in DZ correlations that are larger than half the MZ correlation. Non-shared environmental influences (which include measurement error) can be inferred from MZ twin correlations that are smaller than one. Although phenotypic twin correlations provide a rough estimate of the relative contribution of A, C, D and E, genetic structural equation models are a more elegant and precise way to estimate the variance that is explained by each of the latent factors. The models furthermore enable sophisticated model fitting to describe the complexity of reality. Polderman et al. (2015) have recently summarized virtually all twin studies of the past 50 years.

Quite a few twin studies have investigated the relative contribution of genetic and environmental factors to exercise behavior in adolescents and adults (e.g., de Moor & de Geus, 2013; de Vilhena e Santos, Katzmarzyk, Seabra & Maia, 2012; Stubbe & de Geus, 2009; see Chapter 3 for a review), suggesting a major influence of genes during adolescence and approximately 40% of genetic variance and 60% of non-shared environmental variance in adulthood. My thesis enriches this literature by for the first time disentangling genetic and

environmental influences on differences in exercise behavior during leisure time in children (Chapter 3). Chapter 4 expands upon this study by including data of 14-, 16- and 18-year-old twins. Due to continuous data collection, it also includes a larger dataset for the younger ages and longitudinal data for a large part of the sample. A univariate model was fitted with age as moderator on the means and the variance components. Next, a simplex model was fitted to investigate the (in-)stability of genetic and environmental influences on exercise behavior over time.

It is important to note that genes do not act in isolation and that reality is more complex than summing the effects of genes and the environment. Heritability estimates can be dependent on environmental circumstances. Gene x environment interactions can be investigated by taking environmental covariates into account. In previous studies, it has been shown that the heritability of BMI depends on physical activity levels (Mustelin, Silventoinen, Pietilainen, Rissanen & Kaprio, 2009). Moderators of the genetic and environmental effects on exercise behavior have not been investigated before. In Chapter 5, we examine whether means and the variance (components) of children's and adolescents' exercise behavior vary by different levels of parental education.

Causality testing using twin data

Twin studies not only offer a quantitative solution to the nature-nurture debate, they also allow a better understanding of the true nature of an association between two (or more) phenotypes. More specifically, it is possible to falsify the hypothesis of causality between two traits by calculating genetic and environmental cross-trait correlations in multivariate models and by applying the MZ twin intrapair differences model. These designs have been applied to many phenotypes, amongst others to regular exercise behavior and its association with well-being (Bartels, de Moor, van der Aa, Boomsma & de Geus, 2012; Stubbe et al., 2007), self-rated health (de Moor, Stubbe, Boomsma & de Geus, 2007), symptoms of anxiety and depression (de Moor et al., 2008) and internalizing problems (Bartels et al., 2012). Exercise attitudes and BMI are two of the most intensively studied correlates of exercise behavior. We examine the nature of the correlation of attitudes and of BMI with exercise behavior in Chapters 6 and 7, respectively, and explicitly test the hypotheses that 1) attitudes causally influence exercise behavior and that 2) exercise behavior causally influences BMI.

Genomics of regular voluntary exercise behavior

In the past two decades, genetic studies have become less focused on phenotypic twin (/family) similarity and have been greatly strengthened by the possibility to also investigate genetic effects at the level of DNA. Mapping of the human genome and rapid technological advances (Barrett & Cardon, 2006; International HapMap Consortium, 2005) have made it possible to study the effects of specific genetic loci on virtually any trait. Linkage studies used to be particularly popular in this respect. They exploit the fact that alleles at loci that are closer together on the genome are more likely to be inherited together than loci that are further apart from each other, because recombination events are less likely to occur in the former case. Linkage studies investigate in how far a specific marker co-segregates in families with the trait under study (Ferreira, 2004; Haseman & Elston, 1972). A few linkage studies have been published on physical activity phenotypes (Cai et al., 2006; de Moor, Posthuma et al., 2007; Simonen, Rankinen, Perusse, Rice et al., 2003), without any reproducible hits.

A more direct approach to test the association of genetic variants with a phenotype are association studies. They compare the variation of phenotypes across groups of people with different genotypes for one or more specific genetic variant(s). Nowadays, genetic variants can be investigated across the whole genome, allowing a theory- and hypothesis-free, exploratory approach. Hundreds of thousands of genetic variants are tested simultaneously for their association with a phenotype, thereby covering most of the common genetic variation of the genome (Hirschhorn & Daly, 2005). The main challenge for these genome-wide association (GWA) studies is that due to the number of tests that are carried out, very small p-values have to be handled to correct for multiple testing. At the same time, a trait like exercise behavior is thought to be influenced by many genetic variants with very small effects. Therefore, very large samples are needed to be able to find significant associations. The only GWA study that has been performed on exercise behavior (de Moor et al., 2009) did not include the number of subjects that is now commonly thought to be required for GWA studies.

In contrast to GWA studies, candidate gene studies are theory-based and only test associations with a few pre-defined genetic variants, which reduces the demand on sample size. The candidate gene approach has be adopted a few times in previous studies on physical activity phenotypes (Loos et al., 2005; Lorentzon, Lorentzon, Lerner & Nordstrom, 2001; Salmen et al., 2003; Simonen, Rankinen, Perusse, Leon et al., 2003; Stefan et al., 2002; Winnicki et

al., 2004). However, sample sizes were generally small and replications of significant hits are rare. Not a single genetic variant has been shown to affect regular exercise behavior at the level of "proof beyond reasonable doubt" to date. We attribute this in part to the current lack of studies investigating multiple genetic variants with a similar biological effect in the same pathway. Based on an extensive review of the literature, Knab and Lightfoot (2010) have suggested that the dopaminergic signaling pathway could be a major determinant of physical activity. This is in keeping with the hypothesis that a large part of the heritability of exercise behavior is due to genes that influence the affective reaction to exercise (de Geus & de Moor, 2011), as reward experience is governed by the mesolimbic reward system that involves dopaminergic pathways (Beaulieu & Gainetdinov, 2011). Studies in rodents have shown that acute rewarding effects of exercise were linked to changes in dopaminergic functioning (Greenwood et al., 2011), and dopaminergic gene expression differed in a high-active strain and a low-active strain of mice (Knab, Bowen, Hamilton, Gulledge & Lightfoot, 2009). We therefore investigate in Chapter 8 whether genetic variants in (close proximity to) dopaminergic genes are associated with exercise behavior.

In the closing Chapter 9, the main findings of this thesis are summarized, followed by suggestions for future research. In addition, the fundamental assumptions of the classical twin design, exercise "omics", "deep" phenotyping of exercise behavior and the placement of exercise behavior into the broader context of "physical activity" are discussed, followed by an overall conclusion and main implications of my thesis.

Chapter **2**

SURVEY ITEMS AND DATA COLLECTION

This thesis is mainly based on data of the Netherlands Twin Register (NTR) which was set up more than 25 years ago at the Vrije Universiteit in Amsterdam to investigate human health, lifestyle and behavior (Boomsma et al., 2006; Boomsma et al., 2002; van Beijsterveldt et al., 2013; Willemsen et al., 2013). An enormous amount of data was systematically collected ever since, resulting in a unique dataset for genetic epidemiological studies on psychological and physical health. The NTR did not only answer important questions on the effects of genetic and environmental factors on a wide range of health-related phenotypes, but its longitudinal structure made it also possible to study the change in prevalence and genetic architecture of behaviors over time and across generations.

Data collection of the NTR can roughly be divided into 1) surveys and projects targeting multiples and their families that are recruited shortly after birth of the multiple (the *Young NTR* or *Y-NTR*) and 2) surveys and projects targeting multiples that were recruited via city councils at the start of the NTR and through continuous self-registration (the *Adult NTR* or *A-NTR*). The main difference is that the timing of data collection in the former group is cohort-based, whereas in the latter group, data is collected at fixed time points for all participants every two to three years, irrespective of the participants' dates of birth. Participation in both the Y-NTR and the A-NTR is entirely voluntary. All participants receive a yearly bulletin of the NTR ("*TwInfo*") with information on current research projects and interesting facts about multiples.

Data collection of the *Y-NTR* was initiated in 1987. Through a commercial organization that provides gift boxes for parents of newborns (Felicitas B.V.) and through the "Dutch association for parents of multiples" (Nederlandse Vereniging voor Ouders van Meerlingen, NVOM), mothers of multiples are approached shortly after their multiple delivery to register with the NTR and to fill out a first survey on pregnancy and birth. After registration, mothers receive a subsequent survey on growth and achievement of milestones when the multiples are around two years old. Both mothers and fathers are then invited to report on their multiples' and their own health, lifestyle and behavior when the multiples are approximately 3, 5, 7, 10 and 12 years old. In addition, upon parental consent, teachers of the multiples and their non-multiple siblings are asked to provide information when the multiples are approximately 7, 10 and 12 years old. At the ages of 14 and 16 years, adolescent multiples and their siblings are invited to fill out self-report surveys. When Y-NTR multiples turn 18, they are invited to take part in survey research of the A-NTR. The Y-NTR has collected data on more than 75,000 children and adolescents, with longitudinal

data for a large part of the sample (personal communication, July 2015). Detailed information on the Y-NTR was published by van Beijsterveldt et al. (2013).

Data collection of the *A-NTR* was initiated by recruiting adolescent and young adult twins through city council offices in the Netherlands between 1991 and 1993. Additional multiples and their family members are invited to register with the NTR through advertising on the internet and in the yearly bulletin of the NTR ("*TwInfo*"). They are part of the registry as soon as they fill out the registration form and they will subsequently be approached to fill out self-report surveys. As stated before, Y-NTR multiples are invited to take part in survey research of the A-NTR when they turn 18 years old. The surveys are sent out at fixed time points, irrespective of the participants' dates of birth. The first survey was sent in 1991, followed by surveys in 1993 ("survey 2"), 1995 ("survey 3"), 1997 ("survey 4"), 2000 ("survey 5"), 2002 ("survey 6"), 2004-2008 ("survey 7"), 2009-2012 ("survey 8"), 2011-2013 ("survey 9") and 2013-2015 ("survey 10"). Collection of survey 11 is ongoing and collection of survey 12 is scheduled to start in autumn 2015 (personal communication, July 2015). Exercise behavior was a major topic in survey 6 and part of the resulting data were used in Chapter 6. The A-NTR has collected data on more than 40,000 individuals, with longitudinal data for a large part of the sample (personal communication, July 2015). Detailed information on the A-NTR was published by Willemsen et al. (2013).

As this thesis is mainly based on data of the Y-NTR, this chapter provides a brief overview of the items that were used over time by the Y-NTR to measure voluntary exercise behavior and other traits that are related to exercise behavior and physical activity, as well as the number of multiples, siblings and parents of the Y-NTR that had data available on exercise behavior in May 2014. As part of this thesis, survey data were collected on a large scale in adolescent multiples and their siblings. The data collection will be described in more detail, including the procedures that were followed and response rates. The project was reviewed by the EMGO^{+} science committee and approved by the Medical Research Ethics Committee of the VU University Medical Center (IRB letter May 2007 and letter no. 2003/182).

ITEMS MEASURING EXERCISE BEHAVIOR AND RELATED TRAITS

For 7-, 10- and 12-year-old multiples, parents were provided with a list of common exercise activities (athletics, badminton, ballet/dance, basketball,

fitness training, gymnastics, handball, jogging/running, hockey, netball, horseback riding, (ice-)skating, tennis, martial arts, soccer, swimming and volleyball) and the option to add unlisted activities. They were asked 1) whether or not their children participate in the respective activity and if so 2) for how many years, 3) how many months a year, 4) how many times a week and 5) how many minutes each time. Adolescents were asked to self-report on their own behavior based on very similar items and exercise behavior was assessed fairly consistently over time. All versions of the (Dutch) items assessing voluntary exercise behavior over the years in childhood and youth can be found in Appendix I and an English example of the parental rating is provided below.

EXAMPLE OF PARENTAL RATING

Circle the number(s) of the exercise activity(-ies) that the twins are currently participating in. Indicate for how many years, how many months a year, how many times a week and how many minutes each time the twins participate in the respective activity.

	sport	**years**	**months**	**times per week**	**duration in minutes**
gymnastics at school	**1**	..... yr	..... mth	..... times	..... min
swimming at school	**2**	..... yr	..... mth	..... times	..... min
athletics	**3**	..... yr	..... mth	..... times	..... min
badminton	**4**	..... yr	..... mth	..... times	..... min
ballet/dance	**5**	..... yr	..... mth	..... times	..... min
basketball	**6**	..... yr	..... mth	..... times	..... min
fitness training	**7**	..... yr	..... mth	..... times	..... min
gymnastics	**8**	..... yr	..... mth	..... times	..... min
handball	**9**	..... yr	..... mth	..... times	..... min
jogging/running	**10**	..... yr	..... mth	..... times	..... min
hockey	**11**	..... yr	..... mth	..... times	..... min
netball	**12**	..... yr	..... mth	..... times	..... min
horseback riding	**13**	..... yr	..... mth	..... times	..... min
(ice-) skating	**14**	..... yr	..... mth	..... times	..... min
tennis	**15**	..... yr	..... mth	..... times	..... min
martial arts	**16**	..... yr	..... mth	..... times	..... min
soccer	**17**	..... yr	..... mth	..... times	..... min
swimming	**18**	..... yr	..... mth	..... times	..... min
volleyball	**19**	..... yr	..... mth	..... times	..... min
else…	**20**	..... yr	..... mth	..... times	..... min

In the original question, there are separate columns for the first-born and the second-born twin.

The raw data were cleaned and summarized as a variable representing *voluntary* exercise behavior during leisure time. First, activities related to transportation (e.g., walking, cycling), domestic work (e.g., gardening, house cleaning) and compulsory physical education classes were excluded. If the "unlisted activity" option was used, we excluded activities that barely increase energy expenditure such as playing chess. As we were interested in regular activities, only activities were included that were performed for at least six months at the moment of data collection and for at least three months a year. The majority of the activities that were dropped based on these criteria were holiday specific (i.e., skiing during winter holidays, swimming during summer holidays, sailing camps, etc.). If exercise frequency or duration were missing while the other one was provided, the missing value was replaced with the median of that specific activity within the respective survey.

To quantify exercise behavior, each activity was recoded into its metabolic equivalent of task (MET) score. A MET is defined as "the ratio of work metabolic rate to a standard resting metabolic rate of 1.0 (4.184 kJ)·kg^{-1}·h^{-1}" (Ainsworth et al., 2000; p.498), representing the energy required to perform an activity relative to the energy that is expended during quiet rest. The energy demands of exercise activities are different for children and adults. In general, Ridley, Ainsworth and Olds (2008)'s compendium of energy expenditures was applied to individuals that were younger than 18 years old, whereas for older individuals, MET scores were taken from Ainsworth et al. (2000)'s compendium.

The product of the MET score, weekly frequency and duration was summed over all exercise activities that an individual engaged in. Individuals that did not participate in any exercise activities received a weekly MET hours score of zero. In our previous work, the six-month test-retest reliability of our measure for exercise behavior was found to be 0.91 (Stubbe et al., 2007) and 0.82 (de Moor et al., 2008), and it was significantly associated with other exercise phenotypes such as the sweat index and the frequency of being physically active for at least 20 minutes in the past 6 months in that the average weekly MET hours spent on exercise activities increased simultaneously with those measures (de Moor & de Geus, 2013).

Over the years, the Y-NTR has also collected data on active transportation, dancing whilst going out, perceived exercise ability, the level at which exercise activities are performed, perceived physical condition, the perceived benefits of and barriers towards exercise behavior and the sweat index. Most of these

were not used in the present thesis, but they provide a good opportunity for future research. The specific items that were used over the years are depicted in Appendix II.

LONGITUDINAL STRUCTURE OF EXERCISE DATA COLLECTED AMONG YOUNG MULTIPLES AND THEIR PARENTS AND SIBLINGS

Table 1 provides an overview of the data on exercise behavior in multiples that were available in May 2014, meaning that they were collected up to the year 2013, split by survey (≙ approximate age of the multiples) and birth year. About half of the multiples have repeated data available on at least two measurement occasions. Items measuring children's and adolescents' exercise behavior that allowed the calculation of weekly MET hours spent on exercise activities were for the first time added to the survey targeting approximately 7-year-old ("surveys 7") and 10-year-old ("survey 10") multiples in 2005, and to the survey targeting 12-year-old multiples ("survey 12") in 1999. These data are continuously being collected ever since. It should be noted that between 2007 and 2009, the NTR built an entire new database and the development of and transition to this new software and approach resulted in a decrease in the number of families that were contacted in this period, especially for 7- and 10-year-olds. More recently, the surveys 10 and 12 targeting fathers were shortened and children's exercise behavior was queried in mothers only. Data of parental exercise behavior is available for 3,609 mothers and 2,289 fathers at survey 7, 4,015 mothers and 2,526 fathers at survey 10, and 3,861 mothers and 2,519 fathers at survey 12.

Data collection in adolescent multiples aged approximately 14, 16 and 18 years was initiated in 2004 and included detailed items on exercise behavior from the beginning onwards. In addition to the data of multiples that are depicted in Table 1, 1,944 siblings have provided data on survey 14 and 1,639 siblings have provided data on survey 16. Data collection in multiples aged 14 and 16 years and their siblings is still ongoing, but data in multiples aged 18 years were collected by the Y-NTR for only two waves. After that, the data collection for this age group was moved to the A-NTR. Therefore, data for this age group are available from the *Y-NTR* for 1,174 multiples born between 1984 and 1988 only.

THE DUTCH HEALTH BEHAVIOR QUESTIONNAIRE

Data collection in 14- and 16-year-old multiples and their siblings was initiated in 2004. For details on the data collection of the first four waves (including a pilot study), see Chapter 9 in van der Aa's PhD thesis (2010). Two additional waves were collected as part of the current thesis, one in 2011-2012 and one in 2012-2013. Below, I describe the procedures for these two waves of data collection.

TABLE 1 Available sample sizes of multiples with exercise data for each age group, split by birth cohort: total number of individuals (mothers' reports/ fathers' reports).

Birth year	Survey 7	10	12	14	16
1986	*	*	2 (2/2)	*	*
1987	*	*	234 (230/180)	*	*
1988	*	*	982 (961/721)	*	679
1989	*	*	1099 (1079/805)	2	697
1990	*	*	1339 (1291/1015)	1014	735
1991	*	*	1286 (1264/899)	933	529
1992	*	*	1376 (1346/940)	972	523
1993	*	*	1154 (1142/792)	931	649
1994	*	216 (212/132)	966 (938/658)	1046	753
1995	*	1242 (1206/724)	1162 (1138/544)	824	1136
1996	*	1100 (1074/590)	1202 (1202/2)	934	1290
1997	780 (764/434)	1048 (1026/542)	1112 (1112/0)	1240	141
1998	1370 (1358/778)	4 (4/0)	1216 (1216/0)	1364	2
1999	1312 (1290/744)	352 (352/2)	1290 (1290/0)	822	*
2000	568 (550/324)	1178 (1178/0)	662 (662/0)	*	*
2001	0	1200 (1200/0)	*	*	*
2002	260 (258/166)	1112 (1112/0)	*	*	*
2003	1226 (1154/810)	734 (734/0)	*	*	*
2004	1220 (1154/822)		*	*	*
2005	728 (708/588)		*	*	*
Total	7464 (7236/4666)	8186 (8098/1990)	15082 (14873/6558)	10082	7134

**Surveys collected before detailed items assessing exercise behavior were added or data will be collected in the future.*

Parental consent

Parents or legal guardians of 12- to 13-year-old multiples were asked to provide the NTR with consent to approach their children with a survey. They were also asked to provide basic information and contact details of the siblings of their multiples (if present), as well as consent to approach these children with a survey. In addition, parents were asked whether or not all of their children were able to fill out a survey. If an illness or handicap interfered with filling out a survey, the affected individuals were naturally not approached.

In 2011-2012, the first mailings for parental consent were sent out to families with twins and triplets born between 01-01-1997 and 30-09-1998. Families were approached with an invitation letter containing a link to a website with more information, another link to the online consent form and a user name and password. They were asked to fill out the parental consent form online, but they were offered a paper-and-pencil form upon request. After a few weeks, reminders were sent out to non-responders by e-mail if available, and by mail if no e-mail address was available. The reminders were personalized in that families that had never or just once filled out previous surveys of the NTR received e-mails/letters emphasizing the importance of research, whereas families that had filled out two or more of the previous surveys received e-mails/letters that reminded them of their previous participation. Again, a paper-and-pencil version was offered upon request. As a final reminder, we contacted families by phone.

The whole procedure was repeated in 2012-2013 for the subsequent cohort, born between 01-10-1998 and 30-09-1999 - only this time, reminders were sent more shortly after one another. To start with, all families received the same invitation letter by mail. Approximately one week after the anticipated arrival time of the letters, families with an e-mail address available were sent an e-mail to make sure that they had received the letter and to provide them with their login details again, for their convenience. After a few weeks, reminders were sent out by mail to all participants that had not filled out the parental consent form and one week after the anticipated arrival time of the letters, parents were sent another e-mail. As a final reminder, we contacted families by phone.

It should be noted that for a sub-group of the sample that was subsequently approached with the survey, parental consent had already been obtained (or denied) as part of previous projects. Their parents were naturally not

approached. Of the total number of individuals that were eligible to fill out the survey, parents provided parental consent for approximately 42% in 2011-2012 and 58% in 2012-2013, and they denied consent for approximately 8% and 5%, respectively. The remaining parents did not respond at all.

The survey

Upon parental consent, adolescent multiples and their siblings received the *Dutch Health Behavior Questionnaire (DHBQ)*. Based on standardized questions, the DHBQ assesses various aspects of physical and emotional health, (problem) behavior, family functioning, demographic characteristics and lifestyle, including detailed questions on exercise behavior and physical activity. As part of my thesis, the DHBQ was sent out to multiples that were approximately 14 years old and their siblings aged 12 to 25 years ("DHBQ14"), as well as to multiples that were at least 16 years old and their siblings aged 12 to 25 years ("DHBQ16"). The DHBQ14 and the DHBQ16 slightly differed in that certain questions that were already asked at age 14 (e.g., hair color, eye color), were not repeated in the subsequent questionnaire in order to lower the participant burden, whereas at age 16, some new questions were introduced that better applied to this age group (e.g., the NEO-FFI). Both questionnaires were sent out in 2011-2012 and (a slightly shorter version) in 2012-2013. As opposed to previous years, the survey was only available online. In very rare cases (e.g., severe dyslexia), an older paper-and-pencil version of the survey was sent out upon request.

14-year-old multiples and their siblings

In 2011-2012, the DHBQ14 targeting multiples born between 01-01-1997 (triplets: 01-01-1996, as data in this group were not collected in previous years) and 30-09-1998 and their siblings aged 12 to 25 years was sent out. There were three groups of individuals: 1) individuals with parental consent available or individuals (siblings) that were at least 16 years old, 2) individuals with unknown parental consent (meaning that the parents never responded to our requests for parental consent) and 3) individuals with parents that explicitely did not want their children to fill out a survey. The latter group was naturally not approached. The first group of individuals was directly approached with a letter *or* if available an e-mail, containing the invitation to fill out the DHBQ14, a link to a website with more information, another link to the online questionnaire, a user name and a password. The second group of individuals was approached via their parents. More specifically, the parents received a

mailed invitation letter, a paper-and-pencil parental consent form and additional letters for their children with the link to the online questionnaire, all at the same time. They were asked 1) to fill out the consent form and 2) to then hand over to their children the letters containing the link to the online questionnaire. After a few weeks, only individuals with explicit parental consent and those siblings that were at least 16 years old received a reminder by mail, followed by an e-mail to make sure that they had received the letter and to provide them with their login details again for their convenience. Non-responders were called by phone. Upon completion of the survey, individuals received a postal card from the NTR to thank them.

In 2012-2013, the DHBQ14 was sent out to multiples born between 01-10-1998 and 31-08-1999 and their siblings aged 12 to 25 years. The group with explicit parental consent and siblings that were at least 16 years old were approached with a mailed letter and an e-mail (if available) approximately one week after arrival of the letter to make sure that they had received the letter and to provide them with their login details, for their convenience. Apart from that, the procedure was largely the same as in the previous year.

16-year-old multiples and their siblings

In 2011-2012, the DHBQ16 targeting multiples born between 01-01-1995 (triplets: 01-01-1994) and 01-10-1995 and their siblings aged 12 to 25 years was sent out. All multiples were at least 16 years old, meaning that parental consent was not required. Siblings that were 12 to 15 years old were directly invited to fill out the survey upon parental consent only. Parents of siblings that were younger than 16 years old with unknown parental consent were approached as outlined for the DHBQ14. The invitation letter to the participants was sent out by e-mail if available *or* by mail. It contained a link to a website with more information, another link to the online questionnaire, a user name and a password. After a couple of weeks, mailed reminders were sent out, followed by an e-mail approximately one week after the reminder should have arrived to make sure that the participants had received the letter and to provide them with their login details, for their convenience. Finally, non-responders were called by phone and everyone who had filled out the survey received a postal card by the NTR to thank them.

In 2012-2013, the DHBQ16 targeting multiples born between 02-10-1995 and 11-02-1997 and their siblings aged 12 to 25 years was sent out by e-mail to all individuals with an e-mail address available. Shortly after that, all individuals

that had not filled out the questionnaire (including all individuals without an e-mail address available) were approached by mail (whereby those who had already received an e-mail received a slightly adapted version of the letter). Apart from that, the procedure was largely the same as in the previous year.

The response rates for the DHBQ14 and DHBQ16 are depicted in Table 2. For the DHBQ14, the mailing targeting participants with parental consent (*PC= yes*) and the mailing targeting participants with unknown parental consent (*PC= unknown*) are presented separately. The response rate is considerably higher for individuals with parents that provided their consent beforehand.

TABLE 2 Response rates of DHBQ mailings.

Survey	Wave	Sent	Received	Response rate
DHBQ14	2011-2012	3302	2065	62.5%
PC=yes	2012-2013	2585	1287	49.8%
DHBQ14	2011-2012	3899	420	10.8%
PC=unknown	2012-2013	1621	41	2.5%
DHBQ16	2011-2012	2730	1002	36.7%
	2012-2013	5123	1751	34.2%

PC=parental consent.

Personalized feedback

A personalized feedback function was implemented in the second wave of data collection (2012-2013) to both the DHBQ14 and the DHBQ16. Upon provision of an e-mail address and completion of the final question, participants automatically received an e-mail with their scores on items related to exercise behavior, lifestyle, well-being, leisure time activities and the big five personality items of the NEO-FFI (DHBQ16 only). They were also informed about average scores of other participants who had filled out the survey. The full text of the e-mail following the DHBQ16 is depicted in Appendix III (with random scores). The e-mail for the DHBQ14 was equivalent except that it did not contain information on the big five personality dimensions as these were not queried in the survey.

Part I

HERITABILITY OF REGULAR VOLUNTARY EXERCISE BEHAVIOR

Chapter 3

EFFECT OF SHARED ENVIRONMENTAL FACTORS ON EXERCISE BEHAVIOR FROM AGE 7 TO 12 YEARS

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ABSTRACT

The aim of this study was to investigate the relative influence of genetic and environmental factors on children's leisure time exercise behavior through the classic twin design. Data were taken from the Netherlands Twin Register. The twins were 7- (N= 3,966 subjects), 10- (N= 3,562) and 12-year-olds (N= 8,687), with longitudinal data for 27% of the sample. Parents were asked to indicate the children's regular participation in leisure time exercise activities, including frequency and duration. Resemblance between monozygotic and dizygotic twins for weekly MET hours spent on exercise activities was analyzed as a function of their genetic relatedness. Average weekly MET hours increased with age for both boys (age 7 years: 14.0, SD= 11.8; age 10 years: 22.6, SD= 18.7; age 12 years: 28.4, SD= 24.9) and girls (age 7 years: 9.7, SD= 9.5; age 10 years: 15.3, SD= 15.1; age 12 years: 19.3, SD= 19.8). Around 13% of boys and girls across all age groups did not participate in any regular leisure time exercise activities. Tracking of exercise behavior from age 7 to 12 years was modest ($0.168<r<0.534$). For boys, genetic effects accounted for 24% (confidence interval: 18%-30%) of the variance at age 7 years, 66% (53%-81%) at age 10 years and 38% (32%-46%) at age 12 years. For girls, these were 22% (15%-30%), 16% (9%-24%) and 36% (30%-43%), respectively. Environmental influences shared by children from the same family explained 71%, 25% and 50% of the variance in boys (aged 7, 10 and 12 years) and 67%, 72% and 53% in girls. The shared environment influencing exercise behavior was partially different between boys and girls. Our results stress the important role of the shared environment for exercise behavior in young children.

INTRODUCTION

Regular exercise behavior in leisure time is increasingly accepted to be a main contributor to children's health (Janssen & Leblanc, 2010). Despite this, the proportion of children that are active enough to benefit from exercise is low, with girls being consistently less active than boys (Armstrong & van Mechelen, 1998). A better understanding of why certain children exercise and others do not is important to develop successful health-promoting exercise interventions for children and adolescents. Previous research provides evidence that environmental and social factors are related to being physically active (Biddle & Mutrie, 2008), such as access to exercise facilities, socioeconomic status and support by family and peers (Sallis, Prochaska & Taylor, 2000; van der Horst, Paw, Twisk & van Mechelen, 2007). However, even taking into account these factors, a good deal of variance remains unexplained. More recently, it has

been suggested that irrespective of the surrounding environment, some people may be more predisposed toward exercising than others (de Geus & de Moor, 2011) - because individuals differ concerning their internal "need" to be active, exercise ability and personality factors. These factors, hypothesized to be genetically influenced, may trigger either rewarding or negative physiological responses to exercise (Bryan, Hutchison, Seals & Allen, 2007; de Geus & de Moor, 2011).

Twin studies provide a unique opportunity to disentangle the environmental and genetic influences on exercise behavior. They can be used to decompose environmental factors into those that are shared by the twins (such as the family environment) and those environmental factors that are unique to each child. Several twin studies have investigated leisure time exercise behavior in adolescents (de Moor et al., 2011; Stubbe, Boomsma & de Geus, 2005; van der Aa, de Geus, van Beijsterveldt, Boomsma & Bartels, 2010) and in adults (Beunen & Thomis, 1999; Lauderdale et al., 1997; Simonen, Levalahti, Kaprio, Videman & Battie, 2004; Stubbe et al., 2006). The relative contribution of genetic and environmental influences is different for males and females and it changes vastly across the lifespan (de Geus & de Moor, 2011). For example, van der Aa et al. (2010) investigated leisure time exercise behavior in twins aged 13 to 18 years. For both sexes, heritability estimates at ages 16 to 18 years were very high (80%). For 13- to 14-year-old boys, genetic factors accounted for 80% of the variance in exercise behavior. For girls, genes accounted for only 38% of the variance with the shared environment being more influential (46%). In adult twins, heritability estimates decrease from the peak value in adolescence to values between 40% and 70%. The remaining variance is due to unique environmental factors, and the shared environment is no longer of importance (Stubbe et al., 2006). There are no published twin studies that have specifically investigated the heritability of leisure time exercise behavior in children.

The aim of this study is to bridge this gap by examining the relative influence of genetic and environmental factors on this behavior in children that are age 7, 10 and 12 years. Similar to previous studies (e.g., Stubbe et al., 2005; van der Aa et al., 2010), we have deliberately chosen to focus on the narrow but well-defined trait of leisure time exercise behavior and not on general physical activity. Survey research can be reliably used to query participation in regular exercise activities, but has more difficulty in assessing overall energy expenditure, which should be measured preferentially with objective methods like accelerometry. The shared family environment is expected to be a strong contributor to exercise behavior in children because children are likely to be

dependent on their parents when it comes to exercise activities (e.g., need to get rides to and from facilities). On the basis of the adolescent findings, we also expect a significant genetic contribution to exercise behavior in this period, particularly in boys.

METHODS

Participants

Data were available for young twins registered with the Netherlands Twin Register, which was established by the Department of Biological Psychology at the VU University Amsterdam in 1987 (Boomsma et al., 2006). Young twins are registered by their parents shortly after birth. Mothers and fathers are then invited to complete surveys about their children's health, lifestyle and behavior when the children are approximately 0, 2, 3, 5, 7, 10 and 12 years old (Bartels et al., 2007). The children live in all regions of the Netherlands (Boomsma et al., 2006). Until today, parents of more than 32,000 twin pairs have taken part in research projects. For the present study, data of 209 children with diseases or disabilities that may prevent them from being physically active were excluded. In addition, data from 11 twin pairs were excluded due to missing zygosity information. This resulted in the following samples: age 7 years (N= 3,966 children, mean= 7.45 years, SD= 0.32, 49.6% males), age 10 years (3,562, 10.12, 0.33, 49.0%) and age 12 years (8,687, 12.29, 0.40, 48.8%).

The children's parents were classified as low educated (19.1%), average educated (44.8%) or high educated (33.2%, 2.9% missing), and the large majority was born in the Netherlands (95%), with no differences across zygosity groups. Of the monozygotic (MZ) twins, 92.5% were conceived naturally, 2.4% after hormone treatment and 1.7% with in vitro fertilization (3.4% missing). For the dizygotic (DZ) twins, these were 62.0%, 11.9% and 20.2%, respectively (5.9% missing). The children's body mass index (BMI) was comparable across zygosity groups. For male MZ twins, it was 15.3 (SD= 1.7) for age 7 years, 16.4 (2.2) for age 10 years and 17.5 (2.4) for age 12 years. For female MZ twins, these were 15.3 (1.9), 16.5 (2.2) and 17.6 (2.7), respectively. For male DZ twins, BMI was 15.4 (1.7), 16.4 (2.1) and 17.6 (2.6), and for female DZ twins, 15.5 (1.9), 16.5 (2.3) and 17.8 (2.7). Finally, male DZ twins of opposite-sex pairs had a BMI of 15.3 (1.6), 16.3 (2.0) and 17.4 (2.4). For the girls, these were 15.4 (1.9), 16.6 (2.4) and 17.7 (2.5).

For only a modest part of the children (26.8%), there were data at more than

one age because the detailed survey items on exercise behavior were introduced to the parental surveys in 2004/2005. Therefore, some of the children were already too old to be rated based on these items in the first or second wave of parental data collection, whereas others had not received a second or third survey at the time of data analysis. Zygosity was determined by blood group or DNA typing for 11.8% of the same-sex twin pairs. For the remaining same-sex twin pairs, zygosity was based on survey items on physical similarities and confusion by family members and strangers. This has been shown to result in accurate determination for 93% of same-sex twin pairs (Rietveld et al., 2000). The subjects' parents provided consent to be approached for survey research at enrollment in the Netherlands Twin Register. The data collection protocol was approved by the Medical Research Ethics Committee of the VU University Medical Center. Table 1 summarizes the number of twin pairs by sex and zygosity.

Measures

We provided parents with a list of the 17 most common exercise activities in the Netherlands (athletics, badminton, ballet/dance, basketball, fitness training, gymnastics, handball, jogging/running, hockey, netball, horseback riding, (ice-)skating, tennis, martial arts, soccer, swimming and volleyball), plus the option to add up to two additional unlisted activities. We then asked them to indicate for each activity a) whether or not the child participated in the activity and if so, b) for how many years, c) for how many months a year, d) how many times a week and e) how many minutes each time. If the "unlisted activity" option was used, we excluded activities that barely increase energy expenditure such as playing chess. Activities related to transportation (walking, biking) or compulsory exercise in physical education (PE) classes were also not included because they are often not self-initiated or voluntary. Each activity was recoded into a metabolic equivalent (MET) score based on the compendium of energy expenditures for youth by Ridley et al. (2008). A MET is defined as "the ratio of work metabolic rate to a standard resting metabolic rate of 1.0 (4.184 kJ)·kg^{-1}·h^{-1}" (Ainsworth et al., 2000; p. 498). This standard resting metabolic rate equals quiet sitting. By multiplying the MET score, the frequency and the duration of each exercise activity and then summing all activities that the children undertook, weekly MET hours spent on exercise activities were calculated for each individual. We did not apply a minimum weekly frequency or duration, but we included only those activities in which the children participated for at least 3 months a year, representing regular leisure time exercise behavior. Also, activities had to have been initiated at

least 6 months ago at the time of survey completion. A total of 3.8% of the reported activities were dropped on the basis of these inclusion criteria. Importantly, the majority of these activities were holiday specific (i.e., skiing during winter holidays, swimming during summer holidays, sailing camps, etc.).

Statistical analyses

For age 7, 10 and 12 years, 55.1%, 53.4% and 40.7% of the surveys were filled out by both parents, respectively, and 1.7%, 2.5% and 1.4% were filled out by the fathers only. For the remaining surveys, only the mothers reported on the child. As the correlations between fathers' and mothers' ratings were - for both children - high at all ages with a median correlation of 0.820 (range: 0.779-0.833), averaged weekly MET hours were used when both parents had reported on the same child. If either the frequency or the duration of an activity were not indicated, they were replaced with a median of the age group within the respective exercise activity. In total, 1.54% of the missing data on either frequency or duration was replaced with a median. Missingness in MZ versus DZ twin pairs was very similar (1.6% vs. 1.3%). Different wording of the items within a part of the sample at age 12 years (times a month instead of times a week) led to a slight difference in means ("batch effect"), which was corrected before the analyses. We verified that this correction affected only the means but not the twin correlations.

The correlations between MZ and DZ twins were estimated separately for each sex to evaluate the relative influence of genetic and environmental factors on exercise behavior. MZ twins originate from the same fertilized egg and therefore share (nearly) 100% of their genetic material. DZ twins only share on average 50% of their segregating genes - the same amount as non-twin siblings do. The shared environment includes all factors that the two children of a twin pair share such as the family environment, the neighborhood and recreational environment, and possibly the school and common friends. The shared environment is by definition the same for both MZ and DZ twins (100% resemblance). On the basis of the differing genetic relatedness of MZ and DZ twins, it is possible to estimate the relative influence of genes, the shared environment and the environment that is unique to an individual on an outcome variable (Neale & Cardon, 1992). The last component includes variance due to measurement error. If the MZ correlation is larger than the DZ correlation (and thus MZ twins resemble each other more than DZ twins), this implies genetic influences. If the DZ correlation is larger than half the MZ correlation, the influence of shared environment is likely to be significant as

well.

Twin correlations also allow a rough understanding of quantitative and qualitative sex differences for a trait. Quantitative sex differences are present when the relative contribution of genes, the shared environment and the non-shared environment differs for boys and girls. Qualitative differences are likely when the DZ opposite-sex (DOS) correlation cannot be predicted on the basis of the DZ male-male (DZM) and DZ female-female (DZF) correlations. For instance, if the DOS correlation is lower than the DZM correlation and the DZF correlation, there is a weaker relationship between two children of a different sex than two children of the same sex, suggesting that different genetic or shared environmental factors operate in boys and girls (Falconer & Mackay, 1996).

Twin correlations were estimated with the software package OpenMx (Boker et al., 2011) for each sex by zygosity group (i.e., MZ male twin pair, DZM, MZ female twin pair, DZF and DOS). A model that estimated all parameters freely (saturated model) was fitted to the data. It was tested whether constraining the means and variances to be equal across 1) MZ and DZ twins, 2) MZ, DZ and DOS twins and 3) across sex led to a significant deterioration of the model fit.

To gain further insight into the genetic architecture of exercise behavior, a univariate genetic model was then fitted to the data for each age group. Individual differences in exercise behavior were expected to be due to differences in additive genetic effects (A), common environmental effects shared by twins from the same family (C) and non-shared environmental effects (E). These latent factors are expected to correlate differently for MZ and DZ twins. Because MZ twins share approximately 100% of their genes, the genetic correlation (rg) between twin 1 and twin 2 was fixed to 1 for MZ pairs. For DZ twins that share on average 50% of their genes, this was 0.5. For both MZ and same-sex DZ twins, the shared environmental correlation (rc) was - by definition - fixed to 1. To identify the most parsimonious and best-fitting model, various constraints were stepwise imposed on the model. The various nested models were then compared with the log-likelihood ratio test, which evaluates the difference in minus two times the log-likelihood between two models based on its χ^2 distribution and using the difference in degrees of freedom (df) between those models. As long as the model fit did not significantly decrease ($p>0.05$), constraints were kept to support parsimony of the model.

TABLE 1 Number of (complete) twin pairs and twin correlations for exercise behavior (95% CIs).

	Age 7 years		Age 10 years		Age 12 years	
	N	**r**	**N**	**r**	**N**	**r**
MZM	302 (297)	.94 (.93,.95)	290 (284)	.90 (.88,.92)	732 (719)	.88 (.86,.89)
DZM	350 (345)	.83 (.80,.86)	310 (298)	.56 (.48,.63)	700 (668)	.69 (.65,.73)
MZF	355 (351)	.90 (.89,.92)	339 (336)	.86 (.83,.88)	831 (821)	.88 (.86,.89)
DZF	310 (304)	.80 (.76,.83)	290 (284)	.76 (.71,.80)	689 (670)	.74 (.70,.77)
DOS	681 (671)	.39 (.32,.45)	572 (559)	.48 (.42,.54)	1449 (1408)	.42 (.38,.46)

Separately for each sex x zygosity x age group; MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic of opposite-sex.

RESULTS

Table 2 depicts the average weekly MET hours spent on exercise activities for boys and girls across the three age groups. Exercise behavior did increase over time in both sexes ($p<0.001$) but was lower for girls across all ages ($p<0.001$). Around 13% of all children did not take part in any leisure time exercise activities (Table 2). MET hours spent on PE classes and leisure time exercise activities were only weakly correlated across all ages ($r<0.140$, data not shown). PE was therefore not deemed a confounder and not further included in the analyses. Tracking of exercise behavior from age 7 to 12 years was modest with estimates ranging from 0.28 to 0.51 (Table 3). The means of MZ, DZ and DOS twins were equal within each age group and the MZ and DZ variances were equal within ages 7 and 10 years ($p<0.01$). Sex differences were found across all ages.

Table 1 presents the twin correlations (95% confidence interval, CI) of each sex by zygosity group for MET hours spent on exercise activities, based on the most parsimonious model. The MZ twin correlations were always higher than the DZ twin correlations, suggesting genetic influence. Because the DZ twin correlations were also larger than half the MZ twin correlations across all ages, the shared environment was likely to play a role in children's exercise behavior. Finally, the DOS correlations tended to be lower than the DZ correlations, which implied qualitative sex differences.

Genetic model fitting results are presented in Table 4. The shared environmental correlations between DOS twins (rcdos) were freely estimated in model 1. In model 2, rcdos was fixed to 1, which resulted in a significant deterioration of the model fit for ages 7 and 12 years, but not for age 10 years.

Subsequently, it was tested whether constraining the parameter estimates a, c and e to be equal for boys and girls (model 3), constraining the genetic parameters to zero (boys: model 4a; girls: model 4b) or constraining the shared environmental parameters to zero (boys: model 5a; girls: model 5b) led to a significant deterioration of the model fit. For ages 7 and 12 years, model 1 appeared to be most parsimonious. For age 10 years, this was model 2. Table 5 represents the proportions of variance explained by additive genetic (A), shared environmental (C) and unique environmental factors (E) of the most parsimonious and best-fitting models for the three age groups (95% CIs added in parentheses). To increase comparability over age, both model 1 and model 2 (best-fitting model) are presented for age 10 years. Except for 10-year-old boys, shared environmental factors consistently explained the largest part of the variance in exercise behavior, followed by additive genetic factors.

TABLE 2 Average weekly MET hours spent on regular leisure time exercise behavior (SD) and number (percentage) of children participating in 1) team sports only, b) individual activities only, c) both kinds of activities and d) no exercise activities at all.

A. Boys	**Age 7**	**Age 10**	**Age 12**
Weekly MET hours	13.99 (11.78)	22.57 (18.69)	28.39 (24.93)
a) Team sports only	546 (27.7%)	652 (37.4%)	1569 (37.0%)
b) Individual activities only	744 (37.8%)	497 (28.5%)	1140 (26.9%)
c) Both	398 (20.2%)	390 (22.3%)	926 (21.8%)
d) Non-exercisers	281 (14.3%)	206 (11.8%)	604 (14.2%)
Total number	1969	1745	4239

B. Girls	**Age 7**	**Age 10**	**Age 12**
Weekly MET hours	9.74 (9.47)	15.29 (15.12)	19.33 (19.80)
a) Team sports only	140 (7.0%)	260 (14.3%)	807 (18.1%)
b) Individual activities only	1392 (69.7%)	1034 (56.9%)	2346 (52.7%)
c) Both	203 (10.2%)	296 (16.3%)	677 (15.2%)
d) Non-exercisers	262 (13.1%)	227 (12.5%)	618 (13.9%)
Total number	1997	1817	4448

TABLE 3 Correlations across age groups (N).

	Age 7-10 years	**Age 10-12 years**	**Age 7-12 years**
Boys	.31* (170)	.36* (1223)	.28* (532)
Girls	.51* (179)	.43* (1243)	.28* (522)

*Number of complete pairs in parentheses; * p<0.01.*

TABLE 4 Univariate model fitting results, separately for the three age groups.

Model	Vs.	-2LL	*df*	X^2	*Δdf*	*p*
Age 7						
1. ACE: sex differences, rcdos estimated	**--**	**27861.87**	**3957**	**--**	**--**	**--**
2. ACE: sex differences, rcdos fixed at 1	1	27962.18	3958	100.31	1	<.0001
3. ACE: no sex differences	1	27949.80	3960	87.93	3	<.0001
4a. CE: boys, ACE: girls	1	27955.22	3958	93.35	1	<.0001
4b. ACE: boys, CE: girls	1	27904.97	3958	43.09	1	<.0001
5a. AE: boys, ACE: girls	1	28040.90	3958	179.03	1	<.0001
5b. ACE: boys, AE: girls	1	28010.08	3958	148.20	1	<.0001
Age 10						
1. ACE: sex differences, rcdos estimated	--	28757.33	3553	--	--	--
2. ACE: sex differences, rcdos fixed at 1	**1**	**28761.00**	**3554**	**3.67**	**1**	**.0554**
3. ACE: no sex differences	2	28923.21	3557	162.21	3	<.0001
4a. CE: boys, ACE: girls	2	28959.70	3555	198.70	1	<.0001
4b. ACE: boys, CE: girls	2	28778.86	3555	17.86	1	<.0001
5a. AE: boys, ACE: girls	2	28804.33	3555	43.33	1	<.0001
5b. ACE: boys, AE: girls	2	28867.67	3555	106.67	1	<.0001
Age 12						
1. ACE: sex differences, rcdos estimated	**--**	**74950.01**	**8678**	**--**	**--**	**--**
2. ACE: sex differences, rcdos fixed at 1	1	75066.17	8679	116.16	1	<.0001
3. ACE: no sex differences	1	75212.10	8681	262.09	3	<.0001
4a. CE: boys, ACE: girls	1	75111.73	8679	161.72	1	<.0001
4b. ACE: boys, CE: girls	1	75133.16	8679	183.15	1	<.0001
5a. AE: boys, ACE: girls	1	75078.92	8679	128.91	1	<.0001
5b. ACE: boys, AE: girls	1	75105.02	8679	155.01	1	<.0001

Most parsimonious models are printed in boldface type; -2LL=-2 log likelihood, A=additive genetic factors, C=common environmental factors, E=unique environmental factors.

TABLE 5 Relative contribution of additive genetic, common environmental and unique environmental factors and the environmental correlation between DOS twins (SE) of the best-fitting models to explain exercise participation in three age groups, separately for boys and girls (95% CIs).

		A	C	E	rcdos
Age 7 yr	Boys	.24 (.18, .30)	.71 (.64, .76)	.06 (.05, .07)	.47 (.05)
	Girls	.22 (.15, .30)	.67 (.60, .74)	.11 (.09, .12)	
Age 10 yr, model 1	Boys	.66 (.53, .81)	.25 (.09, .38)	.10 (.08, .12)	.65 (.10)
	Girls	.16 (.09, .24)	.72 (.64, .79)	.11 (.10, .14)	
Age 10 yr, model 2	Boys	.80 (.74, .85)	.10 (.06, .16)	.10 (.08, .12)	1
	Girls	.15 (.08, .23)	.73 (.66, .79)	.12 (.10, .14)	
Age 12 yr	Boys	.38 (.32, .46)	.50 (.43, .57)	.12 (.10, .13)	.45 (.04)
	Girls	.36 (.30, .43)	.53 (.47, .59)	.11 (.10, .12)	

DISCUSSION

The main purpose of this study was to investigate the relative influence of genetic and environmental factors on children's participation in leisure time exercise activities. Average weekly MET hours spent on exercise activities in young Dutch twins doubled from age 7 to 12 years, but this was mainly due to those who were already active increasing their MET hours further. Thirteen percent of boys and girls of all ages were inactive in that they did not participate in any regular leisure time exercise activities. In accordance with previous findings, boys were more active than girls (e.g., Armstrong & van Mechelen, 1998). For boys, additive genetic effects accounted for 23.7%, 65.7% and 38.3% of the variance in exercise behavior at ages 7, 10 and 12 years. For girls, these were 22.1%, 16.3% and 36.1%. Within all three age groups, shared environmental factors explained the largest part of the variance (70.5%, 24.6% and 50.1% for boys; 67.3%, 72.3% and 53.4% for girls). The correlation between shared environmental factors influencing exercise behavior in boys and girls (rcdos) was less than unity, suggesting that boys and girls in the same family do not receive the same level of familial support.

The important role of shared environmental factors for children's regular exercise behavior is consistent with results of smaller-sized twin studies that focused on total physical activity rather than leisure time exercise activities (Fisher, van Jaarsveld, Llewellyn & Wardle, 2010; Franks et al., 2005; Plomin & Foch, 1980). Fisher et al. (2010) measured time spent in moderate and vigorous physical activity by accelerometry in a sample of 234 9- to 12-year-old twins. Shared environmental factors accounted for 61% of the variance, with the remaining 39% being explained by unique environmental effects. No genetic influence was found. Franks et al. (2005) measured physical activity energy expenditure in 200 4- to 10-year-old twins using respiratory gas exchange and doubly labeled water with very similar results (shared environment: 69%, unique environment: 31%). Plomin and Foch (1980) investigated one-week pedometer counts in a sample of 174 7.6-year-old twins (SD= 1.6 years). Again, the shared environment was by far the most important contributor to physical activity (MZ correlation: 0.99, DZ correlation: 0.94).

As previously outlined, the shared environment is made up of all environmental factors that twins share. Thus, the strong shared environmental effect in the present study may be explained by factors such as the neighborhood and recreational environment, school and common friends. These factors may all be related to (accessibility of) exercise opportunities. However, because

parents often act as gatekeepers to children's leisure time activities (Beets, Cardinal & Alderman, 2010; Gustafson & Rhodes, 2006), parenting behavior may be one of the more prominent shared environmental influences on children's exercise behavior. Their support of their children's exercise behavior depends on their attitudes regarding these activities (Anderson, Hughes & Fuemmeler, 2009; Trost et al., 2003), which may vary across families. In a recent review, Beets et al. (2010) identified four categories of parental influence on their children's physical activity. Parents may or may not provide tangible support by organizing transportation to exercise locations and paying for sport clubs and equipment (instrumental support), and by being physically present during their children's exercise activities or even coaching/ participating themselves (conditional support). They may also provide intangible support to increase children's self-efficacy and attitudes toward physical activity by encouragement and praise (motivational support) or by providing advice, suggestions and information about (the benefits of) being active (informational support). This theory predicts that parental influence on their children's exercise activities should wane when the children get older and become less dependent on others for transportation and less willing to imitate their parents' behavior or adopt their attitudes (Mulvihill, Rivers & Aggleton, 2000; Salmon, 2010). The decrease in common environmental influences from age 7 to 12 years is entirely compatible with this prediction and it continues during adolescence as has been shown by van der Aa et al. (2010). The important role of tangible support is further supported by the finding that around two thirds of the twin pairs had at least one type of exercise activity in common (age 7 years: 69.5%, age 10 years: 65.9%, age 12 years: 61.5%), which is much higher than could be expected on the basis of the frequency of each of the types of exercise activities (approximately 20%). It is likely more convenient for parents to organize transportation and cheer their children at a single exercise location as opposed to handling two locations.

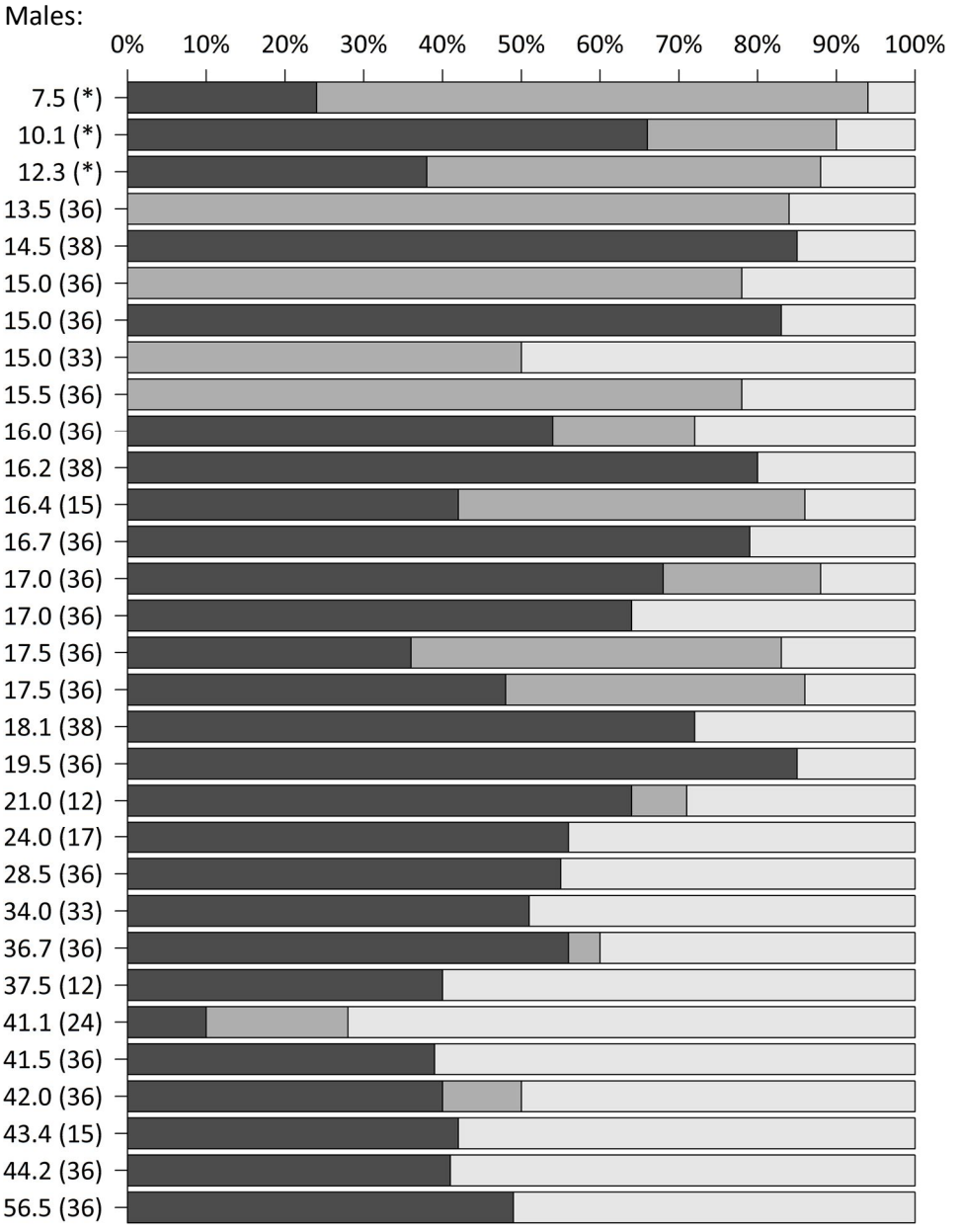
As the environmental correlation between DOS twin pairs was not unity for ages 7 and 12 years, (some of) the shared environmental influences are likely to be qualitatively different for boys and girls. Given the parents' influential role, a look at their differential treatment of sons and daughters concerning exercise activities is warranted. Although the findings are not unanimous, boys tend to receive more parental support than girls (Beets et al., 2010; Gustafson & Rhodes, 2006). In addition, mother-daughter and father-son correlations for physical activity are generally higher than opposite-sex correlations (Gustafson & Rhodes, 2006), indicating a sex-specific influence of parents on their children. Accordingly, Edwardson and Gorely (2010) found a positive association

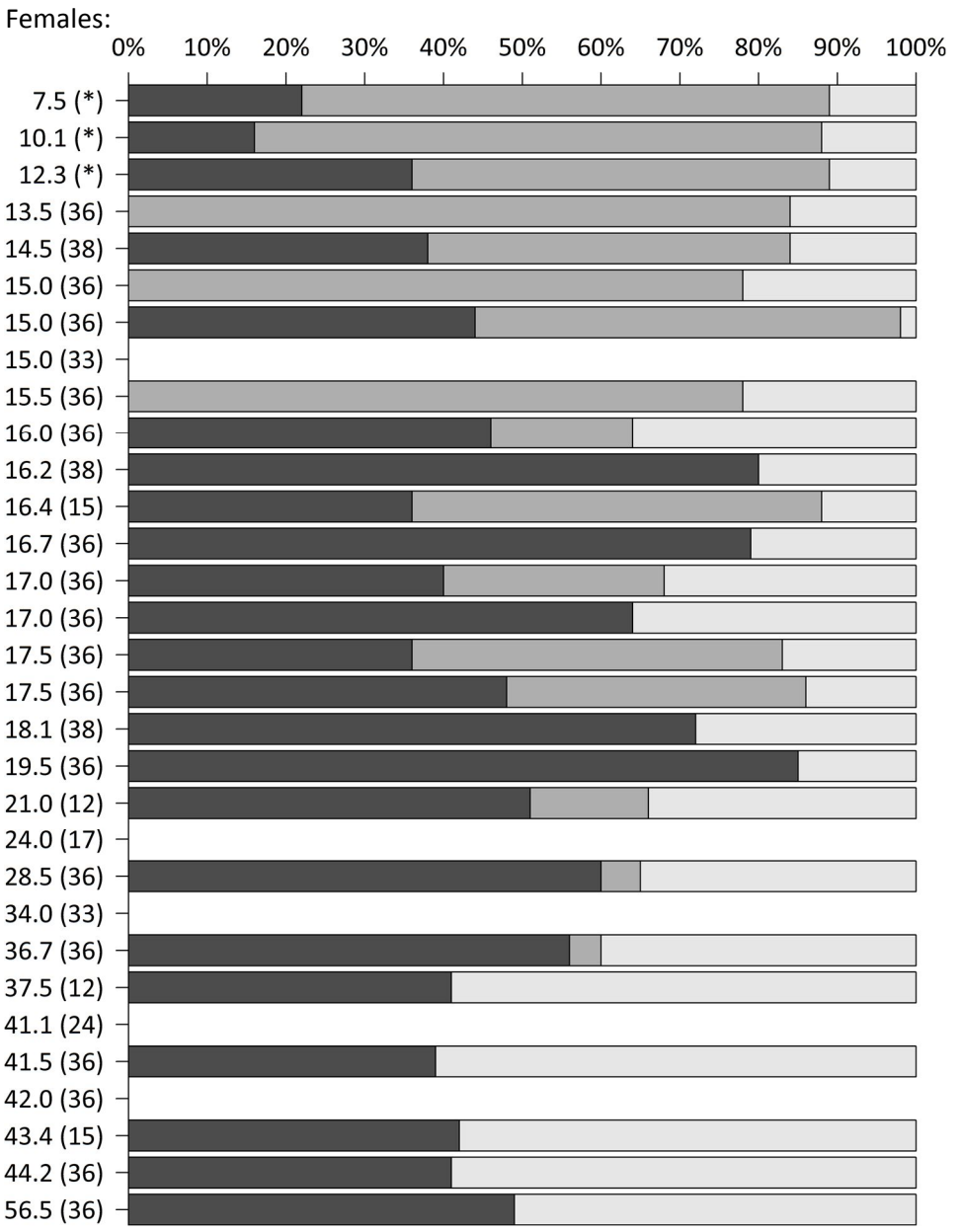
between fathers' explicit modeling and their sons' moderate-to-vigorous physical activity, but no association for girls. Anderson et al. (2009) reported that parents deemed boys' participation in team sports to be more important than girls' participation - for high educated parents, this bias was also apparent for individual activities - and that boys are more similar to their parents concerning the value placed on being active ("parent-child attitude congruence"). Parents may not only provide more support to their sons - they may also differ in the initial choice of which type of activity their sons and daughters should participate in. This may be a main reason why girls in our study engaged predominantly in individual exercise activities, whereas boys participated in all kinds of activities, including the more vigorous team sports (Table 2 and Mulvihill et al., 2000). Accordingly, the percentage of opposite-sex siblings that share at least one activity dropped to 46.5%, which is clearly lower than that for same-sex siblings (75.0%).

The relative contribution of genetic factors was much larger in 10-year-old boys compared with 7- and 12-year-olds. This pattern was not seen in girls. Because we used identical parental surveys, the difference cannot be attributed to a change in study methods. Also, there are no major changes in the educational system at this age (high school starts at least 2 years later). One possible explanation is that most clubs, whether in team sports (e.g., soccer) or individual sports (e.g., tennis), increasingly start selecting for ability around this age. The amount of training is usually larger in the "first teams" compared with the lower ranked teams. Because exercise abilities are strongly heritable (Bouchard & Hoffman, 2011), this may have boosted heritability of participation in these types of activities in boys, who may be more sensitive to their relative ranking among peers than girls. However, it is unclear why this effect has dissipated at age 12 years. Replication in larger samples is needed before drawing definitive conclusions.

After numerous studies using adolescent and adult twin data (e.g., Stubbe et al., 2006; van der Aa et al., 2010), this study is the first to investigate the relative contribution of genes and the environment to exercise behavior in children younger than 12 years old. Our findings fit the existing literature rather well as shown in Figure 1, which summarizes the results of all existing twin studies on leisure time exercise behavior. The figure includes the twin studies that were listed by Stubbe and de Geus (2009), extended with additional studies (Carlsson, Andersson, Lichtenstein, Michaelsson & Ahlbom, 2006; de Moor et al., 2011; Eriksson, Rasmussen & Tynelius, 2006; McCaffery,

FIGURE 1 Summary of (previous) study results showing the relative influence of genes (dark gray), the shared environment (gray) and the unique environment (light gray) - indicated as percentages - on leisure time exercise behavior across the lifespan.





*Y-axis: age in years (reference number, *=the present chapter); The corresponding reference numbers can be found in the published paper.*

Papandonatos, Bond, Lyons & Wing, 2009; Simonen et al., 2004; van der Aa et al., 2010) and the present one. From childhood onward, the heritability of exercise behavior increases to a peak during late adolescence and then decreases again to reach stable proportions in adulthood. The substantial

shared environmental influence is only found in children. Our group (de Geus & de Moor, 2011) has hypothesized that the heritability of leisure time exercise behavior reflects three major sources: individual differences in a homeostatic need for activity, exercise ability and acute psychological effects of exercise (also see Bryan et al., 2007). Personality, itself a heritable trait, may be a fourth important determinant of stable individual differences in exercise participation (de Moor, Beem, Stubbe, Boomsma & de Geus, 2006; Rhodes & Smith, 2006).

A limitation of the present study is the reliance on parental ratings of leisure time exercise behavior. Subjective ratings by the parents may tend to overestimate the actual exercise behavior of the children. However, the correlations between mothers' and fathers' ratings were high and the results were remarkably comparable with similar studies that used objective measurements of general physical activity, to which leisure time exercise activities make an important contribution (Fisher et al., 2010; Franks et al., 2005; Plomin & Foch, 1980). Our use of a fixed list of the most common exercise activities performed by Dutch children probably helped to increase the reliability of parental reporting. It should be noted, however, that by focusing on these structured and well-defined exercise activities, we have ignored an important other contribution to children's leisure-time physical activity, namely active play. Active play probably contributes to overall leisure time physical activity in different proportions across different age groups, with less opportunity for play in 12-year-olds once they enter high school. How this affects the heritability or environmentability of participation in regular structured exercise activities remains uncharted. A specific limitation of using twins, although in general the best design to estimate heritability, is that the findings may not generalize to families with siblings of different ages or a single child. Because twins have the same age, it could be argued that the role of tangible support (a shared environmental factor) is greater, because it is more convenient for the parents to handle the twins as a pair, than it would be for siblings with larger age differences. To balance these limitations, this study had a very large sample size, estimated heritability in groups with a confined age range and deliberately focused on participation in well-defined leisure time exercise activities, which are easier to assess in a standardized way than overall physical activity.

Our analyses confirmed the important role of shared environmental factors for children's exercise behavior that gradually give way to genetic influences when they reach early adolescence. The shared family environment is likely to be an important target for the development of successful interventions on childhood

exercise behavior, but family-based strategies may become less useful in adolescence.

Chapter **4**

INDIVIDUAL DIFFERENCES IN EXERCISE BEHAVIOR: STABILITY AND CHANGE IN GENETIC AND ENVIRONMENTAL DETERMINANTS FROM AGE 7 TO 18

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ABSTRACT

Exercise behavior during leisure time is a major source of health-promoting physical activity and moderately tracks across childhood and adolescence. This study aims to investigate the absolute and relative contribution of genes and the environment to variance in exercise behavior from age 7 to 18, and to elucidate the stability and change of genetic and shared environmental factors that underlie this behavior. The Netherlands Twin Register collected data on exercise behavior in twins aged approximately 7, 10, 12, 14, 16 and 18 years (N= 27,332 twins; 48% males; 47% with longitudinal assessments). Three exercise categories (low, middle, high) were analyzed by means of liability threshold models. First, a univariate model was fitted using the largest available cross-sectional dataset with linear and quadratic effects of age as modifiers on the means and variance components. Second, a genetic simplex model was fitted to the full dataset. Heritability was low in 7-year-olds (14% in males and 12% in females), but gradually increased up to age 18 (79% in males and 49% in females), whereas the initially substantial relative influence of the shared environment decreased with age (from 80% to 4% in males and from 80% to 19% in females). This decrease was due to a large increase in the genetic variance. The longitudinal model showed the genetic effects in males to be largely stable and to accumulate from childhood to late adolescence, whereas in females, they were marked by both transmission and innovation. The shared environmental effects tended to be less stable in both males and females. In sum, the clear age-moderation of exercise behavior implies that family-based interventions might be useful to increase this behavior in children, whereas individually based interventions might be better suited for adolescents. We showed that some determinants of individual differences in exercise behavior are stable across childhood and youth, whereas others come into play at specific ages. In view of the many benefits of regular exercise, identifying these determinants at specific ages should be a public health priority.

INTRODUCTION

Although an active lifestyle is accepted to be a major contributor to health (Garber et al., 2011; Janssen & Leblanc, 2010) and the period of childhood and youth likely constitutes a critical phase of life to establish long-term activity habits (Telama et al., 2014), a large proportion of children and adolescents does not meet physical activity guidelines (Colley et al., 2011; Hallal et al., 2012). Regular exercise behavior in leisure time, due to its higher intensity

compared to habitual physical activity, is a promising target for interventions (Samitz et al., 2011) and a lot of research has therefore been devoted to the determinants of exercise behavior, with studies traditionally focusing on environmental determinants such as socioeconomic status, access to exercise facilities and social support (Biddle & Mutrie, 2008; Sallis et al., 2000; van der Horst et al., 2007). Twin studies provide an important addition to these efforts as they allow for the examination of how much of the population variance in exercise behavior is due to factors shared by family members (as opposed to *non-shared environmental factors*) and the extent to which these familial factors are *shared genetic* factors or *shared environmental* factors.

Most twin studies on exercise behavior have been conducted in adults, with only a handful of studies in younger individuals (for an overview, see Figure 1 in Huppertz et al., 2012). The Netherlands Twin Register (NTR) has conducted studies on regular exercise behavior during leisure time in 7-, 10- and 12-year-old twins (Huppertz et al., 2012), as well as in 14-, 16- and 18-year-old twins (Boomsma, van den Bree, Orlebeke & Molenaar, 1989; de Geus, Boomsma & Snieder, 2003; de Moor et al., 2011; Koopmans, van Doornen & Boomsma, 1994; Stubbe et al., 2005; van der Aa et al., 2010). In childhood, shared environmental effects explained most of the variance in exercise behavior, whereas in late adolescence, genetic effects became more important. However, these studies have only reported the *relative* influence of genes and the environment, while the observed pattern could be caused by different mechanisms. It could arise from a simultaneous decrease in shared environmental variance and an increase in genetic variance, but also from a decrease in shared environmental variance only or an increase in genetic variance only. To elucidate the underlying mechanism, the absolute variance components have to be estimated across ages. Vink et al. (2011) investigated the effect of age on the absolute and relative genetic, shared environmental and non-shared environmental variance in exercise behavior of adult participants of the NTR and found that the genetic variance remained stable from age 19 to 50 years, whereas the non-shared environmental variance increased, giving rise to a gradual decrease in the heritability of adult exercise behavior with increasing age.

Although exercise behavior has been shown to track moderately from childhood to adolescence (Telama, 2009; Telama et al., 2014; Twisk, Kemper & van Mechelen, 2000), the nature of this stability has not been assessed in longitudinal twin studies. Tracking of exercise behavior from adolescence into young adulthood, however, has been assessed previously in Finnish twins with

longitudinal data at the ages of approximately 16, 17, 19 and 26 years (Aaltonen, Ortega-Alonso, Kujala & Kaprio, 2013). The genetic correlations across ages ranged between 0.78 and 0.82 for males and between 0.54 and 0.67 for females. The shared environmental correlations ranged between 0.53 and 0.76 for males and between 0.73 and 0.85 for females, indicating that both stable and new genetic and environmental factors affect exercise behavior in this age range, with some more stability of genetic influences in males and of shared environmental influences in females. The non-shared environmental correlations were lower, in part reflecting that they incorporate measurement error, which may be specific to each measurement occasion.

In the above study by Aaltonen et al. (2013), the genetic and shared environmental correlations over time were retrieved by means of a Cholesky decomposition that does not assume any specific underlying structure of the data. So-called transmission or simplex models instead assume that successive measures of exercise behavior are causally linked so that the behavior at each new age builds upon earlier experiences. In addition to the effects of past behavior ("transmission"), new influences may enter the picture at each phase to account for changes in exercise behavior ("innovation"). In a genetically informative longitudinal study, it is possible to go one step further and to explore transmission and innovation at the level of the variance components (Neale & Cardon, 1992). In such a study, one can account for the fact that genetic and environmental influences may show different patterns of transmission and innovation. For example, the genetic contribution to exercise behavior during leisure time could be largely transmitted from age to age and additionally, new genetic influences could come into play during development. If environmental effects on stability, in turn, were small, this would be reflected in larger innovation compared to the transmission effects. The pattern may be particularly complex for children's and adolescents' exercise behavior in view of the large changes in the genetic architecture over time.

This study aims to 1) investigate the effect of age on the absolute and the relative genetic, shared environmental and non-shared environmental variance of exercise behavior in childhood and adolescence and to 2) elucidate the longitudinal genetic structure of exercise behavior by assessing transmission and innovation of the genetic and the shared environmental components over time. For these purposes, we fitted both an age-moderation model and a simplex model on data of twins aged approximately 7, 10, 12, 14, 16 and 18 years.

METHODS

Participants

The NTR provided data on exercise behavior of twins aged approximately 7 ("survey 7", N= 7,394), 10 ("survey 10", N= 8,111), 12 ("survey 12", N= 14,916), 14 ("survey 14", N= 9,621), 16 ("survey 16", N= 6,585) and 18 years ("survey 18", N= 2,883; van Beijsterveldt et al., 2013; Willemsen et al., 2013). From the total dataset, 375 participants were excluded due to diseases or physical handicaps that may prevent them from being physically active (e.g., congenital heart disease, hemiplegia). For the surveys 14, 16 and 18, an injury at the time of assessment led to exclusion of the exercise data for that specific survey (N= 449 for survey 14, N= 490 for survey 16, N= 69 for survey 18). The top 0.1% of all observations within each survey (that is those with unrealistically high scores on exercise behavior) were excluded as outliers (N= 48 observations). The final sample consisted of 27,332 twins (48.1% males, 51.9% females), with two measurements for 6,861 individuals, three measurements for 4,779 individuals and four measurements for 1,341 individuals. The longitudinal structure included 2-year follow-ups (surveys 10 & 12, 12 & 14, 14 & 16, 16 & 18), a 3-year follow-up (7 & 10), 4-year follow-ups (10 & 14, 12 & 16, 14 & 18), a 5-year follow-up (7 & 12), 6-year follow-ups (10 & 16, 12 & 18) and a 7-year follow-up (7 & 14). Most data were collected around age 12, because 1) items assessing exercise behavior were first introduced to survey 12 in 1999 and to the other surveys approximately five years later (2004/2005) and 2) some participants were too old to provide data on for instance survey 7 at the time that the exercise items were included, whereas others were not old enough yet to provide data on for instance survey 18 at the time that the data were analyzed. The number of twins and complete twin pairs for each survey, split by zygosity, are presented in Table 1.

TABLE 1 Number of twins (complete pairs) with data on exercise behavior after applying exclusion criteria, split by survey and zygosity.

	Survey 7	Survey 10	Survey 12	Survey 14	Survey 16	Survey 18
MZM	1213 (604)	1345 (668)	2451 (1213)	1328 (571)	927 (383)	344 (140)
DZM	1300 (646)	1366 (673)	2335 (1141)	1225 (500)	777 (277)	302 (106)
MZF	1322 (658)	1439 (716)	2830 (1402)	1997 (880)	1393 (576)	774 (318)
DZF	1134 (564)	1210 (599)	2214 (1090)	1589 (680)	1080 (399)	552 (213)
DOS	2362 (1174)	2647 (1310)	4799 (2358)	2891 (1153)	1841 (629)	787 (253)
Total	7331 (3646)	8007 (3966)	14629 (7204)	9030 (3784)	6018 (2264)	2759 (1030)

MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic of opposite-sex.

For 18.5% of the same-sex twin pairs, zygosity was determined by blood group or DNA typing. For the remaining ones, it was determined by survey items on physical similarities and confusion by family members and strangers. Zygosity classification based on these items has shown 93%-97% agreement with DNA polymorphisms (Rietveld et al., 2000; Willemsen, Posthuma & Boomsma, 2005). Parents consented to take part in research of the NTR upon registration. Around the age of 13 years, adolescent twins provided their informed consent to fill out surveys. The data collection protocol was approved by the Medical Research Ethics Committee of the VU University Medical Center (IRB letter May 2007 for parental report and letter no. 2003/182 for adolescent self-report).

Measures

Exercise behavior during leisure time was assessed with similar measures across surveys. In surveys 7, 10 and 12, parents were provided with a list of common exercise activities in the Netherlands (such as athletics, badminton, ballet/dance, basketball, fitness training, gymnastics, handball, jogging/ running, hockey, netball, horseback riding, (ice-)skating, tennis, martial arts, soccer, swimming, volleyball), plus the option to add additional activities, and were asked 1) whether or not their children participated in the exercise activities and if so, 2) for how many years, 3) for how many months a year, 4) how many times a week and 5) how many minutes each time they participated in the respective activity. Adolescents were asked to report on their own behavior in essentially the same way. This study focuses on regular exercise behavior during leisure time. This includes both supervised and unsupervised activities. It excludes physical activities related to transportation (walking, biking), physical education classes and irregular exercise activities that were initiated less than half a year ago or that were performed for less than three months per year (e.g., ski holidays).

Exercise behavior was quantified as weekly metabolic equivalents of task (MET) hours. Each activity was assigned a MET score, based on Ridley et al. (2008)'s compendium of energy expenditures for youth. A MET score represents the energy that is expended to perform a specific activity relative to the standard resting metabolic rate, which would be one MET. For instance, running at a moderate level requires 8.5 times the energy that is used while sitting quietly and thus running has a MET score of 8.5. Individuals who did not participate in any exercise activities received a weekly MET hours score of zero. For the remaining individuals, the product of the MET score, weekly frequency and duration was summed across all exercise activities to obtain "total weekly MET

hours that were spent on regular exercise activities during leisure time".

For the surveys 7, 10 and 12, both parents reported exercise behavior on their children for 59.4%, 23.1% and 42.1% of the sample, respectively. For these cases, the average rating of the parents was used as the correlations between mothers' and fathers' ratings were high (0.74, 0.88 and 0.89, respectively). In addition, 37.5% (survey 7), 75.8% (survey 10) and 56.5% (survey 12) of the ratings were based on maternal report only, and 3.1%, 1.1% and 1.4% on paternal report only, respectively. After survey 12, self-ratings were analyzed.

Statistical analyses

The percentage of non-exercisers (individuals with zero MET hours per week) increased with age (13% for survey 7, 13% for survey 10, 14% for survey 12, 21% for survey 14, 28% for survey 16 and 40% for survey 18). This led to a highly skewed distribution of the phenotype for the older ages which could not be corrected by simple transformation. These censored data would have led to downward biases of the shared environmental components and upward biases of the non-shared environmental components (Derks, Dolan & Boomsma, 2004). Therefore, the data were categorized into three groups (coded 0, 1, 2), based on the following cutoffs: 0) >=0 and <5 weekly MET hours ("low"), 1) >=5 and <20 MET weekly hours ("middle"), and 2) >=20 weekly MET hours ("high"). These cutoffs were chosen based on the condition that for each survey, at least 10% of the individuals should fall into each group. The data were analyzed using liability threshold models (Falconer & Mackay, 1960; Wright, 1934), with two thresholds separating the three groups. These models assume that a latent continuous liability underlies the skewed distribution of the measured phenotype. The resemblance of twins was thus calculated based on this liability. We expected large changes in means and variances with age. These can either be taken into account by constraining the means and variances and allowing the thresholds to vary, or by constraining the thresholds and allowing the means and variances to vary. The second approach was chosen here for a more straightforward interpretation of the results. The thresholds were constrained to -0.64 and 0.23, respectively, which are the z-scores that correspond to the percentage of individuals in the three exercise-categories of the cross-sectional dataset.

The first set of analyses aimed to investigate the effect of age on the absolute and the relative contribution of genetic factors ("A" for "additive genetic"), environmental factors that are shared within twin pairs ("C" for "common

environmental") and non-shared environmental factors ("E", including measurement error) to the total variance in exercise behavior in childhood and youth. To get a rough impression of the genetic architecture, polychoric twin correlations were calculated for each survey (7, 10, 12, 14, 16 and 18) based on so-called saturated models. These models estimate the twin correlations for each sex-by-zygosity group without attempting to model the correlations as a function of genes or the environment.

Next, a model specifying the genetic and environmental architecture of the liability to exercise behavior was fitted to the data, namely a moderation model with linear and quadratic effects of z-transformed age as moderators on the means and variance components (Medland, Neale, Eaves & Neale, 2009; Purcell, 2002; Purcell & Koenen, 2005). A cross-sectional dataset was created by selecting one observation for each individual out of the full, longitudinal dataset. The selection favored data points that were collected for both twins of a pair within the same survey and it was aimed to select approximately the same number of observations for all ages. Based on previous studies, we decided to fit a model to these data that included A-, C- and E- components and that allowed for quantitative and qualitative sex differences. *Quantitative sex differences* were taken into account by estimating separate parameters for males and females. Based on our previous work (Huppertz et al., 2012; Stubbe et al., 2005), *qualitative sex differences* were modelled by freely estimating the correlation between the latent shared environmental components in dizygotic twins of opposite-sex (DOS) instead of constraining them to 1, while leaving the genetic correlation constrained to 0.5. In total, 26 parameters were estimated: 2 grand means (1 for males, 1 for females), 6 variance components (A, C, E, for males and females separately), 1 shared environmental correlation between DOS twins, the linear and quadratic effects of age on the means (4 parameters) and on the latent variance components (12 parameters), and the linear effect of age on the shared environmental correlation between DOS twins (1 parameter). The latter was done to account for changes in qualitative sex differences with age.

The second set of analyses aimed to elucidate the longitudinal structure of exercise behavior. To get a rough impression of the stability of exercise behavior over time, phenotypic polychoric correlations across the surveys 7, 10, 12, 14, 16 and 18 were calculated with the R-package polycor, based on one randomly selected individual per twin pair. To gain insight into the relative contribution of genetic and environmental factors to these longitudinal correlations, the within- and cross-survey twin correlations were calculated for

each of the five sex-by-zygosity groups using the same package.

Finally, a longitudinal genetic model was fitted to the full dataset to decompose the within- and cross-survey (co-)variance into genetic, shared environmental and non-shared environmental effects. The A-components and the C-components were modelled with a simplex structure (Boomsma & Molenaar, 1987), whereas the E-components were modelled with a Cholesky structure, where every latent variable that influences one time point also influences subsequent, but not previous, time points (Neale & Cardon, 1992). The Cholesky structure can thus be thought of as a "full model" and was chosen for the E-component as no specific underlying structure is to be expected since the E-component is a mixture of "real" non-shared environmental influences and measurement error. The simplex structure, in contrast, explicitly differentiates between transmission and innovation. The analyses were conducted based on data of same-sex twin pairs only, while quantitative sex differences were taken into account by estimating separate parameters for males and females. In total, 49 parameters were estimated for each sex: one mean for each survey (6 parameters), genetic transmission (5 parameters), genetic innovation (6 parameters), shared environmental transmission (5 parameters), shared environmental innovation (6 parameters) and non-shared environmental effects (21 parameters). If not stated otherwise, the genetic analyses were conducted in the software package OpenMx in R (Boker et al., 2011).

RESULTS

Table 2 contains the mean age of the participants for each survey, as well as the number and percentage of individuals engaged in the different levels of exercise behavior. For both sexes, the percentage of individuals with low exercise behavior increased from survey 12 to survey 18. The reverse trend was seen for individuals with a moderate level of exercise behavior of which the relative frequency decreased from survey 7 to 18. With the exception of a smaller percentage at survey 7, the percentage of individuals with a high level of exercise behavior remained fairly constant. In all surveys, males exercised significantly more often at a high intensity level than females ($p<0.001$).

Age-moderation model

The polychoric twin correlations of each survey are depicted in Figure 1. The MZ twin correlations were only marginally larger than the DZ twin correlations for survey 7, but the difference between MZ and DZ correlations increased with

increasing age. At the same time, the MZ correlations were generally smaller than twice the DZ correlations, suggesting shared environmental influence. The same-sex correlations within each zygosity were comparable for males and females, suggesting no quantitative sex differences. The DOS correlations were smaller than what would be expected based on the same-sex DZ correlations which implies qualitative sex differences. This difference decreased with increasing age and disappeared in later adolescence.

TABLE 2 Mean age (standard deviation) and the number and percentage of individuals engaged in the different levels of exercise behavior, split by sex and survey.

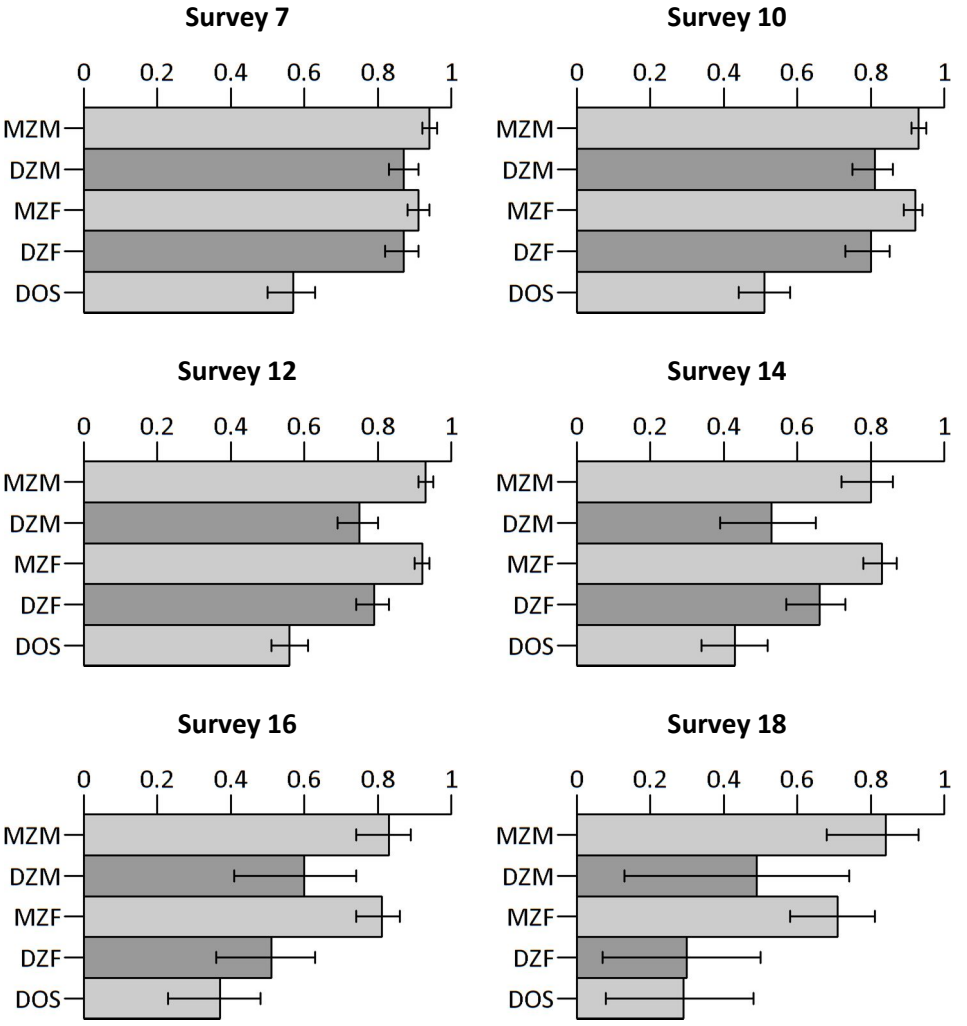
A. Males	Age (SD)*	Low	Middle	High
Survey 7	7.52 (.34)	780, 21.1%	1835, 49.7%	1078, 29.2%
Survey 10	9.84 (.43)	679, 16.8%	1270, 31.5%	2082, 51.6%
Survey 12	12.25 (.40)	1200, 16.7%	1725, 24.0%	4249, 59.2%
Survey 14	14.61 (.60)	809, 20.8%	732, 18.8%	2351, 60.4%
Survey 16	16.87 (.45)	657, 26.2%	376, 15.0%	1479, 58.9%
Survey 18	18.77 (.51)	360, 37.6%	140, 14.6%	457, 47.8%

B. Females	Low	Middle	High
Survey 7	1172, 32.2%	2003, 55.1%	463, 12.7%
Survey 10	1024, 25.8%	1779, 44.7%	1173, 29.5%
Survey 12	1751, 23.5%	3065, 41.1%	2639, 35.4%
Survey 14	1351, 26.3%	1620, 31.5%	2167, 42.2%
Survey 16	1182, 33.7%	993, 28.3%	1331, 38.0%
Survey 18	786, 43.6%	467, 25.9%	549, 30.5%

**Both males and females included; Low=">=0 & <5 weekly MET hours", middle= ">=5 & <20" and high=">=20".*

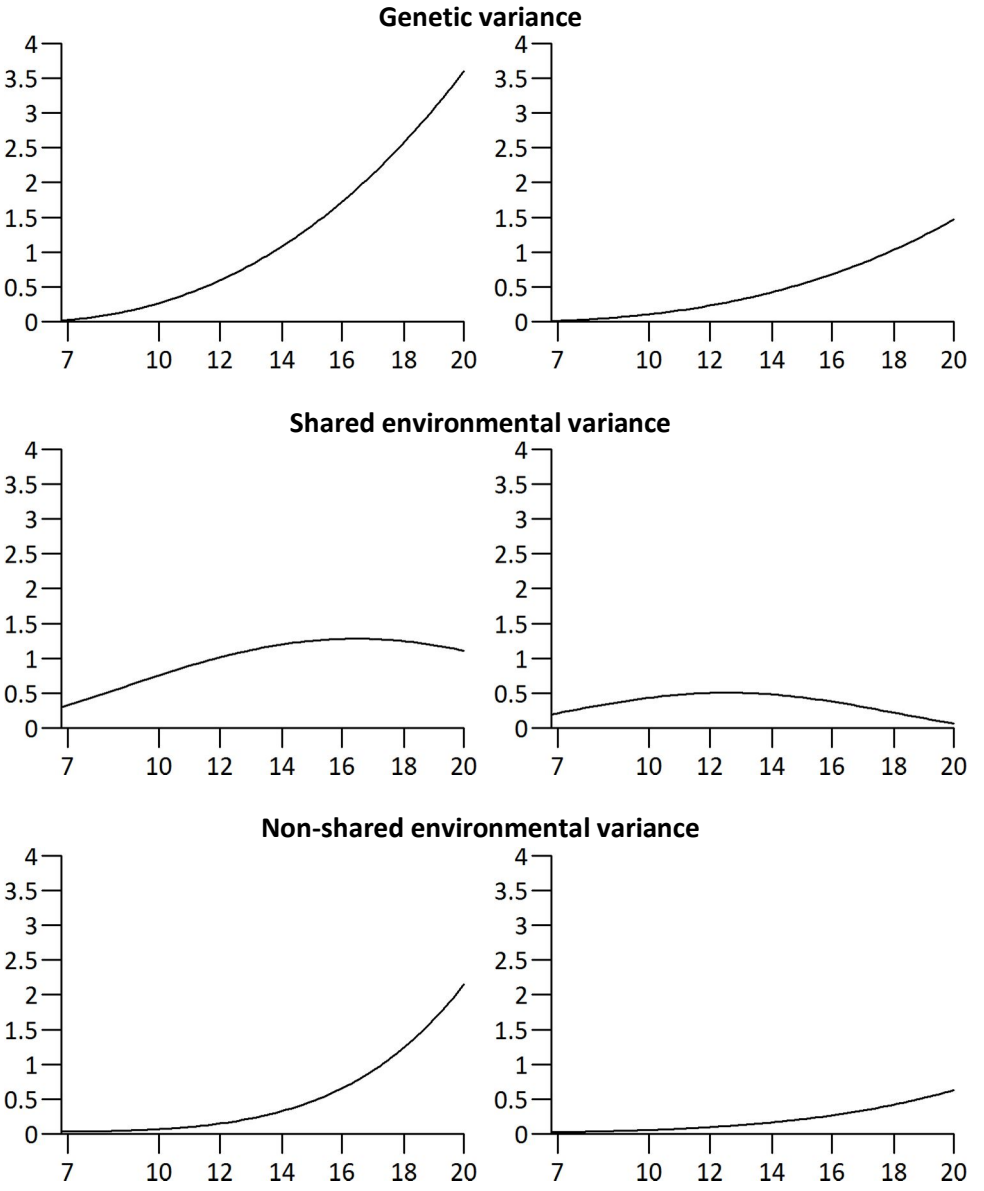
The *unstandardized* genetic, shared environmental and non-shared environmental variance of exercise behavior across surveys is depicted in Figure 2. Although age was z-transformed for the analyses, the x-axes depict age in years, for clarity (age range: 6.78-19.99 years). Based on 99% confidence intervals, the linear effects on the A-components were significant for males (β= 0.50) and females (β= 0.31), whereas the quadratic effects were not. For the C-components, only the quadratic effect in females was significant (β= -0.12). Finally, the linear effects on the E-components were significant for males (β= 0.33) and females (β= 0.17), as were the quadratic effects (β= 0.10 and β= 0.03, respectively). In sum, there was a large increase in genetic variance with age, paired to a more modest increase in non-shared environmental variance. The

FIGURE 1 Twin correlations of exercise behavior and 99% confidence intervals based on fully saturated threshold models.



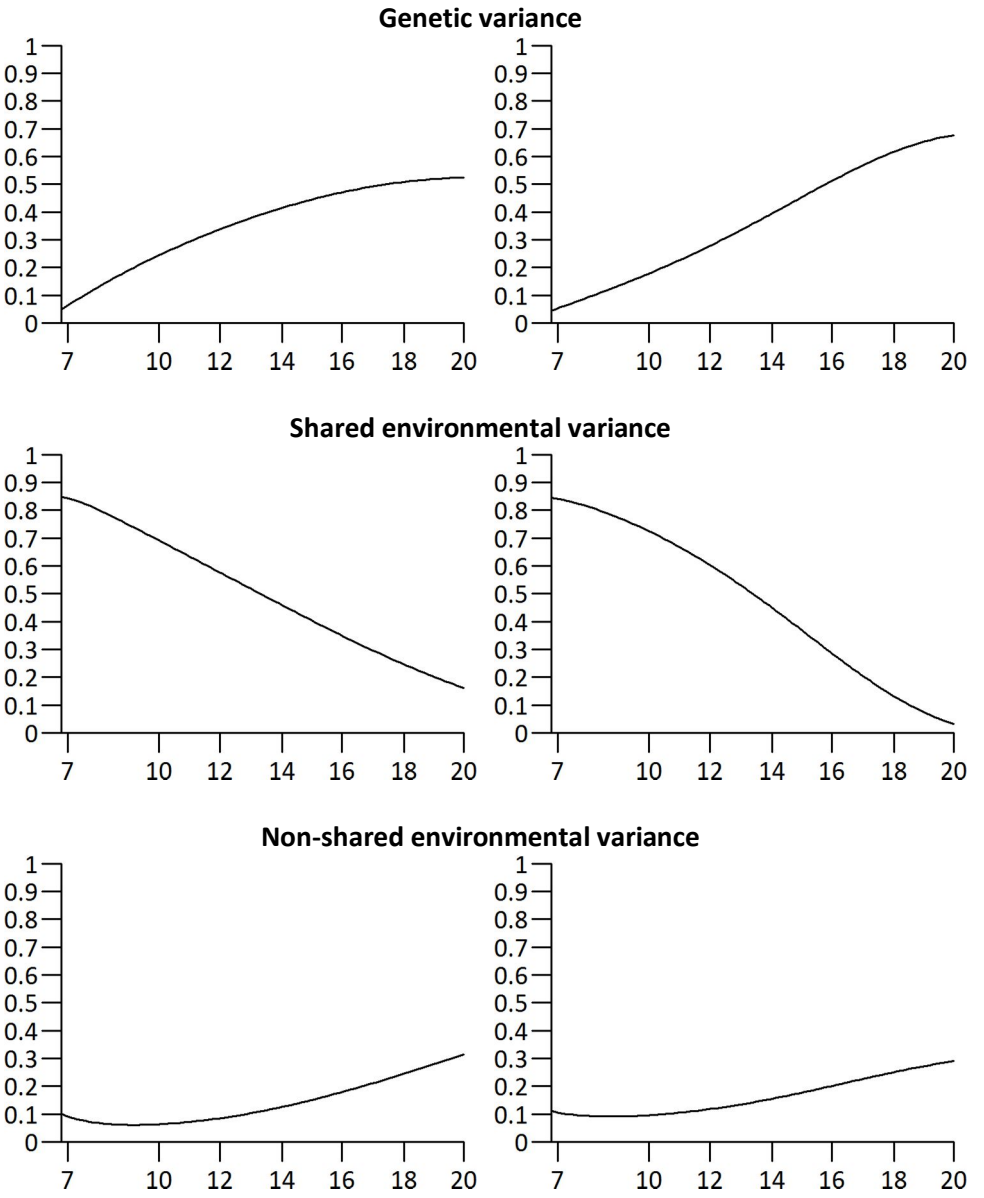
MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic of opposite-sex.

FIGURE 2 Changes in the *absolute* contribution of genetic, shared environmental and non-shared environmental factors to variance in exercise behavior as a function of age, for males (left) and females (right) separately.



Am=A-component for males [linear beta=.50 (99% CI: .30; .69); quadratic beta=.02 (-.13; .16)], Cm=C-component for males [.18 (-.16; .37); -.09 (-.28; .04)], Em=E-component for males [.33 (.26; .40); .10 (.06; .15)], Af=A-component for females [.31 (.23; .38); .02 (-.05; .09)], Cf=C-component for females [-.01 (-.14; .09); -.12 (-.20; -.04)], Ef=E-component for females [.17 (.14; .20); .03 (.01; .06)].

FIGURE 3 Changes in the *relative* contribution of genetic, shared environmental and non-shared environmental factors to variance in exercise behavior as a function of age, for males (left) and females (right) separately.



Am=A-component for males, Cm=C-component for males, Em=E-component for males, Af=A-component for females, Cf=C-component for females, Ef=E-component for females.

influence of shared environmental effects showed an inversed U-shape for females, but the effect was small compared to the increase in genetic variance. It should be noted that the total variance was much larger for males than for females. Next, the genetic, shared environmental and non-shared environmental variances were standardized to obtain their relative contribution, for males and females separately. The standardized estimates are depicted in Figure 3. The A-component increased with age, whereas C decreased and the relative contribution of E remained relatively low at all ages.

Simplex model

The phenotypic polychoric correlations across the surveys 7 to 18 are shown in Table 3. The correlations, which reflect tracking of exercise behavior over time, were mostly moderate, ranging from 0.23 to 0.75, with larger correlations between surveys in closer proximity to each other and in older individuals. Supplementary table I depicts the within- and cross-survey twin correlations. MZ cross-survey correlations were generally larger than DZ cross-survey correlations, implying genetic influences on stability. In combination with the lower longitudinal correlations for surveys that were further apart, this reinforces the use of a genetic simplex model (Boomsma & Molenaar, 1987).

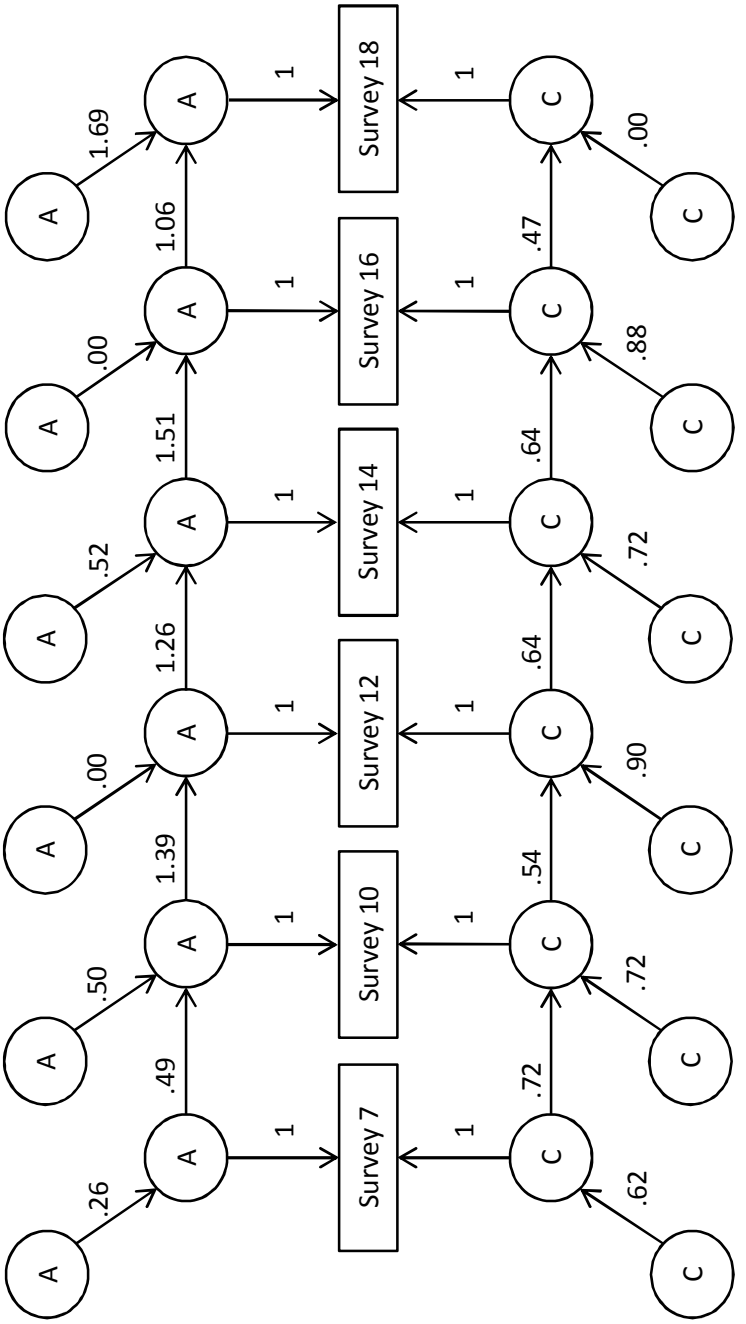
Figure 4 depicts the path estimates of the genetic and shared environmental components based on the simplex model. The depicted parameters were all freely estimated. Table 4 depicts the relative contribution of genetic, shared environmental and non-shared environmental effects to variance in exercise behavior for each age. The genetic and shared environmental variance components are further separated into the part that is due to transmission and the part that is due to innovation. The transmission part is calculated based on all paths that preceded the respective survey, whereas the innovation part is calculated based on the innovation path for the respective survey only. In males, genetic transmission was strong from survey 10 onwards and relatively more important than genetic innovation, with the exception of a strong genetic innovation at survey 18. A different pattern appeared for females in that genetic effects were also transmitted across surveys but new effects consistently emerged for each survey, with approximately the same amount of innovation and transmission for the surveys 16 and 18. The shared environmental effects were marked by both transmission and innovation, with a tendency for innovation being more important in males and transmission in females. For survey 18 in males and the surveys 16 and 18 in females, no new shared environmental effects emerged.

TABLE 3 Phenotypic correlations across repeated measurements, for males and females separately (standard error; number of complete pairs).

Survey	7	10	12	14	16	18
7	-	.46 (.05; 446)	.28 (.05; 603)	.26 (.07; 363)	*	*
10	.49 (.05; 410)	-	.53 (.04; 789)	.52 (.06; 364)	.43 (.07; 279)	*
12	.31 (.05; 621)	.52 (.03; 808)	-	.61 (.03; 1472)	.56 (.03; 926)	.37 (.07; 291)
14	.23 (.06; 415)	.42 (.05; 481)	.59 (.02; 1798)	-	.75 (.03; 619)	.58 (.09; 136)
16	*	.28 (.06; 364)	.47 (.03; 1235)	.70 (.02; 973)	-	.65 (.06; 206)
18	*	*	.42 (.05; 591)	.53 (.06; 300)	.64 (.04; 395)	*

*Above diagonal: males; Below diagonal: females; Based on one randomly selected individual per family; *No data were available yet.*

FIGURE 4 The path estimates of the genetic and shared environmental components of exercise behavior as estimated with the genetic simplex model, for males and females separately. The paths of the non-shared environmental components were omitted for clarity. Males:



Females:

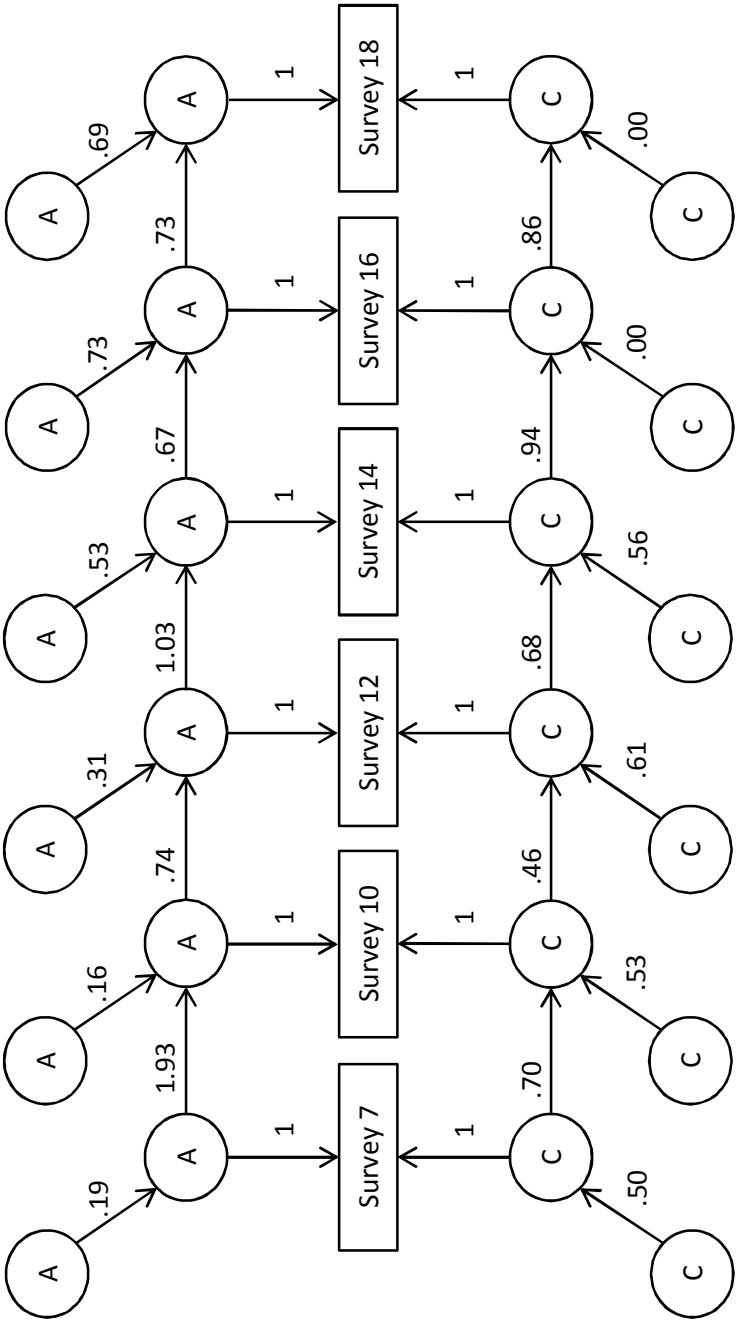

Survey 7
Survey 10
Survey 12
Survey 14
Survey 16
Survey 18
A
C
.19
.16
.31
.53
.73
.69
1.93
.74
1.03
.67
.73
.70
.46
.68
.94
.86
.50
.53
.61
.56
.00
.00
1

TABLE 4 The relative contribution of genetic (A) and shared environmental (C) effects (split by transmission and innovation), as well as non-shared environmental (E) effects to variance in exercise behavior based on the genetic simplex model, split by sex and survey.

A. Males	**A (T, I)**	**C (T, I)**	**E**
Survey 7	.14	.80	.06
Survey 10	.26 (.02 + .24)	.68 (.19 + .49)	.07
Survey 12	.31 (.31 + .00)	.62 (.13 + .49)	.07
Survey 14	.43 (.32 + .11)	.36 (.16 + .20)	.21
Survey 16	.56 (.56 + .00)	.27 (.09 + .18)	.17
Survey 18	.79 (.39 + .40)	.04 (.04 + .00)	.17

B. Females	**A (T, I)**	**C (T, I)**	**E**
Survey 7	.12	.80	.08
Survey 10	.26 (.22 + .04)	.65 (.20 + .45)	.08
Survey 12	.27 (.13 + .14)	.65 (.12 + .53)	.08
Survey 14	.40 (.16 + .24)	.43 (.17 + .26)	.17
Survey 16	.49 (.14 + .35)	.31 (.31 + .00)	.20
Survey 18	.49 (.22 + .27)	.19 (.19 + .00)	.33

In brackets: "due to transmission" + "due to innovation".

DISCUSSION

This study aimed to investigate the impact of genes and the environment on the development of exercise behavior across childhood and adolescence. In this period, the total variance in exercise behavior increased because relative to children, adolescents were less often engaged in moderate levels of exercise behavior and more often in low levels, whereas the frequency of individuals with high levels of exercise behavior remained fairly constant throughout childhood and youth. Two genetic models were fitted to the data: an age-moderation model and a simplex model. The age-moderation model used the largest available cross-sectional dataset and revealed that the absolute genetic variance in exercise behavior increased with age, whereas the absolute shared environmental variance remained relatively stable. Therefore, the *relative* contribution of shared environmental factors decreased across incremental age groups. The simplex model used repeated measures within the same persons to detect the sources of developmental changes in exercise behavior and showed that genetic factors influencing exercise behavior were a main source of stability, particularly in males. Shared environmental factors showed marked innovation around the ages of 10 and 12 years in both sexes. The role of new

shared environmental effects diminished after age 12 and disappeared around the age of 18 years.

Taken together, the age-moderation model and the simplex model converge on a singular pattern. Individual differences in childhood exercise behavior are strongly determined by shared environmental factors with 80% of the variance determined by C around age 7. Throughout the development from age 7 to age 18, genetic factors gradually overwhelm the effects of the shared environment, especially in males. Age 14 is a tipping point where the relative influence of genes definitively trumps that of the shared environment. At age 18, heritability of exercise behavior in young men is very high (79%), whereas it is moderately high in young women (49%), where the effects of the shared environment still linger (19%).

Several previous twin studies have explored the heritability of exercise behavior in childhood and youth. Huppertz et al. (2012) investigated the heritability of exercise behavior for the ages 7, 10 and 12 years, based on a subset of the data that were used for the present study. With the exception of 10-year-old boys (A= 66%, C= 25%), most of the variance in exercise behavior of 7- to 12-year-olds could be explained by shared environmental factors (C= 50-72%). There were significant qualitative sex differences for the ages of 7 and 12 years. At age 10, a drop in heritability was reported, which was not found in the present study, probably due to the larger sample size. The large shared environmental influence in childhood is in line with findings of small-scale studies on total physical activity measured with accelerometers (Fisher et al., 2010), respiratory gas exchange and doubly labeled water (Franks et al., 2005), and pedometers (Plomin & Foch, 1980), although it should be noted that these studies investigated somewhat different phenotypes, which limits comparability to our study.

Shared environmental factors influencing exercise behavior have also been noted in the age range of adolescence before. Maia, Thomis and Beunen (2002) calculated the heritability of the sports participation index in 12- to 25-year-old twins (N= 411 pairs) and found that for males, 68% of the variance was explained by genetic effects and 20% by shared environmental effects. Estimates were 40% and 28% for females. In a larger sample (N= 5,216 individuals at baseline), Aaltonen et al. (2013) found heritability estimates of around 43-52% in approximately 16- to 19-year-old twins, with a shared environmental influence of 18-26%. Two other studies, however, report results that appear not immediately consistent with our finding. We suspect that this

reflects the practice of reporting data on the best fitting AE model rather than a full ACE when dropping C is found to deliver the most parsimonious model. Non-significance of the C-component does not necessarily mean that it is absent, however, but simply that it is relatively hard to pick up with classical twin studies, unless the sample size is very large (Posthuma & Boomsma, 2000). For instance, van der Aa et al. (2010) investigated the heritability of exercise behavior in 14-, 16- and 18-year-old twins on a subset of the dataset that was used for the present study. Genetic effects appeared to be the most important contributors to the total variance in boys and girls (A= 72-85%), with the exception of 14-year-old girls (A= 38%, C= 46%). Likewise, Beunen and Thomis (1999) have investigated sports participation in 15-year-old twins (N= 183 individuals) and found that for boys, most of the variance (83%) was explained by genetic factors after dropping C from the model. For girls, C could not be dropped and only 44% of the individual differences in sports participation were explained by genetic factors, with 54% due to shared environmental factors.

Overall, existing studies are well in line with the general pattern in our study in that individual differences in exercise behavior are strongly determined by shared environmental factors in childhood but that in adolescence, genetic factors gradually overwhelm the effects of the shared environment, especially in males. The shared environmental factors affecting exercise behavior may consist mainly of parental influences in children (Huppertz et al., 2012). Parents often act as gatekeepers to children's exercise activities by providing necessary resources and support. They are also involved in the timing and initial choice of specific exercise activities and might thus affect their children's skill acquisition and, ultimately, their exercise performance (Timmons, Naylor & Pfeiffer, 2007). Parents may be partly responsible for the qualitative sex differences seen in childhood. It has been reported that boys tend to receive more parental support to be physically active than girls, although the findings are not unanimous (Anderson et al., 2009; Beets et al., 2010; Gustafson & Rhodes, 2006). With increasing age, the social support received by peers starts to supersede that of parents (Chan, Lonsdale & Fung, 2012). The influence of peers may well account for the innovation we noted in the shared environmental variance with increasing age as well as the absence of innovation in 18-year-old males and 16- and 18-year-old females when parental influence on exercise behavior may all have disappeared. The quality of coaches and trainers might contribute to both the shared and the non-shared environmental variance throughout childhood and youth (Chan et al., 2012).

The nature of the genetic factors that increasingly affect exercise behavior

throughout childhood and adolescence remains uncharted, but a number of testable hypotheses have been put forward (Bryan et al., 2007; de Geus & de Moor, 2011). The first one suggests genetic effects on a homeostatic "need to be active", which has been operationalized in rodent studies by spontaneous wheel running (Knab & Lightfoot, 2010; Lightfoot, Turner, Daves, Vordermark & Kleeberger, 2004). Large strain differences exist in spontaneous running when animals are granted free access to a wheel, and selective breeding confirms that this "activity drive" is a heritable trait (Garland et al., 2011). In humans, the activity drive may be an integral part of *personality traits* like extraversion, sensation seeking or impulsivity. Other personality traits like neuroticism or conscientiousness may also come into play, e.g., by determining individual differences in attraction to regular exercise behavior and the ability to persist. The personality traits extraversion, sensation seeking and conscientiousness are indeed positively related to exercise behavior, whereas neuroticism is negatively related (de Moor et al., 2006; Rhodes & Smith, 2006). Personality may furthermore play a role in the formation of attitudes towards exercise, in particular the perception of the benefits of and the barriers towards exercise behavior. As personality traits as well as exercise attitudes have a partly genetic basis (de Moor et al., 2012; Huppertz et al., 2014; Jang, Livesley & Vernon, 1996), they are likely to contribute to the genetic variation in exercise behavior (de Geus & de Moor, 2011). Furthermore, as personality is considered to be a rather stable trait from early childhood onwards, it may mainly explain the transmission, but not innovation, of genetic effects across ages.

Apart from personality traits and exercise attitudes, large individual differences have been observed in the *acute mood response to activity bouts* (Ekkekakis, 2008; Parfitt & Hughes, 2009). Low-intensity exercise evokes rewarding reactions in most individuals, whereas high-intensity exercise evokes aversive reactions in most individuals. The responses to intermediate levels of exercise, however, are much more variable, with some individuals reporting rewarding feelings, whilst others report aversive feelings (Ekkekakis, Hall & Petruzzello, 2005). Individual differences in the acute psychological response to exercise are likely to be largely explained by genetic factors (Knab & Lightfoot, 2010). If this response becomes increasingly more important to maintain regular exercise behavior from childhood to adolescence, it could be a source of the genetic innovation that was observed.

Finally, *fitness and exercise ability* (as in endurance, strength, flexibility, motor coordination, training response and similar) have been shown to be highly heritable traits (Bouchard & Hoffman, 2011; Bouchard & Rankinen, 2001). As

adolescents tend to seek out activities that they are good at and to avoid those that they are not good at, an adolescent endowed to be good at exercising (or to improve fast with training) will be more likely to keep pursuing physical exercise on a regular basis (de Geus & de Moor, 2008). In males, strong genetic transmission is seen from age 10 onwards which ultimately results in a very high heritability of exercise behavior at age 18 (79%). The increase in genetic variance is less steep in girls. Exercise ability and trainability might explain part of this difference, as boys are more likely to take part in team sports and competitive sports (implying more comparison among peers) and as perceived athletic ability is culturally more important to boys than to girls. The focus on adolescents here should not detract from the fact that for younger children, perceived competence may also play a role in the maintenance of exercise activities. However, the strong increase in genetic variance suggests that innate differences in competence are more relevant in adolescence than in childhood. Shared environmental influences probably suppress innate differences in the latter group.

As stated in the introduction, the development of exercise behavior from childhood to adolescence has rarely been assessed in twin studies. In part, this may be due to the difficulty of repeatedly assessing exercise behavior in a large set of twins, especially in young twins. Ideally, one would assess exercise behavior with a combination of objective and subjective measures. As this was not feasible for the present study, we relied on subjective reports only, which may have led to biases. In contrast to total physical activity, however, exercise behavior is structured and clearly defined in time and can therefore be recalled with acceptable accuracy. The correlations between mothers' and fathers' ratings of their children's exercise behavior ranged between 0.74 and 0.89 in this study and the six-month test-retest reliability of this measure was found to be 0.91 (Stubbe et al., 2007) and 0.82 (de Moor et al., 2008) in our previous work. Furthermore, it has been associated with the sweat index and the frequency of being physically active for at least 20 minutes in the past 6 months (de Moor & de Geus, 2013), which are likely to be largely affected by exercise behavior. Finally, the results are in line with previous studies that used objective measures of physical activity.

It should be noted that exercise behavior was assessed through parental report for the surveys 7, 10 and 12, and through self-report for the surveys 14, 16 and 18. This may introduce potential rater effects that may mimic some of the patterns that were found. More specifically, self-report, where two individuals report on their own behavior, may lower twin correlations compared to

parental report, where the same individual, namely the parent, reports on both children (Kan, van Beijsterveldt, Bartels & Boomsma, 2014). For the self-reports, genetic models will estimate the genetic effects that are common to both raters as "A" and the genetic effects that are specific to each rater as "E". We indeed found a larger E-component in adolescents compared to children. Unfortunately, we cannot differentiate between the part of the E-component that reflects non-shared environmental effects and the part that reflects measurement error. However, we argue that as opposed to, for instance, ratings on psychopathology (Kan et al., 2014), informant dependency is less of a concern in exercise behavior, as this behavior is less dependent on subjective perceptions, but can be rather objectively reported as weekly frequency and duration. Moreover, in line with a recent study by Telama et al. (2014) and an earlier review (Telama, 2009), we found moderate-to-high tracking of exercise behavior across the entire age range, with larger correlations for surveys that were in closer proximity to each other and higher stability in the surveys targeting older twins, and with no deviant pattern from survey 12 to 14 (from parental report to self-report).

Although, in general, twin studies are the most elegant method to estimate the contribution of genes and the environment to variance in a trait, a number of critical assumptions have to be met to obtain valid results. First, it is assumed that twins are representative of the general population. As stated in our previous work (Huppertz et al. 2012), a specific limitation of using twins to understand the determinants of exercise behavior is that the findings may not generalize to families with siblings of different ages or a single child. Because twins have the same age, it is more convenient for parents to handle their twins as a pair (and thus to promote exercise behavior), than it would be in the case of siblings with a larger age difference. This might have led to a greater role of tangible support (a shared environmental factor) on the part of the parents compared to non-twin families. To confirm that there are no systematic differences in the percentage of non-exercisers and in the means and variances in weekly MET hours between multiples and siblings, we selected a group of multiples and a group of singletons of the NTR aged 13 to 18 years and compared their exercise behavior in narrow age ranges (Supplementary table II). We conclude that exercise behavior of twins is generalizable to the population-at-large. Second, modeling assumed that the twins' parents did not select each other based on the phenotype under study (or a correlated phenotype), whereas various studies have found evidence for significant spousal resemblance in exercise behavior (Aarnio, Winter, Kujala & Kaprio, 1997; Boomsma et al., 1989; Perusse, Leblanc & Bouchard, 1988; Perusse,

Tremblay, Leblanc & Bouchard, 1989; Seabra, Mendonca, Goring, Thomis & Maia, 2008). This may have led to a higher resemblance than expected of DZ twins in genes that affect exercise behavior, which would imply an overestimation of shared environmental variance. Third, the so-called equal environments assumption holds that environmental differences between MZ and DZ twins are not related to the phenotype under study. Otherwise, a higher similarity of MZ twins compared to DZ twins could be due to genetic influences, environmental influences, or both, whereas the classical twin design ascribes a difference in similarity to genetic factors only (Kendler, 1993). The equal environments assumption has been shown to hold for a wide range of phenotypes (Kendler, 1993), including physical activity-related traits (e.g., doing sports; Eriksson et al., 2006).

Notwithstanding its limitations, this study provides an important extension to the literature as it is the largest investigation of exercise behavior in twins aged 7 to 18 years. More than 27,000 individuals have provided data for this study and almost 13,000 individuals have provided data on more than one measurement moment. Exercise behavior was assessed in narrow ranges around the ages of 7, 10, 12, 14, 16 and 18 years, we modeled both the absolute and the relative contribution of genes, the shared environment and the non-shared environment to variance in exercise behavior with age, and we modelled the underlying developmental structure. Our age-moderation analysis confirmed the major role of shared environmental factors in children's exercise behavior and of genetic factors in adolescents' exercise behavior, implying that family-based interventions might work to increase exercise behavior in children, whereas individual-based interventions might be better suited for adolescents. Given the enormous complexity of factors that cause individual differences in exercise behavior, it is not surprising that "one-size-fits-all" interventions do not bring about satisfactory changes in behavior. Age-specific shared environmental and genetic determinants of differences between individuals need to be identified in order to develop personalized interventions that take into account human variation.

SUPPLEMENTARY TABLE I Within- and cross-survey twin correlations for the five zygosity groups separately (standard error; number of complete pairs).

	Survey 7, *Twin2*	**Survey 10**	**Survey 12**	**Survey 14**	**Survey 16**	**Survey 18**
MZM						
Survey 7, *Twin1*	.94 (.01; 604)	.45 (.09; 150)	.37 (.09; 198)	.29 (.12; 114)	*	*
Survey 10	.35 (.10; 150)	.93 (.01; 668)	.61 (.06; 262)	.42 (.11; 115)	.35 (.12; 103)	*
Survey 12	.37 (.09; 198)	.59 (.06; 262)	.93 (.01; 1213)	.61 (.04; 495)	.55 (.06; 357)	.25 (.13; 110)
Survey 14	.26 (.12; 123)	.40 (.11; 125)	.62 (.04; 504)	.80 (.03; 571)	.54 (.07; 226)	.56 (.15; 48)
Survey 16	*	.45 (.11; 103)	.55 (.06; 349)	.69 (.06; 208)	.82 (.03; 383)	.60 (.11; 76)
Survey 18	*	*	.49 (.11; 101)	.46 (.18; 44)	.58 (.12; 70)	.83 (.05; 140)
DZM						
Survey 7	.87 (.01; 646)	.46 (.09; 144)	.20 (.10; 191)	.07 (.14; 110)	*	*
Survey 10	.37 (.10; 144)	.81 (.02; 673)	.37 (.08; 256)	.39 (.12; 113)	.30 (.15; 75)	*
Survey 12	.10 (.10; 191)	.24 (.08; 256)	.75 (.02; 1141)	.31 (.06; 439)	.28 (.09; 257)	.17 (.15; 84)
Survey 14	.21 (.13; 111)	.36 (.11; 121)	.31 (.06; 438)	.52 (.05; 500)	.29 (.11; 168)	-.06 (.22; 43)
Survey 16	*	.38 (.13; 88)	.38 (.08; 272)	.38 (.09; 182	.59 (.06; 277)	.19 (.19; 53)
Survey 18	*	*	.07 (.14; 94)	.13 (.21; 43)	.35 (.17; 58)	.47 (.12; 106)

	Survey 7, *Twin2*	Survey 10	Survey 12	Survey 14	Survey 16	Survey 18
MZF						
Survey 7, *Twin1*	.91 (.01; 658)	.54 (.09; 133)	.29 (.08; 223)	.27 (.10; 151)	*	*
Survey 10	.59 (.08; 133)	.92 (.01; 716)	.45 (.06; 297)	.38 (.08; 185)	.23 (.10; 149)	*
Survey 12	.27 (.08; 223)	.48 (.06; 297)	.92 (.01; 1402)	.52 (.04; 708)	.45 (.05; 504)	.41 (.07; 245)
Survey 14	.22 (.10; 157)	.40 (.08; 202)	.54 (.04; 717)	.82 (.02; 880)	.60 (.05; 393)	.59 (.08; 138)
Survey 16	*	.31 (.09; 157)	.45 (.05; 514)	.61 (.04; 385)	.80 (.02; 576)	.70 (.06; 164)
Survey 18	*	*	.34 (.07; 245)	.54 (.09; 132)	.48 (.08; 160)	.69 (.04; 318)
DZF						
Survey 7	.87 (.02; 564)	.55 (.09; 121)	.30 (.09; 192)	.05 (.11; 131)	*	*
Survey 10	.45 (.10; 121)	.80 (.02; 599)	.45 (.07; 238)	.37 (.09; 141)	.27 (.11; 114)	*
Survey 12	.25 (.09; 191)	.36 (.07; 238)	.79 (.02; 1090)	.44 (.05; 518)	.35 (.06; 356)	.43 (.09; 153)
Survey 14	.17 (.11; 136)	.33 (.10; 147)	.39 (.05; 529)	.65 (.03; 680)	.49 (.06; 280)	.31 (.14; 74)
Survey 16	*	.32 (.11; 118)	.33 (.06; 341)	.47 (.07; 260)	.49 (.05; 399)	.36 (.11; 104)
Survey 18	*	*	.31 (.09; 156)	.31 (.14; 76)	.47 (.10; 107)	.30 (.08; 213)

	Survey 7, *Twin2*	Survey 10	Survey 12	Survey 14	Survey 16	Survey 18
DOS						
Survey 7, *Twin1*	.57 (.03; 1174)	.28 (.07; 297)	.18 (.06; 407)	.07 (.08; 254)	*	*
Survey 10	.22 (.07; 297)	.51 (.03; 1310)	.39 (.05; 508)	.16 (.08; 256)	.28 (.09; 195)	*
Survey 12	.13 (.07; 408)	.32 (.05; 509)	.56 (.02; 2358)	.31 (.04; 1049)	.28 (.05; 694)	.28 (.07; 313)
Survey 14	.29 (.09; 226)	.17 (.09; 227)	.37 (.04; 966)	.43 (.03; 1153)	.39 (.06; 451)	.04 (.11; 147)
Survey 16	*	.17 (.10; 167)	.30 (.05; 580)	.39 (.06; 400)	.36 (.05; 629)	.32 (.09; 175)
Survey 18	*	*	.29 (.09; 184)	.21 (.14; 89)	.31 (.11; 131)	.29 (.08; 253)

*MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic of opposite-sex; *No data were available yet.*

SUPPLEMENTARY TABLE II Percentage of non-exercisers and mean weekly MET hours (variance; N) for multiples versus singletons.

		% non-exercisers*		Weekly MET hours		
Age	**Sex**	**Multiple**	**Singleton**	**Multiple**	**Singleton**	**p****
13	**M**	20.4%	17.6%	28.7 (24.9; 314)	29.7 (24.6; 119)	.77
	F	22.7%	19.1%	20.5 (23.1; 423)	19.2 (18.8; 131)	.82
14	**M**	19.1%	28.3%	29.7 (26.6; 1282)	26.7 (25.8; 60)	.36
	F	21.1%	19.3%	21.4 (22.8; 1688)	23.6 (24.0; 88)	.40
15	**M**	20.1%	22.3%	32.1 (28.5; 528)	27.8 (24.5; 103)	.28
	F	25.2%	23.7%	21.3 (23.3; 686)	24.2 (26.1; 131)	.39
16	**M**	24.5%	24.3%	31.1 (29.3; 912)	30.8 (30.4; 210)	.70
	F	28.3%	26.3%	21.2 (23.9; 1292)	21.3 (25.3; 262)	.84
17	**M**	25.0%	30.1%	31.5 (29.6; 520)	30.6 (29.4; 206)	.75
	F	32.4%	30.4%	19.4 (21.9; 720)	19.6 (23.7; 273)	.91
18	**M**	25.8%	28.8%	31.4 (32.5; 62)	27.0 (29.1; 208)	.44
	F	41.8%	37.6%	16.3 (21.7; 122)	15.6 (19.3; 340)	.79

**<4 weekly MET hours; **p-value of comparing weekly MET hours according to Mann-Whitney U test.*

Chapter **5**

THE EFFECTS OF PARENTAL EDUCATION ON EXERCISE BEHAVIOR IN CHILDHOOD AND YOUTH: A STUDY IN DUTCH AND FINNISH TWINS

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ABSTRACT

Twin studies have estimated the relative contribution of genes and the environment to variance in exercise behavior and it is known that parental education positively affects exercise levels. This study investigates the role of parental education as a potential modifier of variance in exercise behavior from age 7 to 18 years. The study is based on large datasets from the Netherlands Twin Register (*NTR:* N= 24,874 twins; surveys around the ages of 7, 10, 12, 14, 16 and 18 years) and two Finnish twin cohorts (*FinnTwin12*: N= 4,399; 12, 14 and 17 years; *FinnTwin16*: N= 4,648; 16, 17 and 18 years). Regular participation in moderate-to-vigorous exercise activities during leisure time was assessed by survey. Parental education was dichotomized ("both parents with a low education" versus "at least one parent with a high education"). The mean in exercise behavior tended to be higher and the variance tended to be lower in children of high educated parents. Evidence for gene-by-environment interaction was weak. To develop successful interventions that specifically target children of low educated parents, the mechanisms causing the mean and variance differences between the two groups should be better understood.

INTRODUCTION

A wealth of literature supports the notion that regular physical exercise conveys strong health benefits, such as a lower risk of cardiovascular disease, diabetes and cancer, and improved cardiorespiratory, musculoskeletal and neuromotor fitness (Garber et al., 2011; Janssen & Leblanc, 2010; Warburton, Charlesworth, Ivey, Nettlefold & Bredin, 2010). In view of these positive effects that are well-advertised by public health organizations, it is surprising that only a modest proportion of the population engages in regular voluntary exercise. This suggests that we are currently far from understanding the determinants of this important lifestyle behavior. Twin studies can provide a valuable contribution to this understanding as they allow the disentanglement of genetic and environmental influences on behavior. Twin studies have investigated the genetic architecture of exercise behavior across the lifespan (de Geus, Bartels, Kaprio, Lightfoot & Thomis, 2014; Huppertz et al., 2012). For younger children, environmental factors shared by co-twins explain most of the variance in exercise behavior (Huppertz et al., 2012). For adolescents, genetic factors have shown to be a major source of individual differences with heritability estimates between 50% and 85% and with environmental factors specific to each twin individual explaining the remaining variance (van der Aa et al., 2010). In adults, about 40% of the variance is explained by genetic factors

and 60% is explained by unique environmental factors (Stubbe et al., 2006; Vink et al., 2011).

An important limitation of these twin studies is that they have ignored the possibility of interaction between genes and the environment. The expression of genes and thus also the genetic variance, however, may depend on environmental circumstances (Purcell, 2002). A more facilitating environment might increase genetic variance, whereas a more restrictive environment might suppress genetic effects. Parental education constitutes an environmental factor that can be relevant to exercise behavior as both knowledge of health behaviors and the economic position, which is positively correlated with parental education (Harden, Turkheimer and Loehlin, 2007), might facilitate the pursuit of a healthy lifestyle. Parental education has indeed found to be positively associated with physical activity in youth (Ferreira et al., 2006; Hanson & Chen, 2007; Singh, Kogan, Siahpush & van Dyck, 2008). Parental education - as a single measure or combined with occupational status and income - has also been shown to modify the heritability of a whole range of phenotypes in young individuals, including intelligence (Turkheimer, Haley, Waldron, D'Onofrio & Gottesman, 2003), problem behavior (Rosenberg, Pennington, Willcutt & Olson, 2012) and body mass index (Lajunen, Kaprio, Rose, Pulkkinen & Silventoinen, 2012). Its effect on exercise behavior has not been modelled in previous studies. If there is an effect of parental education on exercise behavior, it may work in two directions: *Genetic variance might be lower* in children of high educated parents as their parents might be more inclined to support their children in pursuing physical exercise, thereby leaving less choice to the children of whether or not to participate in this behavior. Or *genetic variance might be higher* in children of high educated parents as more resources, including more alternatives hobbies and interests, might be provided, meaning that the children can freely express their genetic preferences. Although these mechanisms might not be mutually exclusive and could cancel each other out, differences in the magnitudes of effects would make it possible to detect interactions.

This study is based on very large genetically informative datasets obtained from the Netherlands Twin Register and two Finnish twin cohorts. We aim to investigate the role of parental education as a potential modifier of exercise behavior from the ages 7 to 18 years. Genetic models will be fitted conditional on parental education. It is hypothesized that higher parental education will be associated with *higher* means in offspring's exercise behavior and that there will be (genetic) variance differences between the two groups.

METHODS

Participants

The data were derived from the Netherlands Twin Register (Boomsma et al., 2002; van Beijsterveldt et al., 2013; Willemsen et al., 2013) and two Finnish twin cohorts ascertained from the Finnish Population Register Center: FinnTwin12 and FinnTwin16 (Kaprio, 2013; Kaprio, Pulkkinen & Rose, 2002).

The Netherlands Twin Register

The Netherlands Twin Register (NTR) was founded in 1987 to study individual differences in health and behavior. It has since grown to be one of the largest twin registers in the world with around 85,000 twins and their family members registered to take part in research. Parents of twins fill out surveys when their children are born and at the ages of 2 (“survey 2”), 3 (“survey 3”), 5 (“survey 5”), 7 (“survey 7”), 9/10 (“survey 10”) and 12 (“survey 12”) years. From 13 years onwards, the twins are asked to self-report on their health and behavior every two to three years. If individuals decide not to participate in one survey, they will still be approached for subsequent surveys. Participants are mainly Caucasian and live in all regions of the Netherlands.

For this particular study, twins were selected with data on exercise behavior for the surveys around 7, 10, 12, 14, 16 and/or 18 years of age (with a maximum age range of +-2 years) and data on education of at least one parent. Exclusion criteria were a serious illness or disability (e.g., hemiplegia or heart disease, N= 354 individuals) and unknown zygosity (N= 1 pair). If participants reported an injury that interferes with physical activity on surveys 14, 16 and/or 18 years of age, data were excluded for that specific survey (N= 403 individuals for survey 14, 439 for survey 16 and 65 for survey 18). The sample thus consisted of 24,874 twins born between 1986 and 2005 (49% males). Zygosity classification of same-sex twin pairs was based on blood group or DNA typing for 20% of the pairs and it was survey-based for 80%. Classification based on questions on physical similarities and mistaking one twin for another by relatives and strangers has previously shown 93%-97% agreement with DNA polymorphisms in this twin cohort (Rietveld et al., 2000; Willemsen et al., 2005). Participants consented to take part in research of the NTR and the data collection protocol was approved by the Medical Research Ethics Committee of the VU University Medical Center.

The Finnish twin cohorts

The Finnish twin data were collected in two young cohorts (FinnTwin12 and FinnTwin16). Data collection for the FinnTwin12 cohort started in 1994 when the twins born in 1983 were 11-12 years old and eventually targeted all Finnish twins born in 1983-87, with a survey mailed to the twins (N= 5,184 twins, response rate: 94%) and their parents (response rates >86%). The twins received follow-up surveys around the ages of 14 and 17 years. Data collection of FinnTwin16 was initiated in 1991 when the twins born in 1975 were 16 years old and eventually targeted all Finnish twins born in 1975-79. The surveys were sent out to the twins (N= 4,940 twins, response rate: 88%) and their parents (response rates >79%). The twins were approached with follow-up surveys around the ages of 17 and 18 years. All participating families provided written informed consent and the data collection protocol was approved by the ethics committee of the Department of Public Health, University of Helsinki, and the Institutional Review Board (IRB), Indiana University.

For FinnTwin12, individuals were selected with data on education of at least one parent and on exercise behavior at the surveys 12, 14 and/or 17 years of age. After exclusion of twins with unknown zygosity (N= 134 pairs), the sample consisted of 4,399 individuals (51% males). Zygosity classification for 72% of the same-sex twin pairs was based on survey items on physical similarity at school age, supplemented with additional information such as photographs if classification was unclear. Zygosity classification based on survey items has shown 97% correspondence with classification based on DNA polymorphisms in 395 same-sex twin pairs from the FinnTwin12 study (Jelenkovic et al., 2011). For the remaining pairs, zygosity classification was based on DNA typing.

For FinnTwin16, data were selected of individuals with information on parental education and on exercise behavior at the ages 16, 17 and/or 18 years. Exercise measurements were changed to missing when a serious illness or disability was consistently reported over time (N= 33 individuals). Furthermore, 103 pairs were excluded due to missing information on zygosity status. The sample thus consisted of 4,648 individuals of which 48% were males. For 75% of the same-sex twin pairs, zygosity classification was based on validated survey items (Sarna, Kaprio, Sistonen & Koskenvuo, 1978) and for 25%, it was based on DNA typing.

Measures

Parental education

Within the *NTR*, both mothers and fathers were asked to indicate their level of education shortly after their twins were born and when the twins were 3, 7 and 10 years old. This information was used to classify both mothers and fathers into two levels (more recent surveys were preferred): 1) low education (66% of the mothers, 69% of the fathers) and 2) high education (34%, 31%). "High education" corresponds to a university degree or a university of applied sciences degree. In 313 families, one parent was low educated and the other had not provided any information on education. These families were excluded as they could not be clearly assigned to one of the two groups. Next, the parental data were combined into two groups of parental education: families where at least one parent was high educated (the other parent could be low educated, high educated or missing; 43%), and families where both parents were low educated (57%).

Within the *FinnTwin cohorts*, both mothers and fathers indicated their level of education at the baseline assessment when their twins were 12 (FinnTwin12) or 16 (FinnTwin16) years old. In these cohorts, "high education" corresponds to a high school degree that allows entry to further training at a university. Again, both mothers and fathers were grouped into two levels of education (mothers of the FinnTwin12 cohort: 62% low, 38% high; fathers of the FinnTwin12 cohort: 76% low, 24% high; mothers of the FinnTwin16 cohort: 73% low, 27% high; fathers of the FinnTwin16 cohort: 80% low, 20% high). Families where one parent was low educated and the other had not provided any information on education were excluded (N= 260 families for FinnTwin12 and N= 369 families for FinnTwin16). Next, parental data were combined into two groups: for 44% of the families in the FinnTwin12 cohort, at least one of the parents was high educated, whereas for 56%, both were low educated. For the FinnTwin16 cohort, these figures were 34% and 66%, respectively. Table 1 depicts the number of twins and complete twin pairs for each survey of the NTR, the FinnTwin12 cohort and the FinnTwin16 cohort that were included in this study, split by the two levels of parental education and zygosity group.

Exercise behavior

Within the *NTR*, exercise behavior was quantified as weekly metabolic equivalents of task (MET) hours spent on regular exercise behavior during

TABLE 1 Number of twins (complete pairs), split by parental education and zygosity group.

A. Low parental education.

Survey	MZM	DZM	MZF	DZF	DOS
Netherlands Twin Register					
7	542 (269)	647 (321)	623 (310)	549 (273)	1165 (580)
10	677 (335)	701 (345)	760 (378)	684 (339)	1433 (708)
12	1344 (664)	1260 (611)	1588 (785)	1281 (630)	2551 (1250)
14	687 (302)	619 (245)	1046 (466)	801 (342)	1370 (555)
16	472 (193)	370 (133)	753 (316)	601 (234)	867 (302)
18	185 (74)	155 (53)	423 (175)	314 (118)	390 (122)
FinnTwin12					
12	394 (195)	413 (204)	426 (212)	377 (187)	811 (401)
14	364 (180)	371 (182)	398 (196)	336 (164)	726 (347)
17	323 (157)	335 (161)	375 (183)	308 (149)	645 (308)
FinnTwin16					
16	397 (196)	520 (254)	549 (274)	476 (236)	1122 (556)
17	364 (175)	489 (236)	536 (265)	456 (224)	1066 (513)
18	353 (169)	485 (234)	527 (260)	465 (227)	1053 (499)

B. High parental education.

Survey	MZM	DZM	MZF	DZF	DOS
Netherlands Twin Register					
7	603 (301)	580 (289)	643 (320)	535 (266)	1091 (541)
10	644 (321)	644 (318)	645 (321)	502 (248)	1160 (575)
12	954 (474)	944 (466)	1128 (560)	813 (401)	1954 (962)
14	554 (239)	525 (229)	781 (348)	586 (261)	1193 (493)
16	386 (166)	361 (135)	553 (231)	358 (130)	783 (285)
18	140 (59)	141 (51)	271 (111)	187 (78)	320 (111)
FinnTwin12					
12	318 (158)	347 (171)	322 (160)	301 (147)	629 (308)
14	284 (138)	317 (155)	298 (148)	286 (140)	597 (289)
17	256 (126)	281 (136)	291 (143)	275 (137)	542 (262)
FinnTwin16					
16	236 (116)	248 (121)	312 (154)	253 (123)	493 (245)
17	221 (108)	239 (116)	306 (151)	252 (122)	472 (229)
18	220 (106)	238 (114)	308 (152)	247 (118)	478 (233)

MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic of opposite-sex.

leisure time. Exercise behavior was assessed through parental reports in the surveys around 7, 10 and 12 years of age and by self-reports in the surveys around 14, 16 and 18 years of age. Participants were asked to indicate what kind of activities their children (parental report) or they (self-report) participated in and - if any - 1) for how many years, 2) for how many months a year, 3) how many times a week and 4) how many minutes each time. Activities that were done for less than half a year or less than three months a year were excluded (e.g., skiing, sailing camps), as well as activities that merely increase energy expenditure (e.g., playing chess), mandatory physical education at school and activities that are related to transportation (e.g., walking, cycling). If exercise frequency or duration were missing while the other one was indicated, the missing value was replaced with the median of that specific activity within the respective survey (see Huppertz et al., 2012).

All reported activities were assigned a MET score based on Ridley et al. (2008)'s compendium of energy expenditures for youth. The respective value represents the energy that is expended during the activity relative to energy expenditure at rest (which would be 1 MET). Weekly MET hours were calculated by summing the product of frequency, duration and the MET score over all activities that an individual took part in. Good test-retest reliability of this measure has been established in previous studies (de Moor et al., 2008; Stubbe et al., 2007).

For age 7, data were provided by both parents for 62% of the children, by mothers only for 35% of the children and by fathers only for 3% of the children. For age 10, these were 24%, 75% and 1%, respectively, and for age 12, these were 44%, 55% and 1%. As mothers' and fathers' ratings correlated high at all ages (0.73 for age 7, 0.88 for age 10 and 0.89 for age 12), their ratings were averaged when both parents had reported on the same child.

Within the *FinnTwin cohorts*, the twins self-reported on their exercise behavior across all ages. They were asked how often they engage in moderate-to-vigorous exercise or sport activities during their leisure time, with the following answer options: 1) not at all, 2) less than once a month, 3) one or two times a month, 4) about once a week, 5) two or three times a week, 6) four or five times a week and 7) just about every day (or more). For the baseline assessment at age 12 in the FinnTwin12 cohort, the answer options were different, namely: 1) daily, 2) a few times a week, 3) a few times a month, 4) a few times in 6 months and 5) never. They were reversely scored for the analyses so that a higher score corresponds to a higher exercise level, with "1=

never", in order to better match the other assessments. The response categories were treated as continuous scores in all analyses.

Statistical analyses

The analyses were done for each twin cohort and age group separately (six age groups in the NTR, three age groups in FinnTwin12 and three age groups in FinnTwin16). Twin data allow the phenotypic variance to be decomposed into variance that is due to 1) additive genetic factors ("A"), 2) shared environmental factors ("C" for "common") and 3) unique environmental factors ("E"; which includes measurement error). Shared environmental factors are common to both individuals of a twin pair (e.g., growing up in the same family or attending the same school), whereas unique environmental factors are unique to each child (e.g., having different friends or non-shared illnesses).

To get a first indication of the relative contribution of A, C and E to exercise behavior within the two groups of parental education, twin correlations were estimated for each of the five zygosity groups, for children of low versus high educated parents separately. Monozygotic (MZ) twins are genetically virtually identical at the sequence level, whereas dizygotic (DZ) twins share, on average, 50% of their segregating genes. As environmental influences are assumed to be the same for MZ and DZ twins, a higher MZ twin resemblance (rMZ>rDZ) indicates genetic influences. A DZ twin correlation that is larger than half of the MZ twin correlation indicates environmental influences shared by co-twins that make DZ twins more similar to each other than what would be expected based on their genetic similarity alone. An MZ twin correlation that is not unity (rMZ<1) points towards environmental influences that the two twins of a pair do not share and that therefore make them more different from each other. This includes measurement error.

Twin correlations may also indicate quantitative and/or qualitative sex differences. The former denotes that the *same* genetic and/or shared environmental factors operate to *different degrees* in males and females, which is reflected in different twin correlations for males and females. Qualitative sex differences, in contrast, are present if *different* genetic and/or shared environmental factors operate in males and females. This is reflected in correlations of dizygotic twins of opposite-sex (DOS) that cannot be predicted based on the DZM (dizygotic male) and the DZF (dizygotic female) correlations (Falconer & Mackay, 1996). Qualitative genetic and shared environmental sex differences cannot be modelled at the same time. In our previous work, we

found the shared environmental differences to be more relevant in this respect (Huppertz et al., 2012; Stubbe et al., 2005).

The twin correlations were derived from saturated models with separate means and variances for the first-born and the second-born twin and for each sex x zygosity x parental education group. Next, just one mean and one variance were estimated across twin order and zygosity, for both sexes and parental education groups separately (e.g., one mean for sons of low educated parents, one mean for sons of high educated parents, one mean for daughters of low educated parents and one mean for daughters of high educated parents). One-by-one, it was tested whether constraining the 1) means of males, 2) means of females, 3) variances of males or 4) variances of females to be equal across parental education led to a significant deterioration of the model fit. This was done to identify any differences in means or variances between children of low versus high educated parents. A stringent alpha level of 0.01 was chosen to account for the large number of tests in this study.

Next, a series of genetic models were fitted to the data. First, an ACE model was fitted allowing for quantitative and qualitative sex differences in the variance components and sex differences in the means. In order to control for gene-environment correlation, separate means were estimated for children of low and high educated parents (Purcell, 2002). For children of low versus high educated parents separately, the phenotypic variance of exercise behavior was decomposed into additive genetic variance, shared environmental variance and unique environmental variance. The latent A-components were constrained to correlate 1 for MZ twins (100% shared genes) and 0.5 for DZ twins (50% shared genes). The latent C-components were constrained to correlate 1 for both types of (same-sex) twins and the E-components were, by definition, not allowed to correlate.

Separate parameters were estimated for males and females to allow for quantitative sex differences and the shared environmental correlations were initially estimated freely for DOS twins to allow for qualitative sex differences at the outset, whereas the genetic correlations were not allowed to vary, in accordance with our previous work (Huppertz et al., 2012; Stubbe et al., 2005). Next, it was tested whether the shared environmental correlations of DOS twins could be constrained to 1 without a significant deterioration of the model fit (α= 0.01). Only if this did not change the model fit significantly, subsequent tests for differences in the variance components between children of low and high educated parents were performed on the ACE models without qualitative

sex differences.

In order to identify differences in the variance decomposition between children of low versus high educated parents, various constraints were subsequently imposed on the *unstandardized* variance components. For males and females separately, it was tested whether equating the A-, C- and E-components for children of low and high educated parents, simultaneously and one-by-one, led to a significant deterioration of the model fit (Purcell, 2002). For the simultaneous test, again a stringent alpha level of 0.01 was chosen to account for the large number of tests, and for the separate tests, a Bonferroni correction was applied ($\alpha = 0.01/3$). For all analyses, the raw-data maximum likelihood procedure was used to estimate the parameters. Nested submodels were compared with hierarchic χ^2-tests. The -2 log-likelihood (LL) of the constrained model was subtracted from the -2LL of the less constrained model and significance was tested based on the χ^2-distribution and given the difference in degrees of freedom between the respective models. All analyses were run with the software package OpenMx 2.0.1 in R 3.1.2 (Boker et al., 2011).

RESULTS

Table 2 contains the means and variances of exercise behavior for each survey, stratified by sex and parental education, as well as the mean ages with standard deviations. In the NTR, means and variances of exercise behavior tended to increase with age. Mean exercise behavior was lower for children of parents with a low education in 7-, 10-, 12-, 14- and 16-year-old females and for 12-year-old males. Means for males were also lower at other ages, but this did not reach statistical significance. The variance in exercise behavior tended to be larger in children of parents with a low education, but the effect was not consistently significant at all ages. For 18-year-old females, an effect in the opposite direction was found, with a lower variance in the group of low educated parents. In the Finnish twin cohorts, means and variances were relatively stable across ages. The means do not represent “mean frequency”, but rather the mean of the response categories that were assessed. It is important to note that the means at age 12 reflect a 5-point scale whereas at the other ages, they reflect a 7-point scale, which explains the much lower means for age 12. Again, means were consistently lower for children of parents with a low education, whereas the variances were consistently larger for this group, with one exception. However, as indicated by the p-values, these variance differences were partly significant in the FinnTwin12 cohort, but not in

TABLE 2 Means and variances of weekly MET hours (NTR) and items on frequency of exercise behavior (FinnTwin), split by sex and parental education, and p-values that result from equating means or variances (var) to be equal for children of low and high educated parents.

A. Males.

Survey	Mean age (SD)	Low parental education	High parental education	p-value mean*	p-value var*
Netherlands Twin Register					
7	7.53 (.34)	14.31 (151.00), 1772	15.27 (127.40), 1727	.04	2.6e-3
10	9.83 (.44)	22.36 (388.51), 2092	22.88 (363.64), 1867	.46	.20
12	12.24 (.39)	24.54 (437.36), 3872	27.33 (454.48), 2872	3.3e-6	.33
14	14.63 (.59)	29.05 (909.67), 1939	31.18 (734.93), 1648	.04	2.1e-5
16	16.87 (.44)	30.76 (1175.0), 1212	32.67 (944.54), 1114	.19	4.2e-4
18	18.76 (.52)	24.43 (800.51), 491	23.78 (731.59), 410	.75	.37
FinnTwin12					
12	11.42 (.30)	3.39 (2.00), 1215	3.65 (1.55), 980	7.9e-5	1.3e-4
14	14.05 (.09)	5.05 (2.42), 1088	5.13 (2.10), 896	.25	.04
17	17.62 (.22)	4.79 (2.97), 970	5.02 (2.56), 804	9.7e-3	.04
FinnTwin16					
16	16.17 (.13)	4.69 (2.82), 1475	4.81 (2.76), 730	.14	.76
17	17.14 (.08)	4.78 (2.82), 1372	4.99 (2.71), 693	.02	.57
18	18.61 (.17)	4.69 (2.71), 1344	4.78 (2.52), 694	.25	.31

B. Females.

Survey	Mean age (SD)	Low parental education	High parental education	p-value mean*	p-value var*
Netherlands Twin Register					
7	7.51 (.34)	9.47 (92.24), 1754	10.63 (85.97), 1725	1.9e-3	.21
10	9.85 (.43)	14.58 (244.58), 2163	16.61 (229.69), 1728	4.3e-4	.24
12	12.24 (.39)	16.53 (317.13), 4152	19.32 (303.72), 2921	1.9e-8	.28
14	14.63 (.61)	19.58 (625.80), 2584	23.32 (519.88), 1991	2.6e-6	5.2e-5
16	16.88 (.46)	18.16 (567.56), 1851	22.34 (585.12), 1327	1.3e-5	.58
18	18.76 (.49)	14.68 (434.10), 976	17.77 (528.98), 649	.01 n.s.	8.7e-3
FinnTwin12					
12	11.41 (.30)	2.85 (2.11), 1206	3.04 (2.08), 937	9.3e-3	.80
14	14.04 (.08)	4.90 (2.41), 1107	5.08 (1.97), 886	.02	3.5e-3
17	17.61 (.23)	4.80 (2.62), 1016	5.00 (2.19), 841	1.3e-2	1.2e-2
FinnTwin16					
16	16.15 (.13)	4.67 (2.46), 1589	4.68 (2.13), 812	.90	.03
17	17.13 (.07)	4.74 (2.27), 1539	4.84 (1.97), 797	.16	.04
18	18.59 (.16)	4.72 (2.13), 1539	4.70 (2.20), 797	.74	.64

**Compared to the model where means and variances are equal across twin order and zygosity status, but not across sex and parental education.*

TABLE 3 Twin correlations, split by zygosity and parental education (99% CIs).

A. Netherlands Twin Register, surveys 7-12.

Zygosity	Education	Survey 7	Survey 10	Survey 12
MZM	**Low**	.90 (.86, .92)	.90 (.87, .92)	.87 (.85, .90)
	High	.91 (.88, .93)	.89 (.86, .92)	.88 (.85, .90)
DZM	**Low**	.80 (.74, .84)	.57 (.47, .66)	.61 (.54, .67)
	High	.80 (.74, .85)	.70 (.62, .77)	.62 (.54, .68)
MZF	**Low**	.89 (.85, .92)	.91 (.88, .93)	.92 (.91, .93)
	High	.86 (.81, .89)	.85 (.81, .89)	.86 (.83, .88)
DZF	**Low**	.82 (.76, .87)	.67 (.58, .74)	.69 (.64, .74)
	High	.81 (.75, .86)	.80 (.74, .85)	.76 (.70, .81)
DOS	**Low**	.40 (.30, .48)	.38 (.29, .46)	.40 (.34, .46)
	High	.50 (.41, .58)	.45 (.36, .53)	.44 (.37, .51)

B. Netherlands Twin Register, surveys 14-18.

Zygosity	Education	Survey 14	Survey 16	Survey 18
MZM	**Low**	.57 (.46, .66)	.60 (.46, .70)	.64 (.43, .78)
	High	.53 (.40, .63)	.43 (.25, .57)	.47 (.18, .68)
DZM	**Low**	.38 (.24, .51)	.41 (.19, .58)	.45 (.17, .66)
	High	.31 (.15, .46)	.23 (-.01, .44)	.33 (-.01, .59)
MZF	**Low**	.74 (.68, .79)	.64 (.55, .72)	.58 (.45, .69)
	High	.51 (.40, .60)	.61 (.50, .70)	.74 (.59, .83)
DZF	**Low**	.45 (.32, .56)	.26 (.11, .40)	.15 (-.09, .37)
	High	.39 (.24, .52)	.67 (.49, .78)	.16 (-.15, .44)
DOS	**Low**	.13 (.03, .24)	.20 (.04, .34)	.35 (.15, .51)
	High	.28 (.16, .39)	.21 (.05, .35)	.20 (-.05, .42)

C. FinnTwin12.

Zygosity	Education	Survey 12	Survey 14	Survey 17
MZM	**Low**	.70 (.59, .78)	.66 (.54, .76)	.71 (.59, .79)
	High	.59 (.44, .71)	.62 (.46, .73)	.77 (.65, .85)
DZM	**Low**	.50 (.35, .62)	.43 (.26, .57)	.41 (.22, .57)
	High	.46 (.29, .60)	.31 (.11, .48)	.49 (.29, .64)
MZF	**Low**	.70 (.60, .78)	.66 (.55, .75)	.64 (.52, .74)
	High	.70 (.59, .79)	.49 (.31, .63)	.65 (.51, .76)
DZF	**Low**	.61 (.47, .71)	.41 (.23, .57)	.32 (.12, .50)
	High	.66 (.52, .76)	.51 (.33, .65)	.43 (.23, .59)
DOS	**Low**	.28 (.15, .39)	.28 (.15, .40)	.22 (.08, .35)
	High	.34 (.20, .46)	.08 (-.07, .23)	.15 (-.01, .30)

D. FinnTwin16.

Zygosity	Education	Survey 16	Survey 17	Survey 18
MZM	**Low**	.64 (.52, .74)	.59 (.45, .70)	.63 (.50, .74)
	High	.75 (.63, .84)	.75 (.62, .84)	.72 (.58, .82)
DZM	**Low**	.41 (.26, .53)	.37 (.21, .51)	.38 (.22, .52)
	High	.49 (.29, .65)	.44 (.22, .61)	.38 (.16, .57)
MZF	**Low**	.71 (.62, .78)	.70 (.61, .77)	.66 (.56, .74)
	High	.68 (.55, .78)	.79 (.70, .86)	.74 (.63, .82)
DZF	**Low**	.44 (.29, .56)	.50 (.36, .62)	.30 (.13, .45)
	High	.51 (.31, .66)	.43 (.22, .60)	.31 (.09, .51)
DOS	**Low**	.19 (.08, .29)	.20 (.09, .31)	.20 (.09, .31)
	High	.20 (.04, .35)	.24 (.08, .39)	.25 (.09, .40)

Based on the fully saturated model; MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic of opposite-sex.

the FinnTwin16 cohort.

Table 3 depicts the twin correlations and their 99% confidence intervals (CIs). In the NTR, MZ correlations were (with one exception) consistently higher than DZ twin correlations and the same-sex DZ twin correlations were larger than half of the MZ twin correlations in all but a few cases, implying that both genetic effects and shared environmental effects contribute to the variance in children's and adolescents' exercise behavior. DZ correlations tended to be higher for females than for males, implying a larger influence of shared environmental factors in females. Especially for the younger ages, the DOS correlations were lower than what would be expected based on the same-sex DZ correlations, suggesting qualitative sex differences. With a few exceptions, the MZ twin correlations were consistently higher than the DZ correlations in the Finnish twin cohorts and the same-sex DZ correlations were higher than half of the MZ correlations. The twin correlations of males and females were fairly similar, but the DOS correlations were consistently lower than the same-sex DZ correlations.

Figure 1, Table 4 and Figure 2 contain the results of the genetic model fitting. The full ACE models were compared to models that did not allow for qualitative sex differences. In the NTR, qualitative sex differences were present for the ages 7, 10 and 12 years in both groups of parental education, but not for the ages 14, 16 and 18 years. The figures thus depict the A, C and E estimates of the models with a freely estimated shared environmental correlation for DOS twins

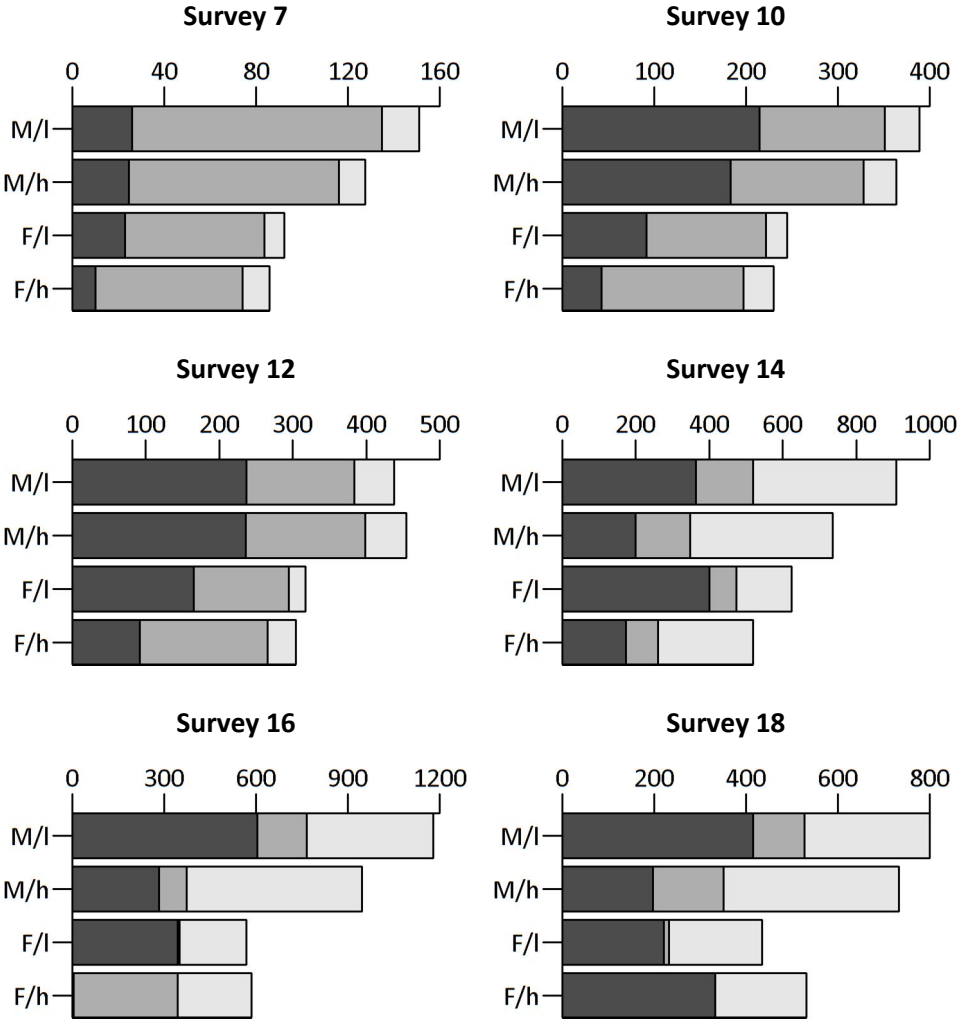
for the first three age groups and with a shared environmental correlation that was constrained to 1 for the last three age groups. In the Finnish twin cohorts, the genetic models revealed that there were no sex-specific shared environmental effects and thus qualitative sex differences were not taken into account in the ACE models. Figure 1 depicts the unstandardized variance components (A, C, E), for children of low versus high educated parents separately. The supplementary material contains the exact numbers with 99% CIs.

In the NTR, the variance of the unstandardized A-components generally tended to be attenuated in children of high educated parents compared to children of low educated parents. The C-component tended to be smaller in daughters of low educated parents with a large and statistically significant effect in 16-year-olds. In the Finnish twin cohorts, no consistent differences in the unstandardized A-components according to parental education could be observed. The genetic variance tended to be lower in children of parents with a high education in FinnTwin12 but higher in FinnTwin16. The shared environmental variance in males of the FinnTwin12 cohort and in both males and females of the FinnTwin16 cohort tended to be lower in high educated parents with one exception. The E-component was consistently lower in children of high educated parents, with the exception of 14-year-old girls.

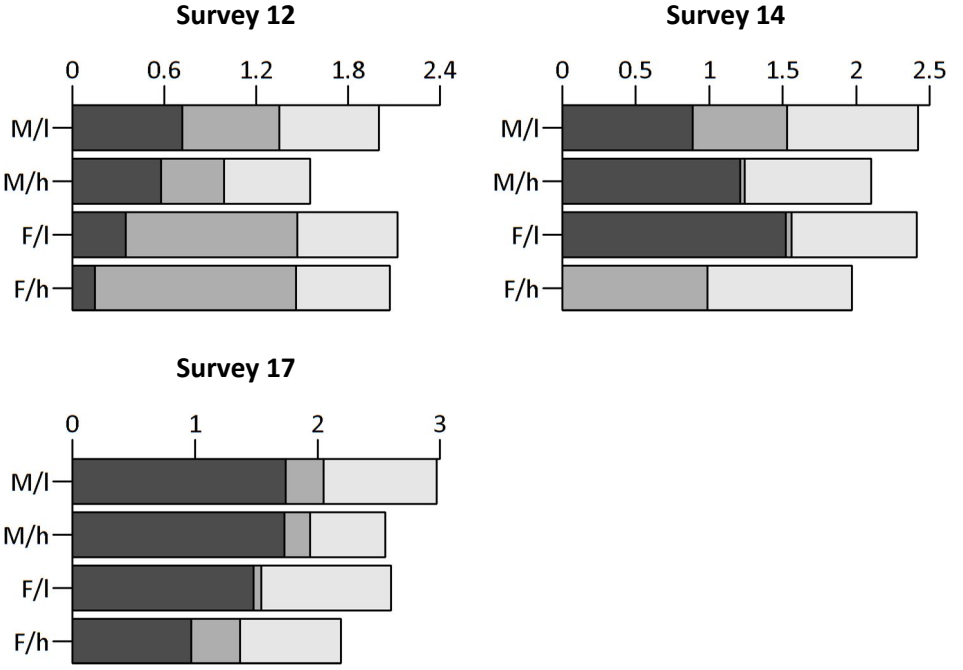
Table 4 depicts the model fitting indices of the models 1) simultaneously setting the *unstandardized* A, C and E to be equal across groups of parental education, 2) only setting the unstandardized A to be equal, 3) only setting the unstandardized C to be equal and 4) only setting the unstandardized E to be equal across the two groups. Comparing the models that estimated A, C and E freely for the two groups and the models that equated the three components at the same time led to significant p-values in the ages 7, 14 and 16 for males and in all but the last age group for females in the NTR. Subsequently constraining each variance component separately indicated significant differences after Bonferroni correction - namely in males, a lower E-component with high educated parents for 7-year-olds, and in females, lower A-components with high educated parents for the ages 10, 12, 14 and 16 years, a higher C-component for age 16, and higher E-components for the ages 10, 12 and 14. In the Finnish twin cohorts, few of the observed differences were significant.

FIGURE 1 Unstandardized additive genetic (dark gray), shared environmental (gray) and non-shared environmental (light gray) variance components, split by sex and parental education (M/l= males, low educated parents; M/h= males, high educated parents; F/l= females, low educated parents; F/h= females, high educated parents).

A. Netherlands Twin Register.



B. FinnTwin 12.



C. FinnTwin16.

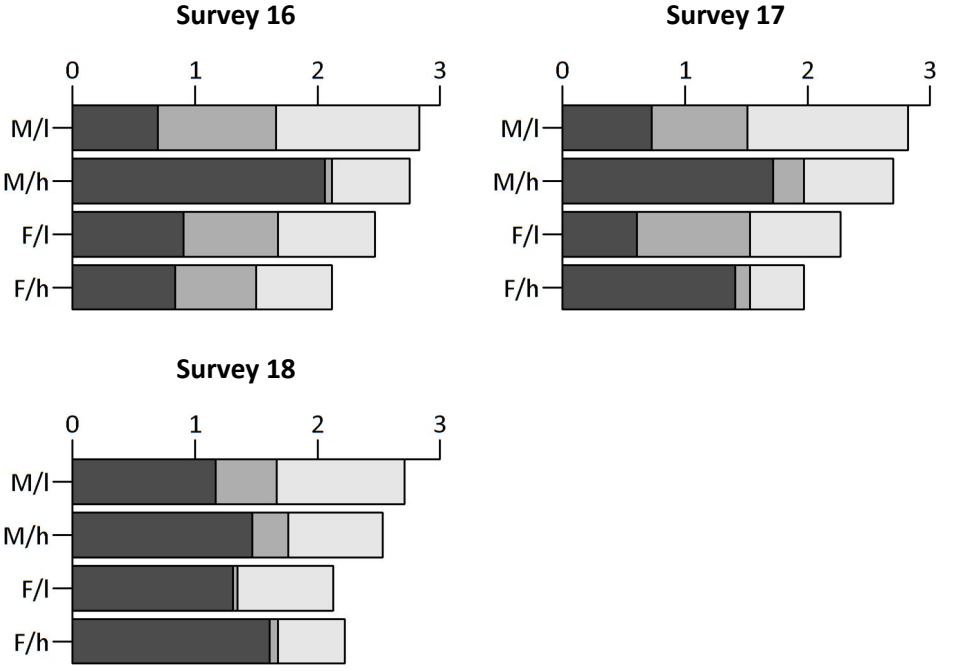


TABLE 4 Model fit indices for constraining the unstandardized additive genetic (A), shared environmental (C) and non-shared environmental (E) variance components to be equal for children of low and high educated parents, split by sex.

A. Netherlands Twin Register, males.

Survey	Model	-2LL	χ^2	Δdf	p-value
7	**Full model***	49321.1	-	-	-
	ACE equal	49342.3	21.2023	3	9.6e-05
	A equal	49321.1	.0431	1	.8355
	C equal	49324.1	2.9934	1	.0836
	E equal	49330.7	9.5878	1	.0020
10	**Full model***	63814.2	-	-	-
	ACE equal	63817.2	3.0086	3	.3903
	A equal	63815.3	1.0721	1	.3005
	C equal	63814.3	.0701	1	.7912
	E equal	63814.4	.2628	1	.6082
12	**Full model***	115286.6	-	-	-
	ACE equal	115287.7	1.1094	3	.7748
	A equal	115286.6	.0004	1	.9835
	C equal	115286.8	.2302	1	.6314
	E equal	115286.8	.1943	1	.6594
14	**Full model***	75413.3	-	-	-
	ACE equal	75432.0	18.7622	3	.0003
	A equal	75414.5	1.2905	1	.2560
	C equal	75413.3	.0024	1	.9607
	E equal	75413.3	.0011	1	.9739
16	**Full model***	51489.3	-	-	-
	ACE equal	51509.8	20.4875	3	.0001
	A equal	51492.6	3.2733	1	.0704
	C equal	51489.5	.1611	1	.6882
	E equal	51494.4	5.1001	1	.0239
18	**Full model***	22933.5	-	-	-
	ACE equal	22936.8	3.3488	3	.3409
	A equal	22934.2	.7744	1	.3789
	C equal	22933.5	.0339	1	.8540
	E equal	22935.6	2.1406	1	.1434

B. Netherlands Twin Register, females.

Survey	Model	-2LL	χ^2	Δdf	p-value
7	**Full model***	49321.1	-	-	-
	ACE equal	49333.0	11.8514	3	.0079
	A equal	49327.6	6.5305	1	.0106
	C equal	49321.3	.2325	1	.6297
	E equal	49329.5	8.4230	1	.0037
10	**Full model***	63814.2	-	-	-
	ACE equal	63830.4	16.2249	3	.0010
	A equal	63824.0	9.8151	1	.0017
	C equal	63815.9	1.7513	1	.1857
	E equal	63824.8	10.5922	1	.0011
12	**Full model***	115286.6	-	-	-
	ACE equal	115340.6	53.9805	3	1.1e-11
	A equal	115304.1	17.5573	1	2.8e-05
	C equal	115291.9	5.2743	1	.0216
	E equal	115333.1	46.4837	1	9.2e-12
14	**Full model***	75413.3	-	-	-
	ACE equal	75462.5	49.2505	3	1.2e-10
	A equal	75422.6	9.3752	1	.0022
	C equal	75413.3	.0452	1	.8317
	E equal	75442.4	29.1015	1	6.9e-08
16	**Full model***	51489.3	-	-	-
	ACE equal	51502.9	13.5651	3	.0036
	A equal	51501.8	12.4781	1	.0004
	C equal	51502.8	13.4432	1	.0002
	E equal	51490.0	.7140	1	.3981
18	**Full model***	22933.5	-	-	-
	ACE equal	22940.9	7.4019	3	.0601
	A equal	22935.6	2.1312	1	.1443
	C equal	22933.7	.2127	1	.6446
	E equal	22933.5	.0129	1	.9097

C. FinnTwin12, males.

Survey	Model	-2LL	χ^2	Δdf	p-value
12	**Full model***	14383.8	-	-	-
	ACE equal	14399.3	15.5043	3	.0014
	A equal	14384.2	.4042	1	.5249
	C equal	14385.1	1.2857	1	.2568
	E equal	14384.9	1.0828	1	.2981
14	**Full model***	14024.6	-	-	-
	ACE equal	14030.2	5.5637	3	.1349
	A equal	14025.3	.6893	1	.4064
	C equal	14026.1	1.4513	1	.2283
	E equal	14024.7	.0625	1	.8026
17	**Full model***	13267.4	-	-	-
	ACE equal	13277.6	10.2019	3	.0169
	A equal	13267.4	1.47e-06	1	.9990
	C equal	13267.5	.0344	1	.8528
	E equal	13273.9	6.4670	1	.0110

D. FinnTwin12, females.

Survey	Model	-2LL	χ^2	Δdf	p-value
12	**Full model***	14383.8	-	-	-
	ACE equal	14385.6	1.8028	3	.6143
	A equal	14384.4	.6510	1	.4198
	C equal	14384.5	.6710	1	.4127
	E equal	14384.0	.2017	1	.6534
14	**Full model***	14024.6	-	-	-
	ACE equal	14038.8	14.1226	3	.0027
	A equal	14030.5	5.8522	1	.0156
	C equal	14026.8	2.1491	1	.1427
	E equal	14026.0	1.3333	1	.2482
17	**Full model***	13267.4	-	-	-
	ACE equal	13277.7	10.3034	3	.0162
	A equal	13268.4	.9438	1	.3313
	C equal	13268.0	.5421	1	.4616
	E equal	13270.7	3.2928	1	.0696

E. FinnTwin16, males.

Survey	Model	-2LL	χ^2	Δdf	p-value
16	**Full model***	16713.3	-	-	-
	ACE equal	16725.0	11.7615	3	.0082
	A equal	16716.4	3.0935	1	.0786
	C equal	16714.6	1.2825	1	.2574
	E equal	16723.6	10.3076	1	.0013
17	**Full model***	15760.7	-	-	-
	ACE equal	15773.0	12.3249	3	.0063
	A equal	15764.2	3.5088	1	.0610
	C equal	15762.3	1.5564	1	.2122
	E equal	15773.0	12.3126	1	.0004
18	**Full model***	15638.5	-	-	-
	ACE equal	15642.2	3.7706	3	.2873
	A equal	15638.9	.4028	1	.5256
	C equal	15638.7	.2720	1	.6020
	E equal	15641.6	3.1229	1	.0772

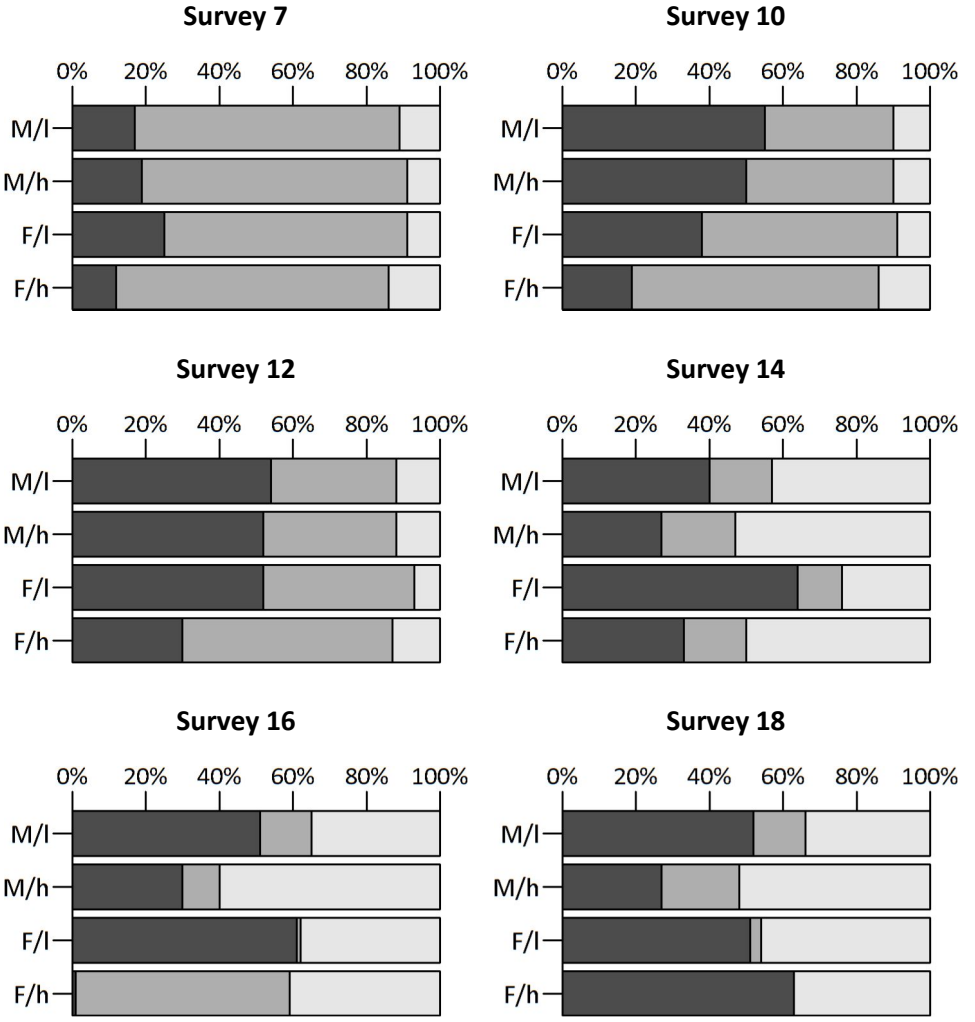
F. FinnTwin16, females.

Survey	Model	-2LL	χ^2	Δdf	p-value
16	**Full model***	16713.3	-	-	-
	ACE equal	16721.0	7.7081	3	.0524
	A equal	16713.3	.0282	1	.8665
	C equal	16713.4	.1037	1	.7474
	E equal	16715.9	2.6574	1	.1031
17	**Full model***	15760.7	-	-	-
	ACE equal	15778.1	17.4459	3	.0006
	A equal	15766.3	5.5907	1	.0181
	C equal	15766.7	6.0525	1	.0139
	E equal	15774.2	13.5205	1	.0002
18	**Full model***	15638.5	-	-	-
	ACE equal	15645.9	7.4005	3	.0602
	A equal	15640.1	1.5935	1	.2068
	C equal	15638.5	.0501	1	.8229
	E equal	15645.3	6.7912	1	.0092

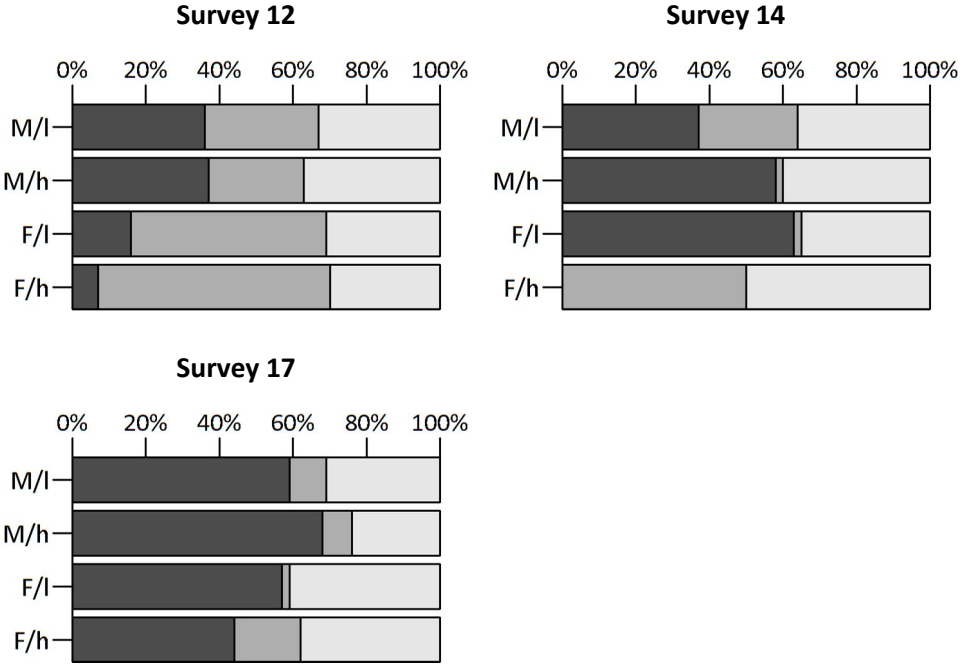
**The unstandardized variance components were equated simultaneously (ACE) and individually (A, C, E).*

FIGURE 2 Standardized additive genetic (dark gray), shared environmental (gray) and non-shared environmental (light gray) variance components, split by sex and parental education (M/l= males, low educated parents; M/h= males, high educated parents; F/l= females, low educated parents; F/h= females, high educated parents).

A. Netherlands Twin Register.



B. FinnTwin12.



C. FinnTwin16.

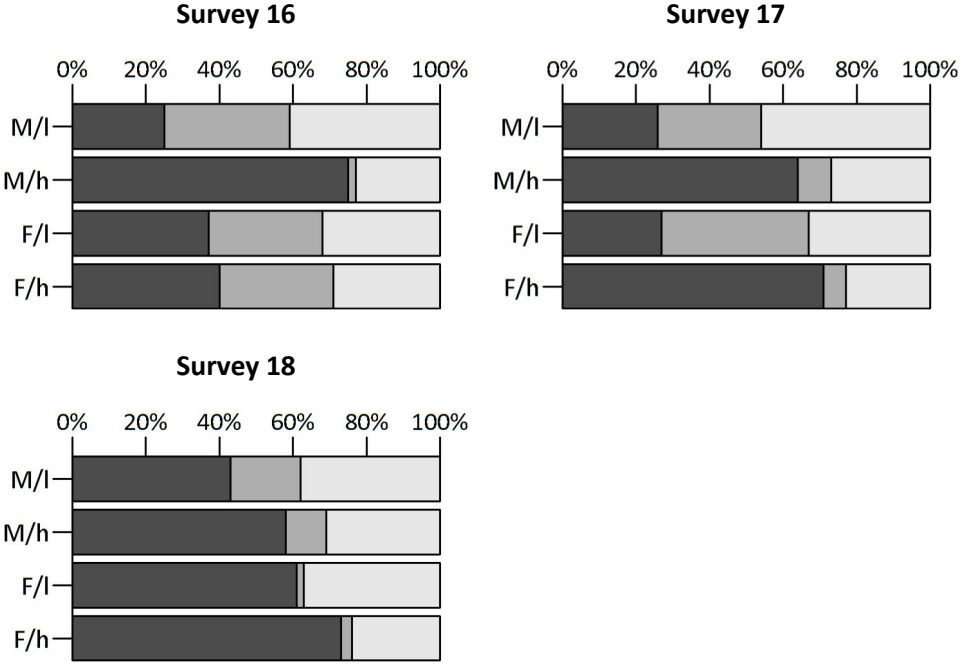


Figure 2 depicts the variance components relative to the total variance (e.g., for A: A/V) as percentages. Again, the exact numbers with 99% CIs can be found in the supplementary material. In accordance with the findings for the unstandardized variance components, the relative contribution of genetic effects to the total variance tended to be lower in children of high educated parents compared to children of low educated parents, whereas the relative influence of shared and non-shared environmental effects was comparable between the two groups in the NTR. Again, patterns were less consistent in the Finnish twin cohorts.

DISCUSSION

This study aimed to investigate the role of parental education as a potential modifier of genetic and environmental effects on exercise behavior in children and adolescents based on data of the Netherlands Twin Register and two Finnish twin cohorts. To this end, means, variances and genetic and environmental variance components were compared between children with two low educated parents and children with at least one high educated parent. Based on twin data, it was tested whether 1) means and variances were different for the two groups and 2) whether the contribution of genetic, shared environmental and unique environmental factors to the variance in exercise behavior differed. It was hypothesized that higher parental education would be associated with a *higher* mean in offspring's exercise behavior which was largely confirmed in both the Dutch and the Finnish data. Total variances tended to be lower in children of high educated parents. Evidence for gene-by-environment interaction was weak. Data in Dutch females partly supported the hypothesis of a reduction in genetic variance in children of high educated parents, but data of males did not. The Finnish data provided no support at all.

Based on a large number of previous studies, we expected that children of high educated parents would exercise more than children of low educated parents (Ferreira et al., 2006; Hanson & Chen, 2007; Singh et al., 2008). This trend was indeed seen both in the Dutch and the Finnish data, but significant differences were mainly seen in Dutch females which is in accordance with previous studies suggesting a stronger association between socioeconomic status and exercise behavior in females than in males (Hanson & Chen, 2007). Hanson and Chen argue that males might be physically more active whilst interacting with their peers, whereas for females, exercise levels might be more dependent on structured activities that in turn are more likely to involve parental influence. As an addition to our analyses, we calculated the percentage of non-exercisers

for both parental education groups separately and found that it was consistently lower in children of high educated parents (Supplementary table III).

It is not known why children of high educated parents exercise more than children of low educated parents, but several possible mechanisms have been proposed. Most obviously, high educated parents might be more aware of the benefits of regular exercise behavior and might therefore be more inclined to promote this behavior in their children. They might also have better financial and other resources to spend more time with their children and promote healthy behavior (Kalil, Ryan & Corey, 2012). This direct effect of parenting might mainly apply to younger children that are more dependent on their parents when it comes to exercise activities as opposed to older children (Huppertz et al., 2012). Adolescents, in contrast, spend less time at home and the direct influence of parents might be outweighed by the influence of peers and the school environment (West, 1997). In this age group, the influence of parents may take a more indirect path. Educational attainment is a heritable trait itself (Gottfredson, 2004; Rietveld et al., 2013), implying that high educated parents tend to have high educated children that in turn might pursue health behaviors to take care of themselves, although their priorities might lie elsewhere. It is important to shed further light on the possible mechanisms causing children of low educated parents to exercise less in order to develop effective interventions.

Interestingly, we also found a consistent trend for a lower variance in children of high educated parents, both in the Dutch and the Finnish data, although only a few differences were significant. Variance differences are hardly even mentioned in studies that assess differences in health behavior by education level. There is no reason to assume that parental education only affects mean levels of exercise behavior and not the variance, however.

Voluntary exercise behavior has been hypothesized to be influenced by genetic effects on the general drive to be physically active, on exercise ability and on the balance of the appetitive and aversive effects in the psychological response during and shortly after exercise (de Geus & de Moor, 2008). We expected these genetic effects to be affected by parental education. There was a tendency for the unstandardized genetic components to be attenuated in daughters of high educated parents in the Dutch data. Combined with the fact that exercise behavior is higher in this group, these children or their parents may be more capable to suppress an unfavorable genetic predisposition that

would prevent engagement in regular voluntary exercise behavior, such as a low innate drive, ability and/or enjoyment. Low educated parents may leave the choice to exercise much more to the children themselves, thereby increasing genetic variance. However, neither in the Dutch data of males nor in the two large Finnish twin cohorts, a clear pattern in the variance decomposition emerged. One possible explanation could be that for part of the sample, the genetic variance was actually *larger* in high educated parents which might have attenuated the effect of a lower genetic variance in the remainder of the sample. Additional covariates such as parenting style and (financial) resources should be assessed and taken into account in the future. The most consistent finding in the Finnish data was that the unique environmental variance tended to be higher in children of low educated parents. Possible reasons for this could be earlier individuation of children in low educated families and/or more twin-specific peer influences in this group.

When interpreting our results, one should bear in mind some fundamental differences between the Dutch and the Finnish data. First of all, the definition of what constitutes a "high education" was largely different in the two datasets. In the Dutch dataset, a high education corresponded to a university degree or a university of applied sciences degree. In the Finnish dataset, this was a high school degree which is a requirement but no guarantee for university education. Although the distribution of low versus high educated individuals turned out to be comparable (about 40% high and 60% low), a relatively high educational level in the "low education" group of the Dutch dataset and a relatively low education level in the "high education" group of the Finnish dataset may have occurred. Second, exercise behavior was quantified as weekly MET hours in the Dutch twins and as frequency of moderate-to-vigorous activity in the Finnish twins. The former takes duration and intensity of the activity into account, the latter does not. A person that exercises twice a week, for instance, might have a weekly MET hours score that, depending on the activity and the duration, could vary between 2 (2x15 minutes at an intensity of 4 MET) and 20 (2x60 minutes at an intensity of 10 MET) or more. In short, the partly differential findings in the Dutch and Finnish datasets may reflect differences in methodology and should not be interpreted as genuine differences between the two countries based on cultural or even genetic effects.

Gene-by-environment (GxE) interaction has been investigated several times with physical activity as a potential modifier of, for instance, body size and/or body composition, with promising results. Both, effects of physical activity on

the heritability of body size/composition (Mustelin et al., 2009) and effects of physical activity on expression of specific genes related to body size/composition have been reported (Vimaleswaran et al., 2009). Taken the substantial genetic contribution to physical activity (den Hoed et al., 2013) and some shared genetic correlations between fitness, exercise and body composition (Mustelin et al., 2011), these studies qualify more as a test of gene-by-gene (GxG) interaction than GxE interaction. This concern could also be voiced with regard to the present study. The exposure to certain environments (such as parental education) is also partly under genetic control (Kendler & Eaves, 1986), which adds complexity to the interpretation of the results. Moreover, there might be gene frequency differences present in the two parental education groups. If the genes in question then also affect children's exercise behavior, this gene-environment correlation (rGE) might lead to results that mimic GxE interaction in absence of any true interaction. One way to control for rGE is estimating separate means for low and high educated parents, which has been done in the present study (Purcell, 2002).

Notwithstanding its limitations, this study constitutes a relevant addition to the literature as it is the first to investigate GxE interaction with exercise behavior as the outcome variable. Identifying GxE interaction is of importance for at least two reasons. From a public health perspective, identifying modifiers of genetic effects on exercise behavior can improve intervention strategies. From a scientific perspective, the identification of GxE interaction might improve gene-hunting studies as these could add an interaction term (Sung et al., 2014). This would not only increase the probability to find significant associations, but it may also lead to the identification of loci or genes that are selectively expressed under certain circumstances.

SUPPLEMENTARY TABLE I Unstandardized additive genetic (A), shared environmental (C) and non-shared environmental (E) variance components, split by parental education (*) and sex (**M=Males, F=Females; 99% CIs).

A. Netherlands Twin Register, surveys 7-12.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
7	**M****	26.1(12.1,42.1)	24.6(13.9,37.8)	108.5(88.1,129.4)	91.4 (74.1,109.0)	16.4(13.3,20.7)	11.4(9.3,14.2)
	F	22.9(14.2,33.6)	10.1(1.3,20.1)	60.8(47.8,73.6)	63.9 (51.9,76.0)	8.6(7.0,10.7)	11.9(9.8,14.7)
10	**M**	214.8(154.9,298.0)	183.1(132.1,250.4)	135.7(49.7,207.2)	144.6(76.0,206.6)	38.0(31.3,47.0)	35.9(29.4,44.7)
	F	91.9(64.0,128.7)	42.8(15.4,75.9)	129.8(88.5,166.9)	154.4(117.6,189.9)	22.9(19.1,27.9)	32.5(26.6,40.3)
12	**M**	236.5(183.6,301.4)	235.8(173.9,314.6)	147.0(81.2,205.6)	162.7(82.9,232.7)	53.9(46.9,62.6)	56.0(47.4,66.9)
	F	165.6(136.4,201.4)	92.3(61.2,130.3)	129.3(90.8,165.1)	173.7(132.4,212.1)	22.2(19.5,25.4)	37.8(32.4,44.5)

B. Netherlands Twin Register, surveys 14-18.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
14	**M****	364.0(.2,578.3)	199.0(0,431.0)	155.2(13.0,521.8)	148.9(.1,393.4)	388.7(314.9,496.0)	387.3(308.0,493.2)
	F	400.6(223.9,519.2)	173.5(0,310.5)	72.8(0,245.6)	88.2(.2,255.1)	150.5(126.2,182.5)	258.2(210.6,324.4)
16	**M**	604.6(27.2,981.1)	283.4(0,550.5)	161.1(0,656.3)	90.3(0,428.7)	412.8(305.1,601.8)	571.0(426.1,799.4)
	F	344.8(200.7,424.5)	5.1(0,363.0)	6.4(0,120.7)	340.0(7.3,434.0)	218.0(177.1,273.3)	239.9(189.1,300.6)
18	**M**	415.1(1.1,770.2)	198.2(.0,616.8)	111.8(0,555.4)	152.3(0,550.8)	272.2(176.0,477.2)	381.1(233.1,673.3)
	F	221.3(37.2,312.2)	332.4(.05,468.9)	11.2(0,160.7)	0(0,325.2)	202.5(154.9,273.5)	198.7(137.2,318.0)

C. FinnTwin12.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
12	**M****	.72 (.30, 1.40)	.58 (.21, 1.06)	.63 (.03, 1.03)	.41 (.03, .75)	.65 (.51, .84)	.56 (.43, .75)
	F	.35 (0, .80)	.15 (0, .63)	1.12 (.69, 1.54)	1.31 (.85, 1.72)	.65 (.52, .83)	.61 (.47, .79)
14	**M**	.89 (.04, 1.85)	1.21 (.64, 1.58)	.64 (0, 1.37)	.03 (0, .51)	.89 (.69, 1.17)	.86 (.65, 1.14)
	F	1.52 (.21, 1.88)	0 (0, .56)	.04 (0, 1.23)	.99 (.40, 1.30)	.85 (.67, 1.08)	.98 (.77, 1.22)
17	**M**	1.74 (.65, 2.46)	1.73 (.90, 2.30)	.31 (0, 1.29)	.21 (0, 1.00)	.92 (.71, 1.25)	.61 (.45, .84)
	F	1.48 (.28, 1.92)	.97 (.08, 1.59)	.06 (0, 1.16)	.40 (0, 1.17)	1.06 (.84, 1.38)	.82 (.62, 1.10)

D. FinnTwin16.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
16	**M****	.70 (.07, 1.29)	2.06 (1.18, 2.57)	.96 (.48, 1.50)	.06 (0, .88)	1.17 (.93, 1.48)	.63 (.46, .89)
	F	.91 (.38, 1.44)	.84 (.09, 1.62)	.77 (.30, 1.26)	.66 (.01, 1.36)	.79 (.64, .99)	.62 (.47, .85)
17	**M**	.73 (.11, 1.34)	1.72 (.67, 2.40)	.78 (.32, 1.34)	.25 (0, 1.21)	1.31 (1.04, 1.65)	.73 (.53, 1.05)
	F	.61 (.10, 1.16)	1.41 (.74, 1.82)	.92 (.41, 1.40)	.12 (0, .78)	.74 (.60, .92)	.44 (.33, .59)
18	**M**	1.17 (.25, 1.90)	1.47 (.56, 2.12)	.50 (0, 1.27)	.29 (0, 1.09)	1.04 (.81, 1.37)	.77 (.56, 1.10)
	F	1.31 (.74, 1.59)	1.61 (.99, 2.03)	.04 (0, .55)	.07 (0, .64)	.78 (.64, .97)	.54 (.40, .74)

SUPPLEMENTARY TABLE II Standardized additive genetic (A), shared environmental (C) and non-shared environmental (E) variance components, split by parental education (*) and sex (**M=Males, F=Females; 99% CIs).

A. Netherlands Twin Register, surveys 7-12.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
7	**M****	.17 (.08, .28)	.19 (.11, .29)	.72 (.62, .80)	.72 (.62, .79)	.11 (.09, .14)	.09 (.07, .11)
	F	.25 (.16, .36)	.12 (.02, .23)	.66 (.55, .75)	.74 (.64, .83)	.09 (.07, .12)	.14 (.11, .17)
10	**M**	.55 (.41, .73)	.50 (.37, .66)	.35 (.17, .49)	.40 (.24, .52)	.10 (.08, .12)	.10 (.08, .12)
	F	.38 (.27, .52)	.19 (.07, .32)	.53 (.39, .64)	.67 (.55, .77)	.09 (.08, .12)	.14 (.11, .18)
12	**M**	.54 (.43, .67)	.52 (.39, .67)	.34 (.21, .44)	.36 (.21, .48)	.12 (.11, .14)	.12 (.10, .15)
	F	.52 (.43, .63)	.30 (.21, .42)	.41 (.30, .50)	.57 (.46, .66)	.07 (.06, .08)	.12 (.11, .15)

B. Netherlands Twin Register, surveys 14-18.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
14	**M****	.40 (.07, .58)	.27 (0, .53)	.17 (.02, .46)	.20 (0, .48)	.43 (.35, .52)	.53 (.43, .64)
	F	.64 (.41, .78)	.33 (.01, .55)	.12 (0, .33)	.17 (0, .43)	.24 (.20, .29)	.50 (.41, .61)
16	**M**	.51 (.19, .72)	.30 (0, .50)	.14 (0, .42)	.10 (0, .36)	.35 (.26, .47)	.60 (.47, .77)
	F	.61 (.43, .69)	.01 (0, .38)	.01 (0, .16)	.58 (.23, .66)	.38 (.31, .47)	.41 (.33, .50)
18	**M**	.52 (.06, .77)	.27 (0, .65)	.14 (0, .52)	.21 (0, .57)	.34 (.23, .52)	.52 (.34, .77)
	F	.51 (.21, .64)	.63 (.13, .74)	.03 (0, .27)	0 (0, .41)	.47 (.36, .60)	.37 (.26, .55)

C. FinnTwin12.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
12	**M****	.36 (.15, .69)	.37 (.14, .66)	.31 (.01, .49)	.26 (.02, .46)	.33 (.25, .42)	.36 (.27, .49)
	F	.16 (0, .37)	.07 (0, .30)	.53 (.34, .68)	.63 (.42, .75)	.31 (.24, .40)	.29 (.22, .38)
14	**M**	.37 (.02, .72)	.58 (.31, .69)	.27 (0, .55)	.02 (0, .24)	.37 (.28, .49)	.41 (.31, .54)
	F	.63 (.09, .72)	0 (0, .28)	.02 (0, .50)	.50 (.25, .60)	.35 (.27, .45)	.50 (.39, .62)
17	**M**	.59 (.22, .76)	.68 (.35, .82)	.10 (0, .42)	.08 (0, .38)	.31 (.23, .42)	.24 (.17, .34)
	F	.57 (.11, .68)	.44 (.04, .68)	.02 (0, .43)	.18 (0, .52)	.41 (.32, .52)	.37 (.28, .51)

D. FinnTwin16.

		A		C		E	
Survey	**Sex**	**Low***	**High**	**Low**	**High**	**Low**	**High**
16	**M****	.25 (.03, .45)	.75 (.43, .83)	.34 (.17, .51)	.02 (0, .31)	.41 (.32, .53)	.23 (.16, .33)
	F	.37 (.15, .58)	.40 (.04, .73)	.31 (.12, .49)	.31 (0, .61)	.32 (.26, .40)	.29 (.22, .40)
17	**M**	.26 (.04, .46)	.64 (.25, .80)	.28 (.11, .46)	.09 (0, .43)	.46 (.36, .58)	.27 (.19, .39)
	F	.27 (.05, .51)	.71 (.37, .83)	.40 (.19, .60)	.06 (0, .38)	.33 (.26, .41)	.22 (.16, .31)
18	**M**	.43 (.09, .67)	.58 (.22, .77)	.19 (0, .46)	.11 (0, .41)	.38 (.29, .51)	.31 (.21, .44)
	F	.61 (.35, .70)	.73 (.45, .82)	.02 (0, .25)	.03 (0, .28)	.37 (.30, .46)	.24 (.18, .34)

SUPPLEMENTARY TABLE III Percentage of non-exercisers, split by sex and parental education.

	Males		Females	
Survey	**Low parental education**	**High parental education**	**Low parental education**	**High parental education**
Netherlands Twin Register				
7	16.3%	9.7%	14.3%	11.1%
10	14.9%	11.0%	15.5%	10.2%
12	16.0%	10.6%	16.3%	10.5%
14	23.1%	14.2%	25.8%	15.2%
16	28.5%	20.6%	35.0%	22.5%
18	36.9%	34.6%	45.4%	34.7%
FinnTwin12				
12	26.0%	16.8%	39.3%	33.9%
14	8.4%	5.7%	7.6%	4.6%
17	13.5%	8.3%	9.9%	6.7%
FinnTwin16				
16	12.5%	11.1%	10.4%	7.4%
17	12.2%	10.1%	9.0%	6.5%
18	12.8%	10.1%	8.6%	7.8%

Part II

CAUSALITY TESTING USING TWIN DATA

Chapter **6**

A TWIN-SIBLING STUDY ON THE RELATIONSHIP BETWEEN EXERCISE ATTITUDES AND EXERCISE BEHAVIOR

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ABSTRACT

Social cognitive models of health behavior propose that individual differences in leisure time exercise behavior are influenced by the attitudes towards exercise. At the same time, large scale twin-family studies show a significant influence of genetic factors on regular exercise behavior. This twin-sibling study aimed to unite these findings by demonstrating that exercise attitudes can be heritable themselves. Secondly, the genetic and environmental cross-trait correlations and the monozygotic (MZ) twin intrapair differences model were used to test whether the association between exercise attitudes and exercise behavior can be causal. Survey data were obtained from 5,095 twins and siblings (18-50 years). A genetic contribution was found for exercise behavior (50% in males, 43% in females) and for the six exercise attitude components derived from principal component analysis: perceived benefits (21, 27%), lack of skills, support and/or resources (45, 48%), time constraints (25, 30%), lack of energy (34, 44%), lack of enjoyment (47, 44%) and embarrassment (42, 49%). These components were predictive of leisure time exercise behavior (R^2= 28%). Bivariate modeling further showed that all the genetic ($0.36<|r_A|<0.80$) and all but two unique environmental ($0.00<|r_E|<0.27$) correlations between exercise attitudes and exercise behavior were significantly different from zero, which is a necessary condition for the existence of a causal effect driving the association. The correlations between the MZ twins' difference scores were in line with this finding. It is concluded that exercise attitudes and exercise behavior are partly heritable, that attitudes and behavior are partly correlated through pleiotropic genetic effects, but that the data are compatible with a causal association between exercise attitudes and behavior.

INTRODUCTION

The prevention of non-communicable diseases, such as coronary heart disease, diabetes and cancer, has become a cornerstone of medical approaches in modern urbanized societies. Increasing regular leisure-time exercise behavior in the general population is a promising strategy to counteract these diseases (Garber et al., 2011; Warburton et al., 2010). Despite numerous attempts to increase exercise participation, the majority of adults does not meet the recommended guidelines (Martinez-Gonzalez et al., 2001; Troiano et al., 2008). To increase the success of interventions, it is important to understand the determinants of exercise behavior. Much research on these determinants has been based on social cognitive models of health behavior that emphasize the role of attitudes and beliefs (Biddle & Nigg, 2000; King et al., 1992), such as the

health belief model (Becker, 1974), the theory of planned behavior (Ajzen, 1985; Hagger, Chatzisarantis & Biddle, 2002) and the health action process approach (Schwarzer, 1992). Attitudes are defined as "a psychological tendency that is expressed by evaluating a particular entity with some degree of favor or disfavor" (Eagly & Chaiken, 1993). A person who perceives the advantages of exercising to outweigh the disadvantages is likely to have a positive attitude towards exercising and to adopt and maintain exercise activities (Becker, 1974).

Many studies have shown that there is a robust association between perceived benefits/barriers and exercise behavior (for reviews and meta-analyses, see Allender, Cowburn & Foster, 2006; Hagger et al., 2002; Petter, Blanchard, Kemp, Mazoff & Ferrier, 2009; Rhodes, Fiala & Conner, 2009; Trost, Owen, Bauman, Sallis & Brown, 2002). However, this association does not necessarily imply the assumed direction of causation from attitude to behavior. A reversed causal mechanism may be in play where attitudes follow behavior. Regular exercisers may increase the perceived benefits of their lifestyle choice and decrease the potential downsides through the social psychological mechanism of cognitive dissonance (Festinger, 1957). Furthermore, the association may (partly) reflect an underlying set of "third" factors, including genetic factors, which affect both exercise behavior and the perception of its benefits and barriers. An increasing number of studies has provided evidence that genetic influences contribute appreciably to individual differences in exercise behavior (Huppertz et al., 2012; Stubbe & de Geus, 2009; van der Aa et al., 2010), with peak heritabilities in late adolescence (≈ 80%) and heritabilities of around 50% across most of the life span, in particular adulthood. Typical perceived benefits of exercising are "meeting new people", "a sense of accomplishment" and "feeling energized". Perception of these may be related to heritable traits like extraversion, exercise ability and the acute psychological mood response to exercise (Bryan et al., 2007; de Geus & de Moor, 2011). Those heritable traits may either underlie the adoption and maintenance of regular exercise behavior through their effects on attitudes (or vice versa), or they may independently influence attitudes and behavior. If this influence is due to shared genes, the latter case is known as genetic pleiotropy, where low level biological variation has independent effects on multiple complex traits at the organ and behavioral level (de Geus & de Moor, 2011).

The hypothesis that exercise attitudes and behavior are related through pleiotropic genetic factors implies that both attitudes and behavior are heritable. Numerous studies have demonstrated the heritability of exercise behavior (de Vilhena e Santos et al., 2012; Stubbe & de Geus, 2009). Little is

known about the heritability of exercise attitudes, but studies have shown a striking heritability for attitudes across various domains such as religious, social and political attitudes (Eaves & Hatemi, 2008; Hatemi et al., 2010; Martin, 1978; Martin et al., 1986; Olson, Vernon, Harris & Jang, 2001). Olson et al. (2001) assessed the general attitude towards three categories of physical activities, with a single item for each category, in a sample of 336 twin pairs. Genetic effects explained 44, 36 and 52% of the phenotypic variance in attitudes towards "doing athletic activities", "exercising" and "playing organized sports", respectively. This suggests that attitudes towards leisure time exercise behavior are likely to be heritable.

De Moor et al. (2008) pioneered two non-experimental tests of the causal hypothesis against the null hypothesis of pleiotropic genetic effects using cross-sectional twin data. First, if perceived benefits and barriers causally influence exercise behavior, all genetic and environmental factors influencing individual differences in the perception of these benefits and barriers will also, through the causal chain, influence individual differences in exercise behavior. The same principle applies to the reverse case where exercise behavior causally influences the perceived benefits and barriers. This can be tested in a bivariate genetic model by computing genetic and environmental correlations between two traits (Neale & Cardon, 1992). The finding that the genetic and the environmental correlations are significant would be consistent with a causal association between the traits. If only the environmental correlation or only the genetic correlation is found to be significant, the causal hypothesis would be rejected, in favor of underlying environmental or genetic factors, respectively, affecting both the perception of benefits/barriers and engagement in exercise activities. Second, if the perceived benefits/barriers are causally related to exercise behavior, within-pair differences in the perceived benefits/barriers of exercise should be associated with within-pair differences in exercise behavior in genetically identical twins. A non-significant association between the two would falsify the hypothesis of causality in either direction and point towards genetic factors driving the association.

The first aim of this study was to investigate the heritability of exercise attitudes based on commonly used survey items on the perceived benefits of and barriers towards physical exercise. The second aim was to better understand the nature of the association by computing 1) the genetic and environmental correlations between exercise attitudes and exercise behavior and 2) the phenotypic cross-trait correlations between the within-trait difference scores in genetically identical twins.

METHOD

Participants

This study is part of ongoing research by the Netherlands Twin Register where twins and their relatives (parents, siblings, spouses and adult offspring) are voluntarily registered (Boomsma et al., 2006; Boomsma et al., 2002; Willemsen et al., 2013). Since 1991, adult participants have been invited to complete surveys about their health, lifestyle and behavior every 2-3 years. Data on both exercise attitudes (perceived benefits and barriers) and exercise behavior (type, frequency and duration) were available for the year 2002. Data of twins and a maximum of two full brothers and two full sisters were selected (N= 5,887). Exclusion criteria were unknown zygosity (53 individuals) and being younger than 18 or older than 50 years old (739). The final sample consisted of 3,906 twins and 1,189 siblings from 2,795 families. The mean age was 30.5 years (SD= 7.0). For 5,060 individuals, data were available on both exercise behavior and at least one of the attitude components that were subsequently derived from principal component analysis (PCA). For 1,273 twin pairs, data on exercise behavior and all the attitude components were available for both individuals. Of these, 189 pairs were monozygotic male (MZM), 81 were dizygotic male (DZM), 512 were monozygotic female (MZF), 248 were dizygotic female (DZF) and 243 were dizygotic of opposite-sex (DOS). Zygosity of same-sex twins was determined by DNA typing (62.7%) or was based on longitudinal assessment of six items on physical similarity and the frequency of confusion of the twins by parents, other family members and strangers (37.3%). Zygosity classification based on these items has shown 97% agreement with DNA polymorphisms (Willemsen et al., 2005). The data collection protocol was approved by the Medical Research Ethics Committee of the VU University Medical Center.

Measures

Exercise participation was assessed in two parts (Stubbe et al., 2006; Stubbe et al., 2007). First, the question “Do you exercise regularly?” could be answered with either “yes” or “no”. If the answer was affirmative, follow-up questions concerned the type of exercise (for example, health club exercise, soccer or running), frequency (times a week) and duration (minutes each time). The trait of interest was leisure time exercise behavior, explicitly excluding non-leisure physical activities (e.g., cycling or walking to get somewhere, gardening, house cleaning, etc.). Ainsworth et al. (2000)’s compendium of physical activities was

used to recode each activity into its metabolic equivalent of task (MET), with one MET corresponding to the rate of energy expenditure of an individual at rest, i.e., approximately one kcal/kg/h. Weekly MET hours were computed as the respective MET score multiplied by the number of hours per week, calculated per reported exercise type and then summed up over exercise types. The six-month test-retest reliability of this measure was 0.82 (de Moor et al., 2008). Individuals that did not participate in any exercise activities received a weekly MET hours score of zero. Because of the skewed distribution of weekly MET hours, data were log-transformed prior to the analyses.

Perceived benefits of exercise behavior were measured by 10 items with a four-point response scale, ranging from "strongly disagree", "disagree", "agree" to "strongly agree". Seven items were derived from a questionnaire by Devereaux Melillo, Williamson, Futrell and Chamberlain (1997). The remaining three items were taken from a questionnaire by Sechrist, Walker and Pender (1987). The internal consistency of the 10 items was high (Cronbach's α= 0.91). Perceived barriers towards exercise behavior were measured by 23 items derived from a questionnaire by Sallis et al. (1989; van Sluijs, van Poppel, Twisk, Brug & van Mechelen, 2005). Each item could be answered on a five-point response scale (ranging from "never", "rarely", "sometimes", "often" to "very often"). Again, the internal consistency of these 23 items was high (Cronbach's α= 0.90, ranging from 0.66 to 0.83 for the subscales that were subsequently derived from PCA, see below). The English back translation of the questionnaire can be found in the supplementary material.

Statistical analyses

A PCA was run in SPSS for Windows (version 20.0, SPSS Inc.) on the 33 attitude items to establish whether they could be reduced to a smaller number of components. Because of the dependency of observations coming from twins and siblings from the same family, we randomly selected one individual per family to confirm results of the PCA. After a direct oblimin rotation, the number of components was determined by selecting all components with an eigenvalue larger than one. An item belonged to a component if the absolute value of the component loading was larger than 0.4 (the absolute was used because components can be scaled negatively). It could therefore happen that one item belonged to more than one component if it loaded high on more than one component. For each component, the mean was computed over those items with an absolute component loading larger than 0.4. Mean scores were coded as missing if more than 25% of the items for the component were missing (as

suggested by van Sluijs et al., 2005). The resulting attitude component scores were used in subsequent analyses, one per individual for each of the six components. A multiple regression analysis was run in STATA to determine the amount of variance in exercise behavior explained by the attitude components, while taking into account familial relatedness.

Modeling of the twin and sibling data was performed using structural equation modeling in OpenMx (Boker et al., 2011). Bivariate analyses were run separately for each attitude component with exercise behavior. These analyses were run in two steps. First, in a series of saturated models, the strength of the relationships between exercise behavior and each of the attitude components (phenotypic correlations) and the twin and sibling resemblances for exercise behavior and each attitude component (twin/sibling pair correlations, within-trait and cross-trait) were estimated in each twin zygosity group separately (MZM, DZM, MZF, DZF and DOS). It was tested whether age should be included as a fixed effect by regressing it on the means and whether separate parameters should be estimated for males and females. Significance was tested against an alpha level of 0.05. In these analyses, full sibling and twin/sibling correlations were equated to dizygotic (DZ) twin correlations as these pairs all share on average 50% of their segregating genes, whereas monozygotic (MZ) twins are genetically identical.

Comparing the MZ with the DZ cross-twin/sibling within-trait correlations provides a first indication of the sources of variation observed for each trait. Possible sources of variation are additive genetic influences (A), dominant genetic influences (D), common environmental influences (C) and unique environmental influences (E). Due to the differences in genetic similarity between MZ versus DZ twins and siblings, additive genetic effects are suggested for a trait if its MZ cross-twin within-trait correlation is substantially larger than the correlation of DZ twins and siblings, whereas dominant genetic effects are suggested if the DZ correlation is smaller than half the MZ correlation. If the DZ correlation is larger than half the MZ correlation, common environmental effects (C) are implied. Finally, as MZ twins share the same genes and the same common environment, an MZ cross-twin within-trait correlation less than unity indicates unique environmental effects (E; including measurement error; Plomin, DeFries, McClearn & McGuffin, 2008). Similar to the comparison of MZ and DZ cross-twin/sibling within-trait correlations, the comparison of the MZ with the DZ cross-twin/sibling *cross-trait* correlations is informative to determine the sources of *co*variation between traits. For example, larger MZ cross-twin cross-trait correlations compared with the DZ

cross-twin/sibling cross-trait correlations suggest that common genetic factors explain part of the observed phenotypic correlation between two traits.

Second, heritability of the attitude components and exercise behavior, and the genetic and environmental correlations between the two were assessed in a series of genetic models. In the first model, the full bivariate ACE model, the variances of the respective attitude components and exercise behavior were decomposed into variance explained by A, C and E. In addition, the covariances among the attitude components and exercise behavior were decomposed into covariance due to A, C and E (i.e., those A, C and E influences that overlap among the traits). Guided by the within- and cross-twin/sibling correlations, the correlational approach to scalar and non-scalar sex limitations was applied, as described by Neale, Roysamb and Jacobson (2006). The A, C and E path coefficients were estimated for both traits, for males and females separately. In addition, the additive genetic correlations between the two traits were estimated for males and females, as well as the common environmental and the unique environmental correlations. The correlation between genotypes of opposite-sex was estimated between traits too. If the cross-twin/sibling within-trait DOS correlation in the saturated model was significantly lower than what would be expected based on the DZ correlation for a trait, its genetic within-trait correlation was freely estimated (for a path diagram, see Fig. 6 in Neale et al., 2006). Finally, means were estimated for the two traits, for each sex separately, and age was included as a fixed effect by regressing it on the mean(s), if it was significant in the saturated model. Again, significance was tested against an alpha level of 0.05.

The full ACE-model was compared to an AE-model in which the common environmental component was dropped. Subsequently, it was tested whether quantitative sex differences could be omitted. Genetic structural equation modeling in OpenMx was used with the raw-data maximum likelihood procedure for estimation of parameters. Nested submodels were compared by hierarchic χ^2 tests. The χ^2 statistic is computed by subtracting -2LL (log-likelihood) of a reduced model from that of the full model: $\chi^2 = -2LL_0 - (-2LL_1)$. This χ^2 statistic is distributed with degrees of freedom (df) equal to the difference in the df between the two models ($\Delta df = df_0 - df_1$). If constraining the model did not give a significant deterioration of fit (using an alpha level of 0.05), the most parsimonious model was accepted as the best fitting model.

It was tested whether dropping the genetic and environmental cross-trait correlations led to a significant deterioration of the model fit. As suggested by

de Moor et al. (2008), testing the significance of the genetic and environmental cross-trait correlations provides a test of whether an association between traits can be causal. If the genetic *and* environmental correlations between the respective attitude component and exercise behavior were significantly different from zero, this would be consistent with the hypothesis that a causal effect drives the association (necessary condition), although it would not constitute a proof of causality. If one of the genetic or environmental correlations was non-significant, this would falsify the hypothesis that exercise attitudes and exercise behavior are causally related for that specific component.

Finally, the within-trait difference scores for the attitude components and for exercise behavior were calculated for MZM and MZF twins. Subsequently, the difference score of each attitude component was correlated with the difference score of exercise behavior. Using MZ twin data removes the possibility of genetic confounding and a significant correlation would therefore show that the relationship between the two phenotypes is not merely due to genes (see MZ twin intrapair differences model in de Moor et al., 2008). This would thus be consistent with (but not proof of) a causal association, whereas a non-significant correlation would falsify the hypothesis of causality.

RESULTS

The PCA including all attitude items yielded six components with an eigenvalue larger than one. The component loadings of all items on the six components are provided as supplementary material. The first component includes items related to available equipment and support and is labeled “Lack of skills, support and/or resources” (Cronbach’s α for these items= 0.80). The second component consists of the 10 perceived benefits items and is labeled “Benefits” (α= 0.91). The third component contains items related to perceived lack of time and is labeled “Time constraints” (α= 0.83). The fourth component includes items that relate to a lack of energy/will-power and having a bad health. This component is labeled “Lack of energy” (α= 0.78). The fifth component includes items about lack of interest and pleasure to exercise and is labeled “Lack of enjoyment” (α= 0.74). Items belonging to the sixth component are related to being overweight and insecurities about physical appearance. This component is labeled “Embarrassment” (α= 0.66). The means and standard deviations of the attitude components and exercise behavior (weekly MET hours) are provided in Table 1.

TABLE 1 Untransformed means and standard deviations of the attitude components and exercise behavior, and phenotypic correlations of the attitude components with exercise behavior (95% CIs).

Variable name	Sex	Means (SD)	Phenotypic correlations
Lack of enjoyment	♂	.71 (.69)	-.44 (-.47, -.42)
	♀	.83 (.73)	-.44 (-.47, -.42)
Time constraints	♂	1.68 (.88)	-.37 (-.41, -.33)
	♀	1.68 (.88)*	-.28 (-.31, -.25)
Benefits	♂	2.13 (.51)	.32 (.29, .35)
	♀	2.16 (.46)	.32 (.29, .35)
Lack of skills, support and/or resources	♂	.58 (.55)	-.36 (-.39, -.31)
	♀	.70 (.60)	-.40 (-.43, -.37)
Embarrassment	♂	.35 (.51)	-.20 (-.23, -.18)
	♀	.69 (.71)	-.20 (-.23, -.18)
Lack of energy	♂	.83 (.60)	-.34 (-.37, -.32)
	♀	1.14 (.68)	-.34 (-.37, -.32)
Weekly MET hours	♂	12.10 (17.14)	
	♀	8.70 (13.03)	

**Males and females were combined as their means were not significantly different from each other.*

Exercise behavior was positively related to the perceived benefits component and negatively related to the perceived barriers components (Table 1). “Lack of enjoyment” was most strongly associated with exercise behavior in both sexes (r= -0.44), “Embarrassment” was least associated (r= -0.20, Table 1). The multiple regression revealed that all six attitude components were significant predictors of exercise behavior ($p<0.01$) and that together they explained 28% of the variance in exercise behavior. The within-trait MZ correlations were larger than the within-trait DZ twin/sibling correlations for all attitude components and exercise behavior (Table 2). This indicates that genetic factors are of importance in all phenotypes. Most DZ twin/sibling correlations were not larger than half the MZ correlations, suggesting that common environmental factors are of minor importance for attitudes towards exercise behavior as well as for actual exercise behavior. For “Lack of skills, support and/or resources”, “Lack of enjoyment”, “Embarrassment” and exercise behavior, the within-trait DOS correlations were significantly lower than what would be expected based on the DZ correlations, indicating qualitative sex differences. All absolute MZ cross-twin cross-trait correlations were larger than the DZ cross-twin/sibling cross-trait correlations, with the exception of “Time constraints” and “Embarrassment” in men (Table 2). The phenotypic correlations between

TABLE 2 Cross-twin/sibling *within*-trait (1) and *cross*-trait correlations (2) of the attitude components and exercise behavior (95% CIs).

	within/cross	MZM	DZM/siblings	MZF	DZF/siblings	DOS/siblings
Benefits	**1**	.26 (.19, .32)	.11 (.07, .16)	.26 (.19, .32)	.11 (.07, .16)	.11 (.07, .16)
	2	.21 (.16, .25)	.07 (.04, .11)	.21 (.16, .25)	.07 (.04, .11)	.07 (.04, .11)
Lack of skills, support and/or resources	**1**	.46 (.41, .51)	.25 (.19, .31)	.46 (.41, .51)	.25 (.19, .31)	.16 (.09, .22)
	2	-.27 (-.31, -.22)	-.13 (-.16, -.09)	-.27 (-.31, -.22)	-.13 (-.16, -.09)	-.13 (-.16, -.09)
Time constraints	**1**	.29 (.23, .35)	.14 (.10, .19)	.29 (.23, .35)	.14 (.10, .19)	.14 (.10, .19)
	2	-.18 (-.23, -.14)	-.19 (-.25, -.13)	-.18 (-.23, -.14)	-.11 (-.15, -.06)	-.09 (-.14, -.05)
Lack of energy	**1**	.42 (.36, .47)	.19 (.15, .24)	.42 (.36, .47)	.19 (.15, .24)	.19 (.15, .24)
	2	-.22 (-.26, -.17)	-.10 (-.14, -.07)	-.22 (-.26, -.17)	-.10 (-.14, -.07)	-.10 (-.14, -.07
Lack of enjoyment	**1**	.46 (.40, .51)	.17 (.11, .23)	.46 (.40, .51)	.17 (.11, .23)	.12 (.05, .18)
	2	-.31 (-.35, -.27)	-.11 (-.15, -.07)	-.31 (-.35, -.27)	-.11 (-.15, -.07)	-.11 (-.15, -.07)
Embarrassment	**1**	.45 (.39, .51)	.29 (.23, .34)	.45 (.39, .51)	.29 (.23, .34)	.08 (.01, .14)
	2	-.16 (-.21, -.12)	-.19 (-.26, -.11)	-.16 (-.21, -.12)	-.10 (-.15, -.05)	-.01 (-.06, .04)
Exercise behavior	**1**	.45 (.39, .50)	.21 (.16, .27)	.45 (.39, .50)	.21 (.16, .27)	.15 (.09, .21)

MZM=monozygotic male, DZM=dizygotic male, MZF=monozygotic female, DZF=dizygotic female, DOS=dizygotic of opposite-sex.

attitudes towards exercise and exercise behavior thus seem at least partly explained by overlapping genetic factors.

In the bivariate ACE models, dropping the C-paths did not lead to a significant deterioration of the model fit, whereas subsequently constraining the A and E parameters to be equal across sex did (Supplementary table II, $\alpha<0.05$). Therefore, AE models that allowed for quantitative and qualitative sex differences were fitted to the data. The heritabilities of the attitude components and exercise behavior in the best fitting models are provided in Table 3. Heritability estimates ranged from 21 to 50%. The attitude components "Lack of skills, support and/or resources", "Lack of enjoyment" and "Embarrassment" were among the most heritable, whereas the heritabilities of "Benefits" and "Time constraints" were lower, indicating a greater role of unique environmental influences on individual differences in these components. In accordance with the cross-twin/sibling within-trait DOS correlations of the saturated model, the within-trait correlations between the latent genetic factors were lower among DOS twins compared to same-sex DZ twins and siblings for "Lack of skills, support and/or resources", "Lack of enjoyment", "Embarrassment" and exercise behavior, indicating that genetic factors influencing those phenotypes are (for a part) qualitatively different in men and women (Table 3).

Table 4 displays how much of the covariance between exercise attitudes and exercise behavior can be explained by genetic and environmental factors (A and E add up to 1). Genetic factors explained considerably more of the association between attitudes and behavior than environmental factors did.

Table 5 displays the estimates and 95% confidence intervals of the additive genetic and the unique environmental cross-trait correlations. None of the genetic correlations could be dropped without a significant deterioration of the model fit. The unique environmental correlations were significant as well, with the exception of the attitude components "Benefits" and "Embarrassment" for males. Similarly, the phenotypic cross-trait correlations between the intrapair MZ difference scores were significant for all attitude components but "Benefits" and "Embarrassment" for males (Table 6).

TABLE 3 Heritabilities of attitude components and exercise behavior in six bivariate models, separately for males and females, and the genetic within-trait correlations across DOS twins (95% CIs).

Variable name	Males		Females		DOS correlations
	A	E	A	E	
Benefits	.21 (.11, .31)	.79 (.69, .89)	.27 (.20, .33)	.73 (.67, .80)	.5[b]
Lack of skills, support and/or resources	.45 (.35, .54)	.55 (.46, .65)	.48 (.42, .54)	.52 (.46, .58)	.26 (.12, .41)
Time constraints	.25 (.15, .35)	.75 (.65, .85)	.30 (.23, .36)	.70 (.64, .77)	.5[b]
Lack of energy	.34 (.23, .44)	.66 (.56, .77)	.44 (.38, .49)	.56 (.51, .62)	.5[b]
Lack of enjoyment	.47 (.36, .56)	.53 (.44, .64)	.44 (.38, .50)	.56 (.50, .62)	.19 (.03, .35)
Embarrassment	.42 (.31, .52)	.58 (.48, .69)	.49 (.42, .54)	.51 (.46, .58)	.16 (.02, .30)
Exercise behavior[a]	.50 (.41, .58)	.50 (.42, .59)	.43 (.37, .49)	.57 (.51, .63)	.31 (.16, .45)

A=proportion of variance explained by additive genetic factors (=heritability), E=proportion of variance explained by unique environmental factors.

[a]*Taken from the first model (benefits).*

[b]*Model with genetic twin/sibling resemblance fixed to .5 fitted the data.*

TABLE 4 Proportions of the phenotypic covariances between exercise attitude components and exercise behavior that can be explained by additive genetic (A) and unique environmental (E) effects, separately for males and females (95% CIs).

Attitude component	Males		Females	
	A	E	A	E
Benefits	.82 (.59, 1.04)	.18 (-.04, .41)	.57 (.42, .71)	.43 (.29, .58)
Lack of skills, support and/or resources	.65 (.45, .83)	.35 (.17, .55)	.71 (.61, .81)	.29 (.19, .39)
Time constraints	.59 (.39, .78)	.41 (.22, .61)	.69 (.53, .84)	.31 (.16, .47)
Lack of energy	.67 (.43, .89)	.33 (.11, .57)	.64 (.52, .75)	.36 (.25, .48)
Lack of enjoyment	.78 (.63, .92)	.22 (.08, .37)	.66 (.56, .76)	.34 (.24, .44)
Embarrassment	.99 (.59, 1.43)	.01 (-.43, .41)	.82 (.63, 1.00)	.18 (0, .37)

TABLE 5 Estimated genetic (r_A) and unique environmental (r_E) cross-twin cross-trait correlations with exercise behavior, separately for males (m) and females (f; 95% CIs).

Attitude component	r_{Am}	r_{Af}	r_{Em}	r_{Ef}
Benefits	.80 (.57, 1.00)	.54 (.42, .68)	*.09 (-.02, .20)*[a]	.22 (.15, .28)
Lack of skills, support and/or resources	-.47 (-.60, -.33)	-.64 (-.73, -.56)	-.23 (-.34, -.11)	-.22 (-.29, -.15)
Time constraints	-.61 (-.80, -.42)	-.55 (-.68, -.43)	-.24 (-.34, -.13)	-.14 (-.21, -.07)
Lack of energy	-.50 (-.68, -.33)	-.54 (-.64, -.44)	-.18 (-.29, -.06)	-.23 (-.29, -.16)
Lack of enjoyment	-.70 (-.83, -.58)	-.68 (-.76, -.59)	-.19 (-.30, -.07)	-.27 (-.33, -.20)
Embarrassment	-.36 (-.54, -.20)	-.40 (-.50, -.29)	*0 (-.13, .12)*[a]	-.07 (-.15, 0)

r_{Am}=additive genetic correlation for males, r_{Af}=additive genetic correlation for females, r_{Em}=unique environmental correlation for males, r_{Ef}=unique environmental correlation for females; [a]Can be dropped without a significant deterioration of the model fit (α<.05).

TABLE 6 Cross-trait correlations between the cross-twin within-trait difference scores for monozygotic male (MZM) and female (MZF) twins (number of pairs).

	MZM	MZF
Benefits	.11 (196)	.22** (532)
Lack of skills, support and/or resources	-.27** (194)	-.25** (527)
Time constraints	-.32** (194)	-.16** (527)
Lack of energy	-.25** (194)	-.26** (528)
Lack of enjoyment	-.24** (194)	-.28** (528)
Embarrassment	-.08 (192)	-.12** (522)

***Significant at the .01 level (two-tailed).*

DISCUSSION

The main aims of this study were 1) to test the heritability of perceived benefits of and barriers towards exercise behavior and 2) to test whether a causal effect could be a valid explanation for the phenotypic association. Six main attitude components emerged from commonly used items by means of a PCA: "Benefits", "Lack of skills, support and/or resources", "Time constraints", "Lack of energy", "Lack of enjoyment" and "Embarrassment". The perceived benefits component was positively related to exercise behavior, whereas the perceived barriers were negatively related to exercise behavior. The attitude components explained 28% of the variance in exercise behavior. Heritability of the attitude components ranged from 21 to 49%.

What can give rise to the heritability of attitudes? Personality is a first possible trait that may mediate attitude formation after initial exposures to exercise activities (Olson et al., 2001). Personality traits are known to have a partly genetic basis (e.g., de Moor et al., 2012; Jang et al., 1996) and are also known to be associated with exercise behavior (de Moor et al., 2006). A meta-analysis concluded that there is a positive association of exercise behavior with extraversion and conscientiousness and a negative association with neuroticism (Rhodes & Smith, 2006). Courneya and Hellsten (1998; also see Davis, Fox, Brewer and Ratusny, 1995) investigated the relationship between the big five personality dimensions and exercise motives and barriers. They found that conscientious individuals were more likely to exercise for health reasons and were less likely to report barriers such as a lack of energy or a lack of motivation. This makes intuitive sense as conscientiousness is related to being ordered and self-disciplined (Rhodes & Smith, 2006). Extraverts, in turn, mainly exercised for social reasons and enjoyment of physical activity, which is compatible with the tendency of extraverts to be sociable and to seek excitement (Rhodes & Smith, 2006). Finally, individuals who scored highly on neuroticism mainly exercised to improve their physical appearance and for weight control. They reported a lack of energy, lack of motivation and embarrassment as barriers to exercise. Neurotic individuals tend to be emotionally unstable and self-conscious (Rhodes & Smith, 2006) and are probably predisposed to be worried about their physical appearance and the judgmental reactions of others (Courneya & Hellsten, 1998).

The affective responses to acute exercise may form another link between attitudes and exercise behavior. Individual differences in the acute mood effects of exercise have long been neglected, but recent research has produced

interesting findings (Ekkekakis, 2008; Parfitt & Hughes, 2009). Ekkekakis et al. (2005) have proposed an evolutionary-based model on the affective reactions to exercise. They argue that low-intensity exercise is likely to evoke rewarding reactions in most individuals, whereas high-intensity exercise is likely to evoke aversive reactions. However, with respect to intermediate levels of exertion, there is large variability in reactions between individuals, with some of them reporting a positive response (pleasure) and others reporting a negative response (displeasure). Differences in the acute mood effects of exercise are likely to be (for a part) genetically determined (de Geus & de Moor, 2008). Perceiving exercise benefits or a lack of energy and enjoyment, in turn, is likely to be affected by the acute mood effects of exercise.

Whether or not exercise activities make someone feel better may also depend on fitness and exercise ability as most people favor doing things they are good at (de Geus & de Moor, 2008). Numerous studies have looked at individual differences in sport performance (for an overview, see Bouchard and Hoffman, 2011). Even for highly standardized training interventions, large individual differences have emerged for changes in fitness indices such as VO_2max, heart rate, cholesterol levels and blood pressure (Bouchard & Rankinen, 2001) and genetic factors are likely to play a major role in causing those differences (Bouchard & Hoffman, 2011). Depending on their genetic predisposition, some people may improve fast in a given exercise activity, whereas others may improve slowly or not at all. Slow-improvers may conclude that they are “not the sporty type” or even feel embarrassed and drop out, whereas fast-improvers may enjoy the activity and further improve through training (Brutsaert & Parra, 2006; de Geus & de Moor, 2008). Olson et al. (2001) have indeed found a large genetic correlation (r= 0.63) between “attitudes toward athleticism” and self-reported athletic ability. Similar mechanisms may lead to individual differences in activity-induced weight loss (Hainer et al., 2008), again influencing exercise attitudes. Finally, a genetic predisposition to have a higher (baseline) level of fitness should positively affect an individual’s capacity to take on many tasks before succumbing to physical and mental fatigue which could be related to the perception of time constraints, an often cited barrier to regular exercise.

Thus, there are various mechanistic connections between traits with a known or plausible heritable component and exercise attitudes. The above overview is not exhaustive and the suggested mechanisms are probably interrelated. It should be noted that, despite our focus on genetics, the largest amount of variance in exercise attitudes could be explained by unique environmental

factors, providing support for the assumption that individuals form their attitudes based on their experiences (although this component does include measurement error as well; Olson et al., 2001).

There were significant phenotypic correlations between all the attitude components and exercise behavior for both men and women. The strength of the associations is in line with previous studies (Hagger et al., 2002; Rhodes et al., 2009). The second aim of this study was to better understand the nature of this relationship. Based on the above it might be expected that genetic factors influencing personality, acute mood effects of exercise and fitness/exercise ability affect the formation of exercise attitudes and increase the chance of adoption and/or maintenance of exercise behavior. However, the main question is whether these genetic factors influence exercise behavior independently of attitude formation (pleiotropy) or whether the data are compatible with the main hypothesis held by exercise interventionists stating that attitudes causally contribute to exercise behavior. Given that not only the genetic correlations, but also the environmental correlations and the cross-trait correlations between the MZ twin difference scores were significant (the latter two with the exception of “Benefits” and “Embarrassment” in males), the data are indeed compatible with a causal effect of attitudes on exercise behavior. A different pattern might have led to falsification of this hypothesis. Such falsification was for instance illustrated for the effects of exercise behavior on mental health (de Moor et al., 2008) and subjective well-being (Bartels et al., 2012). However, it should be noted that the data do not constitute a proof of causality and that due to the cross-sectional design, the direction of any causality remains unknown. Adding longitudinal follow-up data is needed to reveal the direction of causality and to provide more definitive support for the common practice of targeting attitudes in exercise intervention programs (Hagger et al., 2002). For males, the non-significant environmental correlation and the non-significant cross-trait correlations between the MZ twin difference scores for “Benefits” and “Embarrassment” point towards genetic pleiotropy in the absence of causality for these specific attitude components. However, this finding should be treated with caution as the unique environment also contains measurement error, which does not need to be correlated across traits.

The limitations of this study should be addressed. First of all, a single compound exercise score was used. However, it is likely that perceived benefits and barriers affect subcomponents of exercise behavior such as adoption/maintenance and frequency/duration to different degrees. More than two decades ago, Dishman (1990) stated that “knowledge and belief in

the health benefits of physical activity may motivate initial involvement and return to activity following relapse, but feelings of enjoyment and well-being seem to be stronger motives for continued participation" (p.83). Studies have shown that the predictors of exercise adoption indeed tend to differ from the predictors of exercise maintenance (Buckworth, Lee, Regan, Schneider & DiClemente, 2007; Nigg, Borrelli, Maddock & Dishman, 2008). Schwetschenau, O'Brien, Cunningham and Jex (2008) investigated the difference between internal (e.g., embarrassment) and external (e.g., inadequate exercise facilities) barriers at an on-site corporate fitness center. External barriers mainly accounted for not joining the fitness center, whereas internal barriers mainly accounted for frequency of fitness center visits. Secondly, the assumption that DZ twins and siblings are genetically about half as similar as MZ twins only holds true under the assumption of random mating. As soon as spouses select each other based on their phenotypic similarity (e.g., when exercisers are attracted by other exercisers), DZ correlations are higher than expected which would overestimate common environmental effects (de Moor et al., 2011; Eaves, 1977). However, no significant impact of the common environment was found in this study, suggesting that the potential distortion through assortment was limited. Finally, the classical twin design is based on the equal environments assumption which posits that non-genetic sources of differential treatment of MZ versus DZ twins do not inflate the MZ twin resemblance for the phenotype under study (Derks, Dolan & Boomsma, 2006; Scarr & Carter-Saltzman, 1979). A more similar treatment of MZ versus DZ twins might arise when MZ twins resemble each other more in athletic appearance than DZ twins. This differential treatment may influence their attitude formation. However, the equal environments assumption is violated only if the differential treatment is caused simply by zygosity status, but not when it is indirectly caused by, for instance, exercise ability, which itself has been shown to be substantially heritable (Bouchard & Hoffman, 2011).

Despite these limitations, the present study provides an important extension of the literature as it is the first one to investigate the heritability of perceived exercise benefits and barriers and their (genetic) association with exercise behavior in a large group of twins and their siblings. Health promotion strategies often aim to change the populace's attitudes towards exercise behavior by educating people on the health benefits of regular exercise and ways to reduce barriers to engage in exercise activities. This study showed that the perception of exercise benefits and barriers will partly depend on an individual's genetic makeup, but that substantial environmental influences are present as well. Furthermore, after taking genetic pleiotropy into account, our

data were compatible with a causal association between exercise attitudes and exercise behavior. Replication in longitudinal studies is now needed to more firmly establish this causality and its direction.

SUPPLEMENTARY TABLE I Component loadings of the items measuring the perceived benefits of and barriers to exercise behavior on the six extracted components revealed with PCA (oblimin rotation).
A. Benefits.

Item	Component:	1	2	3	4	5	6
a. Exercise improves the way my body looks.		-.04	**.73**	.06	-.04	.12	.05
b. I feel better when I exercise regularly.		-.01	**.81**	0	-.06	-.08	-.07
c. Exercising gives me more energy.		0	**.83**	.03	-.03	-.03	-.07
d. Exercising gives me a sense of accomplishment.		.03	**.73**	-.01	.04	-.10	.05
e. Exercise keeps my mind active.		.04	**.79**	0	.02	-.10	-.01
f. Exercise is good for my heart.		-.01	**.79**	0	-.04	.15	.02
g. Exercise lifts my spirits.		.03	**.83**	-.02	-.04	-.09	-.05
h. I exercise to stay healthy.		-.05	**.63**	-.02	0	.19	.13
i. Exercise decreases feelings of stress and tension.		.02	**.75**	-.03	-.02	-.12	-.07
j. Exercising is a way to meet new people.		.03	**.55**	-.05	.14	-.05	.05

B. Barriers.

Item	Component:	1	2	3	4	5	6
a. I am insecure about my appearance when I am active.		0	.06	.05	-.08	.07	**.67**
b. I am not interested in physical activity.		.13	-.17	.01	-.31	**.51**	.13
c. I do not have self-discipline or will-power.		.12	.05	-.04	**.55**	.30	.02
d. I do not have the time for it.		.03	0	**.56**	-.35	.12	-.24
e. I do not have the energy for it.		-.05	-.01	.10	**-.85**	0	.01
f. I do not have anybody to exercise with.		**.48**	.02	.04	-.21	.14	-.07
g. I do not enjoy exercise or physical activity.		.25	-.18	0	-.17	**.55**	.20
h. I do not want to fail, so I do not try it.		**.48**	.02	-.05	.05	.04	.35
i. I do not have the required materials for exercising.		**.83**	-.01	.04	.15	0	.01
j. I often think that the weather is too bad.		**.67**	.02	.03	-.15	-.01	-.25
k. I do not have enough skills as a sportsman or woman.		**.61**	-.06	.01	-.04	.17	.20
l. I am too tired to exercise.		-.02	-.02	.11	**-.80**	-.06	.04

Item	Component:	1	2	3	4	5	6
m. I do not have enough knowledge on how to exercise.		**.70**	-.04	0	.02	-.01	.14
n. I have a poor health.		.11	-.05	-.05	**-.45**	-.37	.37
o. I am scared of injuries.		.34	-.04	-.03	-.18	**-.44**	.26
p. I think moving is too difficult.		.16	-.04	-.04	**-.41**	-.05	**.42**
q. I do not have easily accessible facilities in the area.		**.60**	0	.08	.03	-.08	-.05
r. I am too fat.		-.02	0	.16	-.01	.02	**.76**
s. I think exercising is boring.		.24	-.15	.07	-.09	**.52**	.27
t. I have obligations at work.		.06	0	**.83**	.03	-.01	-.02
u. I have social obligations.		.02	.01	**.89**	.02	-.02	.09
v. I have obligations in my family.		.01	-.03	**.85**	.05	-.07	.14
w. I think exercising is too expensive.		**.40**	-.04	.17	-.08	.11	.11

Component loadings in bold were selected for a component (threshold=.4).

SUPPLEMENTARY TABLE II Bivariate model fitting results, separately for the six attitude components.

Attitude component	Model	Vs.	-2LL	df	X^2	Δdf	p
Benefits	1. ACE model		23940.99	10085	-	-	-
	2. AE model	**1**	**23941.89**	**10090**	**.90**	**5**	**.97**
	3. AE model, m=f	2	23988.76	10096	46.87	6	0
Lack of skills, support and/or resources*	1. ACE model		25440.84	10033	-	-	-
	2. AE model	**1**	**25444.83**	**10038**	**3.98**	**5**	**.55**
	3. AE model, m=f	2	25500.31	10044	55.49	6	0
Time Constraints	1. ACE model		29982.40	10035	-	-	-
	2. AE model	**1**	**29984.53**	**10040**	**2.13**	**5**	**.83**
	3. AE model, m=f	2	30010.65	10046	26.12	6	0

Attitude component	Model	Vs.	-2LL	df	X^2	Δdf	p
Lack of energy	1. ACE model		26867.46	10034	-	-	-
	2. AE model	**1**	**26870.30**	**10039**	**2.84**	**5**	**.72**
	3. AE model, m=f	2	26932.22	10045	61.92	6	0
Lack of enjoyment*	1. ACE model		27324.10	10038	-	-	-
	2. AE model	**1**	**27325.39**	**10043**	**1.29**	**5**	**.94**
	3. AE model, m=f	2	27357.88	10049	32.49	6	0
Embarrassment*	1. ACE model		26910.59	10000	-	-	-
	2. AE model	**1**	**26919.89**	**10005**	**9.30**	**5**	**.10**
	3. AE model, m=f	2	27160.99	10011	241.10	6	0

-2LL=-2 log likelihood, df=degrees of freedom, X^2=chi-square test statistic, Δdf=degrees of freedom of X^2 test, p=p-value; Most parsimonious models are printed in boldface type; The genetic within-trait DOS correlation is freely estimated for exercise behavior and for the attitude components that are marked with an asterisk.

Chapter 7

REGULAR EXERCISE BEHAVIOR IN YOUTH IS NOT RELATED TO CURRENT BODY MASS INDEX OR BODY MASS INDEX AT 7-YEAR FOLLOW-UP

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ABSTRACT

This population-based study aimed 1) to test the presence of an association between regular voluntary exercise behavior that is performed in leisure time and body mass index (BMI) in childhood and youth and 2) to investigate the causal nature of this association using a longitudinal design in genetically informative subjects. Both exercise behavior and BMI were assessed repeatedly over time in 21,458 twin individuals from the Netherlands Twin Register (47.5% male) - first by parental report (ages 7, 10 and 12) and subsequently through self-report surveys (ages 14, 16 and 18). Exercise behavior was quantified as weekly metabolic equivalent of task hours. Correlations over time were higher for BMI than for exercise behavior ($r \approx 0.70$ vs. $r \approx 0.35$) across 12 different follow-up periods. Cross-sectionally, regular involvement in exercise behavior was not associated with lower BMI and in genetically identical twin pairs discordant for exercise behavior, the exercising twin did not have a lower BMI than the non-exercising twin. Longitudinally, linear and quadratic relationships between exercise behavior and BMI were non-significant. Changes in exercise behavior over time did not induce opposite changes in BMI. No consistent association between regular exercise behavior and BMI was observed from ages 7 to 18 years.

INTRODUCTION

Childhood obesity is a major health concern of our time. Olds et al. (2011) estimated the average prevalence of overweight in almost 500,000 children and adolescents of nine countries to range between 13.5% and 37.4%. Being overweight as a child not only impairs health in the short run (Reilly et al., 2003), but also increases morbidity and premature mortality in adulthood (Reilly & Kelly, 2011). Attempts to intervene and curb the obesity epidemic have aimed at increasing energy expenditure and/or decreasing energy intake, particularly the consumption of high-caloric food. Interventions that target children and youth tend to favor an increase in energy expenditure as opposed to a decrease in energy intake, owing to its positive effects on general health and possible risks associated with energy intake modification, such as compromise of growth and facilitation of eating disorders (Flynn et al., 2006; Janssen & Leblanc, 2010).

A wealth of studies on the relationship between energy expenditure and body composition in childhood and youth has focused on daily physical activity. These observational studies have produced rather mixed outcomes and studies

with null findings might be underrepresented as a result of a publication bias towards significant results (Bleich, Ku & Wang, 2011; Jiménez-Pavón, Kelly & Reilly, 2010; Must & Tybor, 2005; Wareham, van Sluijs & Ekelund, 2005). Admittedly, the majority of studies have methodological flaws such as a cross-sectional design that prohibits conclusions on cause-effect relationships and small sample sizes (Must & Tybor, 2005; Wareham et al., 2005). However, a systematic review on the effect of physical activity interventions in over 36,000 children did not support an obesity-reducing effect (Dobbins, Husson, DeCorby & LaRocca, 2013). The use of suboptimal measurement instruments is another potential flaw. Survey-based assessments of physical activity have shown poor agreement with actual measures of energy expenditure, which may reduce power to detect a relation between physical activity and body composition (Adamo et al., 2009). However, even studies using objective physical activity measurements, including doubly labeled water, step counts or accelerometers, have not produced systematic evidence for this link (Wilks, Besson, Lindroos & Ekelund, 2011), although sample sizes in these studies have by necessity been far more modest than those in survey studies.

Measuring physical activity by survey in childhood and youth is particularly challenging because of complex activity patterns that include spontaneous physical activity (e.g., fidgeting), physical activity related to transportation (bicycling), school or work, physical activity related to indoor and outdoor play and all structured and unstructured exercise activities (Adamo et al., 2009). These are even difficult to capture by surveys that rely on subjective recall of a complex set of activities. The present study, therefore, uses a different approach by focusing only on regular voluntary exercise behavior that is performed in leisure time and in structured settings, like health clubs, recreational outdoor activities and team sports. Because the exercise activities are both voluntary and often scheduled at regular times, recall is easier than less salient activities like the amount of walking or moderately intensive household activities during the day. Self-reported exercise behavior measured with surveys has indeed been shown to have high test-retest reliability (de Moor et al., 2008).

Obesity can be defined in a myriad of ways, but body mass index (BMI) has become the standard for defining and assessing overweight in both adults and children, and it is directly associated with negative long-term health consequences (Barlow, 2007; Bjorge, Engeland, Tverdal & Smith, 2008). Although not a perfect indicator of body fatness in thin children (Freedman & Sherry, 2009), it has been shown to strongly correlate with skinfold thickness,

body fat percentage and total fat mass in children and adolescents (Mei et al., 2002). BMI is therefore the most feasible approximation of body composition that can be assessed in large population-based samples.

Cross-sectional and longitudinal relationships were assessed between regular exercise behavior and BMI in a very large genetically informative longitudinal dataset, with data of monozygotic (MZ) and dizygotic (DZ) twins aged 7, 10, 12, 14, 16 and 18 years. The expectation of this study is that higher levels of exercise behavior in childhood and adolescence will lead to lower levels of BMI. This would be reflected in significant cross-sectional and longitudinal associations between (changes in) the two traits, even when accounting for genetic pleiotropy or confounding by latent environmental factors.

METHODS

Participants

The study is based on longitudinal research of the Netherlands Twin Register (NTR; van Beijsterveldt et al., 2013). Registered twins and family members are primarily Caucasian and live in all regions of the Netherlands (rural and urban areas). The large majority of twins are registered with the NTR as newborns. Both mothers and fathers (the latter after age 2) are invited to complete surveys about their twins' health, lifestyle and behavior at birth and when the children are approximately 2 (= "survey 2"), 3, 5, 7, 10 and 12 years old. The twins are subsequently approached to complete self-report surveys when they are 14 (= "survey 14"), 16 and 18 years old. Individuals with diseases or disabilities that may prevent them from being physically active (e.g., hemiplegia or heart disease) were excluded from the analyses (N= 346). Subsequently, an injury at the time of assessment led to an exclusion of the exercise data for that specific survey (N= 419 for survey 14, N= 371 for survey 16 and N= 72 for survey 18). The final dataset comprised 21,458 individuals born between 1984 and 2001 (47.5% males).

Table 1 presents the number of individuals with within-trait and cross-trait data on exercise behavior and BMI for the entire study. Data on both exercise behavior and BMI were available for 3,089 individuals on survey 7 (522 complete MZ and 1,005 complete DZ twin pairs), 4,444 on survey 10 (759 complete MZ and 1,425 complete DZ twin pairs), 10,261 on survey 12 (1,855 complete MZ and 3,153 complete DZ twin pairs), 7,171 on survey 14 (1,120 complete MZ and 1,759 complete DZ twin pairs), 4,256 on survey 16 (669

complete MZ and 875 complete DZ twin pairs) and 2,949 on survey 18 (464 complete MZ and 606 complete DZ twin pairs). The longitudinal structure included 2-year follow-ups (surveys 10 and 12, 12 and 14, 14 and 16, 16 and 18), a 3-year follow-up (7 and 10), 4-year follow-ups (10 and 14, 12 and 16, 14 and 18), a 5-year follow-up (7 and 12), 6-year follow-ups (10 and 16, 12 and 18) and a 7-year follow-up (7 and 14). Twin individuals and/or their parents provided informed consent to take part in research. If individuals decide not to participate in a specific survey, they can always re-enter on a subsequent survey. The main reason given for non-participation is "time constraints". The data collection protocol was approved by the Medical Research Ethics Committee of the VU University Medical Center (no. 2010/284).

TABLE 1 The number of individuals with within-trait (A) /cross-trait (B) data on weekly MET hours and BMI, both cross-sectionally and across different follow-up periods, for male (M) and female (F) individuals separately.

A. Within-trait		**MET hours**		**BMI**	
	Surveys	**M**	**F**	**M**	**F**
Cross-sectionally	**7**	1947	1971	1504	1593
	10	2941	2970	2221	2229
	12	6033	6313	5063	5247
	14	3480	4611	3234	4339
	16	1966	2796	1854	2757
	18	1106	1988	1085	1930
2-year follow-up	**10&12**	1205	1231	827	854
	12&14	2597	3238	2168	2653
	14&16	1027	1584	955	1547
	16&18	495	904	491	891
3-year follow-up	**7&10**	497	489	325	326
4-year follow-up	**10&14**	750	980	580	779
	12&16	1523	2105	1331	1867
	14&18	294	614	278	552
5-year follow-up	**7&12**	525	514	351	349
6-year follow-up	**10&16**	198	300	156	246
	12&18	681	1252	614	1085
7-year follow-up	**7&14**	413	505	309	412

B. Cross-trait		MET hours and BMI		ΔMET hours and ΔBMI*	
	Surveys	M	F	M	F
Cross-sectionally	**7**	1500	1589	-	-
	10	2216	2228	-	-
	12	5031	5230	-	-
	14	3071	4100	-	-
	16	1742	2514	-	-
	18	1057	1892	-	-
2-year follow-up	**10&12**	951	970	824	853
	12&14	2464	3101	2054	2525
	14&16	969	1570	867	1334
	16&18	483	871	453	813
3-year follow-up	**7&10**	375	365	323	324
4-year follow-up	**10&14**	672	932	543	724
	12&16	1459	2084	1250	1704
	14&18	280	584	268	535
5-year follow-up	**7&12**	408	387	351	349
6-year follow-up	**10&16**	174	292	143	217
	12&18	669	1202	602	1067
7-year follow-up	**7&14**	369	477	288	382

For example, for the 2-year follow-up "10&12", the following variables were available: MET hours for survey 10, MET hours for survey 12, BMI for survey 10 and BMI for survey 12; MET=metabolic equivalent of task, BMI=body mass index.

Measures

Exercise behavior was consistently assessed by parental report for surveys 7, 10 and 12, and by self-report for surveys 14, 16 and 18. A list of common exercise activities was provided, plus the option to add activities. Individuals were asked to indicate for each activity a) whether or not their child/ they participated in the activity and if so, b) for how many years, c) for how many months a year, d) how many times a week and e) how many minutes each time. Participants had to have been active in the activity during the past half year and only activities that were conducted for a minimum of three months a year were included (thereby excluding ski holidays, sailing camps and similar). In addition, activities related to transportation (walking and biking) were excluded. Activities during compulsory physical education classes were also excluded.

Each activity was subsequently recoded into its metabolic equivalent of task (MET), reflecting the energy expended during a specific activity as a multiple of energy expenditure at rest (approximately one kcal/kg/h). For individuals younger than 18 years, METs were taken from Ridley et al. (2008)'s compendium of energy expenditures for youth, whereas for individuals of 18 years or older, they were taken from Ainsworth et al. (2000)'s compendium of physical activities. The product of the MET score, weekly frequency and duration was summed over all exercise activities that an individual engaged in. If participants indicated an unrealistically large number of MET hours a week (>250), these were truncated at 250 MET hours (N= 16). For all surveys, if either exercise frequency or duration was missing while the other was provided, it was replaced with the median of that activity within the respective age group.

BMI (weight in kg/(height in m)^2) was calculated based on reported height and weight. BMI was standardized with the software package Growth Analyser RCT (2011, Version 4.0.28., Rotterdam, the Netherlands: Growth Analyser B.V.), based on sex-specific and age-specific BMI scores of the Dutch population (sdsBMI). As the correlations between mothers' and fathers' ratings were high at all ages (ranging from 0.82 to 0.88 for exercise behavior and from 0.98 to 1 for BMI), their average rating was used. If mean sdsBMI values were outside the range of +-5, height, weight, BMI and sdsBMI were excluded for that person at the respective survey (N= 16).

Statistical analyses

In a first set of analyses, tracking over time of both exercise behavior and sdsBMI were examined by test-retest correlations across the 2-, 3-, 4-, 5-, 6- and 7-year follow-up periods. So-called saturated models were fitted in the structural equation software OpenMx (Boker et al., 2011). Obviously, twin pairs are more similar to each other than strangers. This affects not only the correlations between twins but also the variances. Saturated models make it possible to calculate correlations while taking into account familial relatedness and even differences in genetic relatedness between MZ and DZ twins.

In a second set of analyses, the cross-sectional association between exercise behavior and sdsBMI was computed in each of the six age groups. Generalized estimating equations (GEE) were used to correct for familial relatedness. Sex was entered to the model as a first predictor. To allow for a possible threshold effect, where exercise behavior is effective only above a certain exercise intensity, both linear and quadratic relationships with BMI were tested. Weekly

MET hours were z-transformed (second predictor), then squared (third predictor), and added to the model as additional predictors.

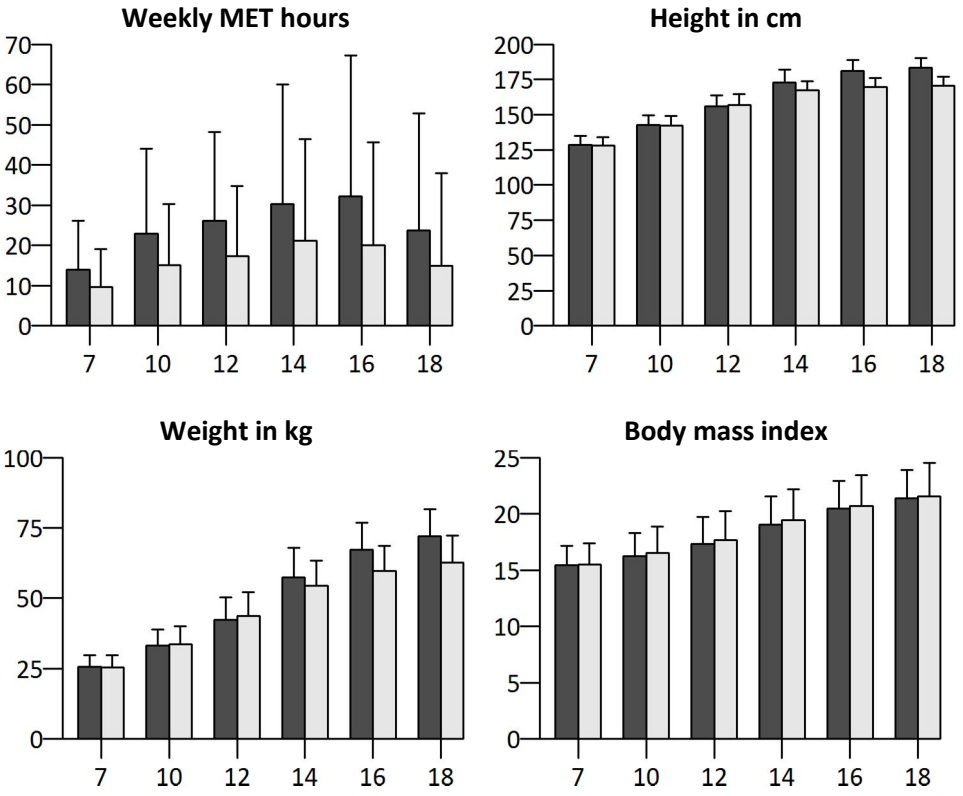
Under the causal hypothesis, MZ twins discordant for exercise behavior should also be discordant for BMI. In these pairs, there is complete matching of age, sex and full genetic background, and even the part of the environment that is shared by siblings (such as family, neighborhood and parental socioeconomic status) is better matched than in any other possible design. Therefore, in a third set of analyses, it was tested whether the MZ twins that were discordant for exercise behavior (one twin exercised much more than the other) also showed intrapair differences in BMI. To optimize statistical power either by increasing sample size or by increasing the expected effect size, discordant twin pairs were selected in each of the six age groups by two different methods. For method 1, exercise behavior-discordant MZ pairs were selected for which the amount of weekly MET hours of one individual was equal to or greater than the median within survey and sex, whereas the other individual scored lower than this median. For method 2, we divided all individuals into five categories based on the amount of exercise behavior they engaged in. The first category comprised non-exercisers (zero MET hours), and the remaining individuals were divided into quartiles. Next, exercise behavior-discordant MZ pairs with a minimum difference of two categories were selected (e.g., one member in category two and the co-twin in category four or five). Paired t-tests were used to test the hypothesis that the twin with the lower exercise behavior would have a higher sdsBMI compared with his or her co-twin.

The fourth set of analyses tested the longitudinal prediction of sdsBMI by exercise behavior across the 2-, 3-, 4-, 5-, 6- and 7-year follow-up periods. A final set of analyses focused on the correlations between *changes* in exercise behavior (weekly MET hours at time point 2 - weekly MET hours at time point 1) and *changes* in sdsBMI (sdsBMI at time point 2 - sdsBMI at time point 1) across all available time lags. Significance was tested with a liberal alpha level of 0.001 throughout.

The use of longitudinal twin data allows a more robust test of causal hypotheses about the nature of an association between two traits than the standard longitudinal study (Bartels et al., 2012; de Moor et al., 2008; Huppertz et al., 2014). While it is usually assumed that exercise behavior causally influences BMI, causality could also run the other way around (Richmond et al., 2014), e.g., because overweight individuals might not enjoy exercising. More importantly, there may be underlying (genetic and/or environmental) factors

influencing exercise behavior at baseline but also BMI at follow-up, which could create the illusion of causality in a standard longitudinal approach. In a genetically informative study, the true causal nature of these associations can be additionally tested by significance of both the genetic and environmental correlations (Bartels et al., 2012; de Moor et al., 2008; Huppertz et al., 2014). Therefore, in case systematic regression effects were found, causality would be tested by confirming that effects of all the latent genetic and environmental factors on baseline exercise behavior were transmitted to follow-up sdsBMI.

FIGURE 1 Means and standard deviations of weekly MET hours, height, weight and body mass index, split by survey, for males (dark bars) and females (light bars) separately.



RESULTS

Figure 1 depicts the means and standard deviations (SD) of weekly MET hours, height, weight and BMI, split by survey and sex (the exact numbers can be found in the supplementary material, including the means and SDs of age and sdsBMI). Both the means and the SDs of exercise behavior increased from childhood up to survey 16 for boys and survey 14 for girls, but the means were lower at survey 18. Boys spent more MET hours a week on exercise behavior than girls and overall they were taller and heavier than females. The sdsBMI was close to the expected mean of zero and standard deviation of one across all surveys. Tracking over time tended to decrease with increasing time intervals for both traits but was consistently higher for sdsBMI compared with exercise behavior (Table 2). For exercise behavior, the median cross-time correlation was 0.43 for the 2-year interval, 0.42 for the 3-year interval, 0.34 for the 4-year interval, 0.26 for the 5-year interval, 0.32 for the 6-year interval and 0.16 for the 7-year interval. For sdsBMI, these were 0.78, 0.67, 0.72, 0.69, 0.62 and 0.60, respectively.

TABLE 2 Cross-time correlations for weekly MET hours and sdsBMI, for male and female individuals separately (99% CIs).

Longitudinal follow-up time	Sur-veys	MET hours		sdsBMI	
		Male	Female	Male	Female
2-year interval	**10&12**	.34 (.26, .40)	.38 (.31, .44)	.79 (.76, .82)	.79 (.76, .82)
	12&14	.39 (.34, .44)	.43 (.39, .47)	.74 (.72, .77)	.77 (.74, .78)
	14&16	.43 (.35, .49)	.58 (.53, .62)	.74 (.70, .77)	.81 (.79, .83)
	16&18	.53 (.44, .61)	.45 (.39, .52)	.77 (.72, .81)	.85 (.83, .87)
3-year interval	**7&10**	.41 (.28, .51)	.42 (.31, .51)	.66 (.58, .72)	.67 (.59, .74)
4-year interval	**10&14**	.27 (.17, .37)	.30 (.21, .38)	.72 (.67, .76)	.73 (.68, .76)
	12&16	.38 (.31, .44)	.40 (.34, .46)	.64 (.60, .68)	.71 (.68, .74)
	14&18	.23 (.09, .36)	.42 (.32, .50)	.72 (.64, .78)	.75 (.70, .79)
5-year interval	**7&12**	.29 (.16, .41)	.23 (.11, .34)	.67 (.59, .73)	.71 (.64, .76)
6-year interval	**10&16**	.18 (-.02, .35)	.27 (.08, .43)	.54 (.35, .67)	.68 (.58, .75)
	12&18	.36 (.25, .45)	.40 (.32, .47)	.57 (.49, .63)	.67 (.63, .71)
7-year interval	**7&14**	.18 (.02, .33)	.14 (0, .26)	.59 (.48, .67)	.60 (.52, .67)

Correlations are corrected for family relatedness; CI=confidence interval, MET=metabolic equivalent of task, BMI=body mass index.

Table 3 depicts the cross-sectional association of sdsBMI by the linear (-0.07<B<0.10) and quadratic (-0.02<B<0.01) effects of weekly MET hours. The linear effect of exercise behavior on sdsBMI was significant for survey 12 with a

negative sign (B= -0.07). Thus, more exercise behavior was associated with lower BMI. At survey 18, however, a significant positive (linear) effect was found (B= 0.10), signaling higher BMI in adolescents with more exercise behavior. Effect sizes were very small. All other linear and quadratic relationships across the surveys were non-significant. The overall absence of a relationship between exercise behavior and sdsBMI was reconfirmed in the MZ twin pairs discordant for exercise behavior as shown in Table 4. Whether discordance was defined as exercise behavior at/above or below the sex-specific median of each survey or as a difference of at least two exercise categories, the sdsBMI of the twin who exercised more was not significantly different from that of the twin who exercised less at any age.

The longitudinal linear and quadratic relationships between exercise behavior and sdsBMI can be found in Table 5. Linear effects ranged from B= -0.03 to 0.14, and quadratic effects from -0.03 to 0.01. All but one of the relationships were non-significant. Counter our expectation, the one significant relationship

TABLE 3 Cross-sectional association of sdsBMI by the linear and quadratic effects of weekly MET hours (99.9% CIs).

Survey	Predictors	Unstandardized beta	p-value
7	**Sex***	.04 (-.08, .17)	.28
	MET hours	-.02 (-.11, .07)	.37
	MET hours squared	0 (-.03, .03)	1.00
10	**Sex**	.03 (-.07, .14)	.29
	MET hours	-.01 (-.08, .07)	.79
	MET hours squared	0 (-.02, .02)	.73
12	**Sex**	-.06 (-.13, .01)	.01
	MET hours	-.07 (-.12, -.03)	1.34e-7
	MET hours squared	.01 (-.01, .02)	.04
14	**Sex**	.01 (-.07, .10)	.61
	MET hours	-.01 (-.06, .04)	.55
	MET hours squared	0 (-.01, .01)	.97
16	**Sex**	-.03 (-.15, .08)	.34
	MET hours	.07 (-.01, .15)	4.28e-3
	MET hours squared	0 (-.02, .02)	.80
18	**Sex**	.01 (-.13, .16)	.74
	MET hours	.10 (.01, .19)	3.51e-4
	MET hours squared	-.02 (-.04, 0)	1.63e-3

**0=male, 1=female; Dependent variable: sdsBMI; BMI=body mass index, MET=metabolic equivalent of task, CI=confidence interval.*

TABLE 4 Comparison of sdsBMI in monozygotic twin pairs discordant for exercise behavior (EB).

A. Discordance based on median split.

Survey	Status of twin	MET hours (SD)	sdsBMI (SD)	N pairs	p-value*
7	**Higher EB**	14.34 (6.08)	-.05 (1.24)	34	.22
	Lower EB	4.52 (3.16)	-.21 (1.41)		
10	**Higher EB**	26.07 (11.27)	0 (1.06)	65	.16
	Lower EB	8.43 (5.01)	-.08 (1.10)		
12	**Higher EB**	28.62 (13.78)	-.26 (1.10)	166	.17
	Lower EB	10.03 (6.93)	-.21 (1.13)		
14	**Higher EB**	38.71 (25.82)	-.04 (.98)	184	.30
	Lower EB	9.77 (7.85)	.02 (1.04)		
16	**Higher EB**	40.04 (29.36)	.01 (.96)	120	.19
	Lower EB	7.51 (7.73)	-.06 (.92)		
18	**Higher EB**	29.13 (26.33)	-.02 (1.06)	99	.21
	Lower EB	2.33 (4.06)	-.12 (1.03)		

B. Discordance based on ≥2 quintiles.

Survey	Status of twin	MET hours (SD)	sdsBMI (SD)	N pairs	p-value*
7	**Higher EB**	11.93 (4.44)	0 (1.08)	13	1.00
	Lower EB	2.63 (2.64)	.01 (1.04)		
10	**Higher EB**	29.10 (9.51)	.21 (1.04)	25	.50
	Lower EB	6.59 (4.72)	.15 (1.17)		
12	**Higher EB**	32.09 (15.51)	-.16 (1.17)	68	.92
	Lower EB	6.31 (6.83)	-.15 (1.29)		
14	**Higher EB**	44.54 (28.76)	.09 (1.07)	69	.61
	Lower EB	7.19 (8.28)	.04 (.95)		
16	**Higher EB**	46.89 (25.58)	.01 (.88)	60	.81
	Lower EB	9.45 (11.07)	.03 (.89)		
18	**Higher EB**	39.89 (27.04)	.05 (1.13)	37	.49
	Lower EB	5.54 (9.66)	-.04 (1.05)		

**p-value of the comparison of sdsBMI between the higher EB and the lower EB twin; The p-value for EB was consistently <.001; BMI=body mass index, MET=metabolic equivalent of task, SD=standard deviation.*

TABLE 5 Longitudinal prediction of sdsBMI by the linear and quadratic effects of weekly MET hours (99.9% CIs).

Longitudinal follow-up time	Surveys	Predictors	Unstandardized beta	p-value
2-year interval	**10&12**	**Sex***	0 (-.16, .17)	.95
		MET hours	-.01 (-.14, .11)	.71
		MET hours squared	.01 (-.03, .05)	.60
	12&14	**Sex**	.03 (-.07, .13)	.26
		MET hours	-.03 (-.09, .04)	.20
		MET hours squared	.01 (-.01, .04)	.07
	14&16	**Sex**	-.06 (-.21, .08)	.17
		MET hours	.08 (-.02, .17)	.01
		MET hours squared	-.01 (-.04, .02)	.18
	16&18	**Sex**	.14 (-.07, .35)	.02
		MET hours	.14 (.01, .27)	5.92e-4
		MET hours squared	-.01 (-.04, .01)	.09
3-year interval	**7&10**	**Sex**	.02 (-.22, .26)	.80
		MET hours	-.02 (-.18, .13)	.64
		MET hours squared	-.03 (-.09, .02)	.05
4-year interval	**10&14**	**Sex**	.02 (-.17, .20)	.78
		MET hours	.03 (-.10, .16)	.51
		MET hours squared	0 (-.06, .05)	.81
	12&16	**Sex**	-.03 (-.15, .10)	.48
		MET hours	.03 (-.05, .11)	.24
		MET hours squared	.01 (-.03, .05)	.54
	14&18	**Sex**	.04 (-.23, .30)	.66
		MET hours	.03 (-.14, .20)	.55
		MET hours squared	.01 (-.03, .04)	.64
5-year interval	**7&12**	**Sex**	-.06 (-.31, .19)	.45
		MET hours	.01 (-.17, .19)	.81
		MET hours squared	-.02 (-.08, .05)	.38
6-year interval	**10&16**	**Sex**	.07 (-.24, .39)	.45
		MET hours	.08 (-.15, .32)	.24
		MET hours squared	-.03 (-.14, .08)	.35
	12&18	**Sex**	.06 (-.12, .25)	.25
		MET hours	.05 (-.06, .15)	.17
		MET hours squared	-.01 (-.05, .04)	.51
7-year interval	**7&14**	**Sex**	.08 (-.15, .32)	.25
		MET hours	.05 (-.11, .20)	.32
		MET hours squared	-.02 (-.07, .04)	.34

**0=male, 1=female; Dependent variable: sdsBMI; BMI=body mass index, MET=metabolic equivalent of task, CI=confidence interval.*

TABLE 6 Correlations between *change* in weekly MET hours (MET hours at time point 2 - MET hours at time point 1) and *change* in sdsBMI (sdsBMI at time point 2 - sdsBMI at time point 1), for male and female individuals separately (99% CIs).

Longitudinal follow-up time	Surveys	Correlation between ΔMET hours and ΔsdsBMI*	
		Male	Female
2-year interval	**10&12**	-.01 (-.11, .09)	.02 (-.08, .12)
	12&14	-.01 (-.07, .06)	-.03 (-.09, .03)
	14&16	-.02 (-.11, .07)	.01 (-.06, .08)
	16&18	.01 (-.11, .14)	.05 (-.04, .14)
3-year interval	**7&10**	-.03 (-.19, .12)	.01 (-.14, .15)
4-year interval	**10&14**	.10 (-.02, .21)	.03 (-.09, .14)
	12&16	.05 (-.03, .13)	.07 (.01, .14)
	14&18	.06 (-.10, .21)	-.01 (-.12, .11)
5-year interval	**7&12**	-.06 (-.24, .12)	-.14 (-.30, .02)
6-year interval	**10&16**	.09 (-.14, .31)	.10 (-.09, .27)
	12&18	.05 (-.06, .16)	-.01 (-.10, .07)
7-year interval	**7&14**	.05 (-.13, .23)	.01 (-.13, .14)

**For example, for "10&12", this would be (MET hours at age 12 - MET hours at age 10) × (sdsBMI at age 12 - sdsBMI at age 10); CI=confidence interval, MET=metabolic equivalent of task, BMI=body mass index.*

(B= 0.14) suggested that high exercise behavior at survey 16 predicted *higher* BMI at survey 18. Finally, Table 6 depicts the correlations between the change in exercise behavior and change in sdsBMI for all the possible longitudinal combinations. Based on 99% confidence intervals, all but one of the correlations were not significantly different from zero (-0.14<B<0.10). An increase in exercise behavior in girls aged 12 to 16 years led to an unexpected increase in sdsBMI (B= 0.07). As there was no consistent relationship between exercise behavior and sdsBMI, investigating the possibility of a causal relationship with longitudinal multivariate genetic modelling was deemed to be redundant.

DISCUSSION

This study examined the relationship between regular exercise behavior and BMI in childhood and youth, using repeated surveys in a population-based sample of 7-, 10-, 12-, 14-, 16- and 18-year-old twin individuals. Based on the hypothesis that regular exercise is a causal determinant of obesity, higher levels of exercise behavior in childhood and adolescence were expected to be

associated with lower levels of BMI at all ages and changes in exercise behavior across time were expected to predict opposite changes in BMI. The availability of twin data would have allowed an explicit test of the causal nature of these associations (Bartels et al., 2012; de Moor et al., 2008; Huppertz et al., 2014). Under the causal hypothesis, exercise behavior-BMI associations should derive from significant cross-trait correlations between the genetic and environmental factors influencing either trait. Moreover, genetically identical twins discordant for exercise behavior should also be discordant for BMI, such that the twin with the highest level of exercise should be leaner than the co-twin, in spite of an identical genome and a shared family environment.

None of our expectations were borne out by the data. Cross-sectionally, the linear and quadratic effects of exercise behavior on sdsBMI were mostly non-significant. In addition, there was no compelling evidence for a longitudinal association between exercise behavior and BMI. Increases in exercise behavior across time were not paralleled by decreases in sdsBMI, nor were decreases in exercise behavior paralleled by increases in sdsBMI. Further twin modelling of the causal nature of the association was considered moot, as no association was present.

Notwithstanding their counterintuitive nature, the results are rather well aligned with previous work. The few longitudinal studies focusing specifically on exercise behavior (as opposed to general physical activity) in large population-based samples found no robust association between exercise behavior and BMI (Boone, Gordon-Larsen, Adair & Popkin, 2007; Haerens, Vereecken, Maes & de Bourdeaudhuij, 2010; Lajunen et al., 2009), with one exception (Gordon-Larsen, Adair & Popkin, 2002). Taken together, the current evidence does not point towards a lack of regular leisure time exercise as a major source of obesity in childhood and youth.

This does not, of course, preclude that other forms of physical activity have an effect on BMI. We deliberately choose to focus on the narrow trait of voluntary exercise behavior in leisure time. This salient voluntary behavior can be reliably assessed by self-report through surveys on a scale of tens of thousands of participants, which is a major asset for causal modelling in a twin design. Furthermore, exercise behavior presents a well-defined and feasible target for intervention. From adolescence onwards, exercise activities in leisure time are the major source of exercise bouts for the majority of people with sufficient intensity and duration to increase or maintain cardiorespiratory fitness and to induce positive health outcomes. Habit formation in this domain can be

maintained across the life course (in contrast to school-based physical education or free child play) and a large meta-analysis of 80 prospective studies in adults testing the effects of exercise on mortality in 1,338,143 participants (118,121 deaths) showed that the risk reduction per unit of time increase was largest for (moderate-to-) vigorous exercise (Samitz et al., 2011).

Nonetheless, (high-intensity) leisure time exercise activities may only account for up to 25% of the total daily activity-induced energy expenditure (Westerterp, 2003). Other aspects of physical activity could still prove to be possible determinants of BMI in childhood and adolescence. Obvious aspects are the parts of daily physical activity in children and adolescents that were excluded here, including standing time, light activities and moderate-to-vigorous activities like cycling or walking to school, physical education classes (between one and three hours a week in the Netherlands), free play, dance and household- or job-related physical activity (Dutch children can work up to four hours a week on non-schooldays from age 13 onwards). There might be no difference in calories burned between a child that participates in scheduled exercise activities but is largely sedentary the remaining time and a child that does not participate in scheduled exercise but is actively playing and commuting to school. The effect of each aspect of physical activity on BMI, their change over time (e.g., free play might be more important in younger children) and their relationships with each other should be investigated more closely (Churilla & Fitzhugh, 2012).

A second obvious aspect of physical activity that could influence BMI in childhood and adolescence is the amount of sedentary behavior. Sedentary behaviors are defined as activities that are performed sitting or reclining and cost ≤1.5 times the basal metabolic rate. In adults, many negative health outcomes, including high BMI, have been reported to follow from sedentary behavior, independent of physical activity levels (Altenburg, Lakerveld, Bot, Nijpels & Chinapaw, 2014; Chau et al., 2013; van der Ploeg, Chey, Korda, Banks & Bauman, 2012). Similar detrimental effects appear to occur already in children and adolescents, although the evidence is still incomplete (Chinapaw, Altenburg & Brug, 2014). Importantly, the association between sedentary behavior and exercise behavior itself is weak at best, at least in adults (Pate, O'Neill & Lobelo, 2008). Hence, high exercise behavior can co-occur with high levels of sedentary behavior and vice versa, distorting potential causal effects of exercise behavior on BMI.

Although future investigation may reveal an effect on BMI of these aspects of

daily physical activity other than exercise behavior, the results of the present study are also rather well aligned with a major role of the alternative determinant of BMI: eating behavior patterns. Bleich et al. (2011) reviewed the literature on the relative contribution of energy intake and energy expenditure to obesity in childhood and adolescence, with mixed results. A main reason for the blurry picture were the large differences between studies in terms of methods used and populations studied. Furthermore, the assessment of food intake is prone to reporting errors. To overcome this problem, Waxman and Stunkard (1980) observed four obese boys, their non-obese brother and a peer for four to five months in their natural environments and monitored their eating behavior and physical activity. They concluded that - compared with their controls - energy intake was higher, but energy expenditure was not lower in the obese boys. Model-based equations on the association between energy intake, energy expenditure and energy balance, both at the population level and at the individual level, confirm energy intake as the factor driving the obesity epidemic (Swinburn, Sacks & Ravussin, 2009). Westerterp (2010) reviewed studies that were based on the doubly labelled water method from the early 1980s onwards and concluded that energy expenditure has not decreased since then in spite of the substantial increase in the prevalence of obesity. Moreover, activity-related energy expenditure in modern day humans does not deviate from that of other terrestrial mammals, after taking differences in body size into account (Westerterp & Speakman, 2008).

In adults, there is now good evidence that increases in energy intake can come about as a compensatory reaction to exercise behavior itself (Melanson, Keadle, Donnelly, Braun & King, 2013). Exercise may increase the amount of food that an individual eats and it may amplify the preference for high-fat, energy-dense foods. Moreover, starting an exercise program might lead to less non-exercise activity - either owing to physiologically caused fatigue or because of a feeling that one can afford to rest more because of the activity. Westerterp (2010) highlights that humans are better at compensating for a negative energy imbalance compared with a positive energy imbalance. It is not known whether compensatory eating occurs in children or adolescents, but if the twins participating in this study indeed show increased eating with higher levels of exercise behavior, this could have caused the absence of an exercise behavior-BMI relationship. Unfortunately, no food intake was assessed in any of the survey waves.

Apart from the absence of food intake data, this study had further limitations that should be noted. First of all, individual differences in basal metabolic rate

(BMR) were not assessed, although in sedentary subjects, they can account for around 60% of the total energy expenditure (Westerterp, 2003). BMR is not currently a feasible target for intervention, but it may be a determinant of individual differences in BMI (McMurray, Soares, Caspersen & McCurdy, 2014). Secondly, we did not correct for growth and maturation. Body height and body weight change dramatically as children develop from age 7 into adulthood. Growth and maturation may affect both BMI and physical activity (and vice versa), thereby undermining the detection of a relationship between the two. This limitation was somewhat attenuated by using sdsBMI, which provides a standardized ranking of the participants using sex-specific and age-specific BMI scores of the Dutch population, but such standardization does not remove the effects of variance in growth and maturation that can exist within each sex/age stratum. In addition, BMI contains both fat mass and fat-free mass, and we could not separate the effects of exercise on these compartments here. Although large effects of exercise behavior on fat-free mass are not anticipated in children and adolescents, they could potentially have masked parallel reductions in fat mass. Finally, we assessed height and weight by parental report and self-report, which can induce a reporting bias. However, self-reported body height and weight have been shown to be strongly correlated to actual body height and weight (Strauss, 1999). Also, the use of self-report allowed us to create the largest genetically informative longitudinal dataset in the world uniformly assessing exercise behavior and BMI across the entire age range from childhood to young adulthood, which is a major strength of the study.

In conclusion, we found no evidence for a cross-sectional or longitudinal association between exercise behavior and BMI across childhood and adolescence. Alternative determinants of BMI such as BMR, other aspects of daily physical activity and sedentary behavior, but prominently also energy intake, are likely to be more important. It should be explicitly mentioned here that this does not detract from the value of encouraging regular exercise behavior in childhood and youth, as it has been shown to have many other favorable effects on health, even in the absence of an effect on body weight, and should thus still be promoted (Melanson et al., 2013). Claiming a primary role of exercise behavior in the variation of BMI in childhood and adolescence, however, may foster false expectations.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE I Means and standard deviations (SD) for age, weekly MET hours, height, weight, BMI and sdsBMI, for males and females separately.

		Mean (SD)	
Trait	**Survey**	**Males**	**Females**
Age	**7**	7.45 (.32)	7.44 (.32)
	10	9.87 (.45)	9.90 (.45)
	12	12.24 (.40)	12.24 (.40)
	14	14.73 (.62)	14.72 (.64)
	16	16.96 (.55)	16.99 (.57)
	18	18.99 (.78)	18.95 (.71)
Weekly MET hours	**7**	14.06 (12.09)	9.70 (9.43)
	10	22.99 (20.98)	15.09 (15.22)
	12	26.16 (22.10)	17.30 (17.53)
	14	30.25 (29.83)	21.18 (25.26)
	16	32.19 (34.95)	20.02 (25.67)
	18	23.70 (29.06)	15.01 (22.99)
Height	**7**	128.74 (6.09)	128.06 (5.94)
	10	142.69 (6.95)	142.37 (6.86)
	12	155.85 (7.95)	156.83 (7.87)
	14	172.99 (9.10)	167.16 (6.59)
	16	181.22 (7.42)	169.71 (6.49)
	18	183.36 (6.87)	170.47 (6.38)
Weight	**7**	25.61 (4.20)	25.52 (4.36)
	10	33.18 (5.79)	33.68 (6.40)
	12	42.33 (8.06)	43.62 (8.62)
	14	57.31 (10.54)	54.46 (8.97)
	16	67.28 (9.46)	59.62 (8.95)
	18	71.92 (9.77)	62.64 (9.70)
BMI	**7**	15.43 (1.74)	15.50 (1.90)
	10	16.23 (2.08)	16.54 (2.35)
	12	17.35 (2.37)	17.64 (2.62)
	14	19.03 (2.49)	19.46 (2.72)
	16	20.47 (2.46)	20.70 (2.72)
	18	21.38 (2.52)	21.53 (2.99)
sdsBMI	**7**	-.09 (1.26)	-.09 (1.17)
	10	-.09 (1.19)	-.08 (1.18)
	12	-.13 (1.15)	-.19 (1.17)
	14	-.01 (1.09)	0 (1.05)
	16	.10 (1.04)	.04 (1.00)
	18	.04 (1.09)	.03 (1.05)

Part III

GENOMICS OF REGULAR VOLUNTARY EXERCISE BEHAVIOR

Chapter **8**

THE DOPAMINERGIC REWARD SYSTEM AND REGULAR VOLUNTARY EXERCISE BEHAVIOR: A CANDIDATE ALLELE STUDY

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ABSTRACT

Twin studies provide evidence that genetic influences contribute strongly to individual differences in exercise behavior. We hypothesize that part of this heritability is explained by genetic variation in the dopaminergic reward system. Eight single nucleotide polymorphisms (SNPs in DRD1: rs265981; DRD2: rs6275, rs1800497; DRD3: rs6280; DRD4: rs1800955; DBH: rs1611115, rs2519152 and in COMT: rs4680) and three variable number of tandem repeats (VNTRs in DRD4, upstream of DRD5 and in DAT1) were investigated for an association with regular leisure time exercise behavior. Data on exercise activities and at least one SNP/VNTR were available for 8,768 individuals aged 7 to 50 years that were part of the Netherlands Twin Register. Exercise behavior was quantified as weekly metabolic equivalents of task (MET) spent on exercise activities. Mixed models were fitted in SPSS with genetic relatedness as a random effect. None of the genetic variants were associated with exercise behavior ($p>0.02$), despite sufficient power to detect small effects. We did not confirm that allelic variants involved in dopaminergic function play a role in creating individual differences in exercise behavior. A plea is made for large genome-wide association studies to unravel the genetic pathways that affect this health-enhancing behavior.

INTRODUCTION

Despite its well-known health benefits both in childhood and youth (Janssen & Leblanc, 2010) and in adults (Garber et al., 2011; Warburton et al., 2010), regular leisure time exercise behavior drops from childhood to adolescence and reaches unacceptable low proportions in adulthood, with the majority of people in the United States and Europe not engaging in regular exercise activities at the recommended level (Armstrong & van Mechelen, 1998). Twin studies have shown that a substantial part of the variation in exercise behavior between individuals can be explained with genetic factors (Stubbe & de Geus, 2009). However, there is no definite evidence on which genes are implicated in the take-up and maintenance of exercise behavior (Bouchard & Hoffman, 2011; Rankinen et al., 2010). A few significant associations have been found, but replication studies are scarce and the functional meaning of those genes is often not straightforward (de Geus & de Moor, 2008).

It is likely that a large part of the heritability of leisure time exercise behavior is due to genes that influence the affective reaction to exercise (de Geus & de Moor, 2011). Feelings of reward and punishment have been hypothesized to be

crucial agents in the take-up and maintenance of exercise behavior (Bryan et al., 2007; de Geus & de Moor, 2011). The net rewarding effects of exercise may have to outweigh the net aversive effects to a substantial degree for the behavior to be repeated (de Geus & de Moor, 2011). As part of an intervention study, Williams et al. (2008) investigated the relationship between acute affective responses during a moderate-intensity exercise test on a treadmill and subsequent exercise behavior 6 months and 12 months after the baseline assessment. They found large individual differences in the affective reactions to the exercise test, with some of the participants reporting a more positive affect during (versus before) the test, some of them reporting a more negative affect and some showing no change. Importantly, individuals characterized by a positive affect during the exercise test were more likely to be engaged in exercise behavior at 6 and 12 months of follow-up.

Reward is governed by the mesolimbic reward system that involves dopaminergic pathways (Beaulieu & Gainetdinov, 2011). Associations between those pathways and physical activity behavior have been found both in animal models and in humans. It is well established that physical activity affects the dopaminergic system in some way. For instance, Greenwood et al. (2011) showed that in rodents, acute rewarding effects of exercise were linked to changes in dopaminergic functioning. The reversed case, where dopaminergic functioning affects physical activity behavior and thus acts as a potential determinant of exercise behavior, has been less studied and deserves closer attention. Knab et al. (2009) examined voluntary wheel running in mice. Both a high-active strain of mice (C57L/J) and a low-active strain of mice (C3H/HeJ) were divided into two groups: one group had free access to running wheels for 21 days and the other did not. After 21 days, the high-active strain and the low-active strain differed in the expression of two dopaminergic genes (drd1 and th), irrespective of access to the running wheels. Assuming that expression was controlled in part by cis-acting variants, this suggests that innate differences in dopaminergic functioning can affect physical activity behavior. A review on the role of the dopamine system as a determinant of physical activity can be found in Knab and Lightfoot (2010).

There are not many studies in humans that have investigated the effect of genetic variants in dopaminergic genes on physical activity. Jozkow, Slowinska-Lisowska, Laczmanski and Medras (2013) found no significant association between two polymorphisms and the level of physical activity in a group of adult men. Two variants were investigated: rs6275 in the DRD2 gene (N= 371) and a 48-base pair variable number of tandem repeat (VNTR) in the DRD4 gene

(N= 397). Simonen, Rankinen, Perusse, Leon et al. (2003) examined the association between rs6275 in DRD2 and physical activity in participants of the Quebec Family Study (QFS, N= 721) and replicated it in participants of the HERITAGE Family Study (N= 275 African American and 497 Caucasian participants). They found that Caucasian women that were homozygous for the T allele had been significantly less active during the past year than CT heterozygotes and CC homozygotes. Thomson, Hanna, Carlson and Rupert (2013) examined the association between rs1800955 in the DRD4 gene and risk-taking behavior in sports by measuring general and ski/snowboarding-specific sensation seeking behavior in 503 male and female skiers and snowboarders. They found a significant association between the studied polymorphism and sport-specific sensation seeking, with higher sensation seeking scores in the CC homozygotes. Thus, part of the genetic variation that causes differences in exercise behavior may indeed reside in the dopaminergic midbrain reward systems, although the evidence is not compelling.

There are currently several strategies to detect genetic variants involved in the heritability of behavioral traits - the two most frequently used techniques are (i) genome-wide association studies (GWAS) where markers are placed across the length of the entire genome, ranging in density from a few hundreds of thousands to millions (Flint, 2013; Visscher, Brown, McCarthy & Yang, 2012), and (ii) candidate gene studies (Tabor, Risch & Myers, 2002), where polymorphisms are typed in genes of putative biological relevance. Both techniques have strengths and weaknesses - for instance, a GWAS allows for unexpected gene discovery by taking an agnostic approach to the selection of single nucleotide polymorphisms (SNP); it is limited, however, by requiring very large samples to overcome the multiple testing penalty and by the difficulty of explaining association results when identified SNPs are intergenic. Candidate gene studies, on the other hand, rely on polymorphisms in (close proximity to) genes of interest, ideally with known effects on gene function. While this limits the ability to discover novel polymorphisms, it provides interpretability within an a priori theoretical framework and greatly reduces the multiple testing burden.

For the present study, we selected the latter approach. Eight SNPs (rs265981, rs6275, rs1800497, rs6280, rs1800955, rs1611115, rs2519152 and rs4680) and three VNTRs (a 48-bp VNTR in exon III of DRD4, a dinucleotide repeat 18.5 kb upstream of DRD5 and a 40-bp VNTR in the 3' untranslated (UTR) region of DAT1) were chosen based on their known function in the dopaminergic reward system.

Dopamine receptors relay signals from one nerve cell to a neighboring nerve cell. At least five subtypes have been identified (dopamine receptors D1 to D5) that are encoded by dopamine receptor genes (DRD1 to DRD5, respectively). The receptors D1 and D5 are grouped in the D1-like family and increase the cellular response (increased cyclic adenosine monophosphate (cAMP) production), whereas D2, D3 and D4 are grouped in the D2-like family and decrease the cellular response (decreased cAMP production). We selected four SNPs and two VNTRs that affect the dopamine receptors for this study: rs265981 is located within the DRD1 gene and has two possible alleles, A (minor) and G (major). The A allele has been associated with a decrease of DRD1 expression levels and thus worse dopamine transmission compared to the G allele (Zhu et al., 2011). Rs6275 (minor allele A and major allele G) is a synonymous SNP located within the DRD2 gene. The G allele has been associated with increased DRD2 expression levels (Doehring et al., 2009). The rs1800497 polymorphism (minor allele A and major allele G) lies within the ankyrin repeat and kinase domain containing one gene (ANKK1) downstream of and in linkage disequilibrium with the DRD2 gene (Mota, Araujo-Jnr, Paixao-Cortes, Bortolini & Bau, 2012; Neville, Johnstone & Walton, 2004). The A allele has been associated with a reduced number of dopamine D2 receptors and thus increased dopamine transmission (Laakso et al., 2005; Zhang et al., 2007) and higher reward responsiveness (Lee, Ham, Cho, Lee & Shim, 2008). Rs6280 lies within the DRD3 gene and is translated to one of two amino acids in the D3 receptor protein: glycine (minor allele C) or serine (major allele T), with glycine having a higher affinity for dopamine compared to serine (Lundstrom & Turpin, 1996) and thus decreasing dopamine transmission. Rs1800955 (minor allele C and major allele T) is located in close proximity to the DRD4 gene and has been shown to influence promoter activity, with the C allele potentially enhancing activity compared to the T allele (Okuyama, Ishiguro, Toru & Arinami, 1999; Shi, Gershon & Liu, 2008). A VNTR in exon III of the DRD4 gene was investigated consisting of 48 base pairs with varying repeats ranging from 2 to 11. The 7-repeat allele has been shown to have a lower affinity for dopamine compared to the other repeats (Asghari et al., 1995), thus increasing dopamine transmission (Guo, North, Gorden-Larsen, Bulik & Choi, 2007). A VNTR 18.5 kb upstream of the DRD5 transcription start site consists of a dinucleotide polymorphism with alleles ranging from 130 to 166 base pairs and has been hypothesized to be in strong linkage disequilibrium with one or more functional variants in the DRD5 gene. The 148 allele has been associated with decreased DRD5 expression levels (Lowe et al., 2004).

Dopamine β-hydroxylase (DBH) converts dopamine to norepinephrine and is

encoded by the DBH gene. Rs1611115 (minor allele T and major allele C) is located in the promoter region of the DBH gene. This polymorphism has been shown to account for 30-50% of the variance in DBH activity. More specifically, the C allele has been associated with higher plasma levels of DBH and thus lower dopamine levels (Cubells & Zabetian, 2004; Zabetian et al., 2001). The rs2519152 polymorphism (minor allele C and major allele T) is situated within the DBH gene and the T allele has been associated with lower DBH activity and thus higher dopamine levels compared to the C allele (Tang et al., 2006).

Finally, two genes were selected based on their association with *dopamine reuptake* and *dopamine degradation*: the DAT1 (= SLC6A3) gene and the COMT gene, respectively. The dopamine active transporter is encoded by the DAT1 gene and clears dopamine from the synapse by depositing it back into the cells. A VNTR in the 3' UTR region of the DAT1 gene was investigated that consists of a 40-base pair repeat with three alleles: 440, 480 and 520. We investigated the effect of the 480 allele in the present study as it has been associated with higher expression of the transporter, resulting in higher dopamine reuptake and thus lower levels of dopamine (Faraone, Spencer, Madras, Zhang-James & Biederman, 2013; Yacubian et al., 2007). Catechol-O-methyltransferase is encoded by the COMT gene and degrades dopamine. The SNP rs4680 (minor allele A and major allele G) lies within the COMT gene and is either translated to methionine (Met) or valine (Val), depending on the allelic variant that an individual has (G versus A, respectively). The COMT-Met enzyme degrades dopamine slower than the COMT-Val enzyme does and therefore results in higher dopamine levels (Chen et al., 2004), thereby increasing reward responsiveness and reward seeking (Lancaster, Linden & Heerey, 2012).

The aim of the present study was to specifically test candidate alleles with a known function in the dopaminergic reward system for their association with regular leisure time exercise behavior, assuming that higher dopamine levels and stronger dopamine transmission are associated with higher reward sensitivity and thus more exercise behavior. The specific hypotheses are summarized in Table 1.

METHODS

Participants

Data originated from twins and their family members that agreed to participate in longitudinal research of the Netherlands Twin Register (NTR) which has been

TABLE 1 Allele-specific hypotheses (1=effect on dopamine level*, 2=effect on exercise behavior*).

Gene	Variant	Allele	Expected effect	Ref. number	1	2
DRD1	**rs265981**	**A**	Decreased DRD1 expression levels	24	↓	↓
DRD2	**rs6275**	**G**	Increased DRD2 expression levels	25	↓	↓
	rs1800497	**A**	Reduced number of (inhibitory) D2 receptors	28, 29	↑	↑
DRD3	**rs6280**	**C**	Higher affinity for dopamine → decreased transmission	31	↓	↓
DRD4	**rs1800955**	**C**	Increased DRD4 expression levels	32, 33	↓	↓
	VNTR: 7 allele		Lower affinity for dopamine → increased transmission	34, 35	↑	↑
DRD5	**VNTR: 148 allele**		Decreased DRD5 expression levels	36	↓	↓
DBH	**rs1611115**	**C**	Higher DBH activity	37, 38	↓	↓
	rs2519152	**T**	Lower DBH activity	39	↑	↑
DAT1	**VNTR: 480 allele**		Higher DAT activity → higher reuptake	40, 41	↓	↓
COMT	**rs4680**	**G**	Methione → slower degradation of dopamine	42	↑	↑

**↑=increase, ↓=decrease; The corresponding reference numbers can be found in the published paper.*

set up to investigate individual differences in human behavior. The data collection protocol was approved by the Medical Research Ethics Committee of the VU University Medical Center. The final sample consisted of 8,768 individuals (3,900 families), of which 38% were males and 62% were females, with a mean age of 32.5 years (SD= 12.3, age range= 7-50 years).

Twins and their families are involved in research projects: for 7-, 10- and 12-year-olds, both mothers and fathers are invited to fill out surveys on their twins' health, lifestyle and behavior. From 13 years onwards, the twins and their siblings are invited to complete self-report surveys. When reaching adulthood (18 years), the twins are asked to fill out surveys every 2-3 years and additional family members are invited to take part in research projects (siblings, parents, adult offspring and spouses). Characteristics and recruitment of participants were described by van Beijsterveldt et al. (2013) and Willemsen et al. (2013). Individuals with diseases or disabilities that may prevent them from being physically active were excluded from the present study. Only individuals with a Dutch/Western European background were included that had genotype data available and at least one measure of exercise behavior (see following pages).

Measures

Phenotyping

For this study, we focused on regular leisure time exercise behavior since we were interested in voluntary (leisure time) physical activity that might be affected by individual differences in reward sensitivity. Participants (or their parents, for <13-year-olds) were asked to indicate 1) which exercise activities they participated in and (if any) 2) for how many years, 3) how many months a year, 4) how many times a week and 5) how many minutes each time they participated in the respective activity. Test-retest reliability of this questionnaire was high (>0.82) in previous studies (de Moor et al., 2008; Stubbe et al., 2007) and it has been associated with other exercise phenotypes (de Moor & de Geus, 2013). Our focus was regular leisure time exercise behavior, explicitly excluding irregular activities such as sailing camps or ski holidays (by requiring activities to be conducted for at least 3 months a year and for at least half a year), non-leisure activities such as transportation (e.g., cycling or walking to get somewhere), gardening, house cleaning and - for younger participants - compulsory physical education classes. Each activity was recoded into its metabolic equivalent of task (MET), reflecting energy expenditure during a specific activity as a multiple of energy expended at rest (approximately one kcal/kg/h). For individuals younger than 18 years old, Ridley et al. (2008)'s compendium of energy expenditures for youth was applied. For individuals of 18 years or older, Ainsworth et al. (2000)'s compendium of physical activities was used. The product of the MET score, weekly frequency and duration was summed over all exercise activities that an individual engaged in, resulting in one summary score, namely "weekly MET hours spent on exercise activities." If an individual participated in more than 120 MET hours a week, the score was truncated at 120 MET hours (N= 31 of the final sample).

Exercise data of several longitudinal assessments were combined into one score. First, exercise data of individuals that were >50 years old were changed to missing and exercise data (of the respective assessment only) were removed when participants were injured at the time of survey completion. Subsequently, the data were combined by creating a new "weekly MET hours"-variable based on the most recent questionnaire of adults. Missing values were then replaced with older data of those individuals - preferentially, with data at an adult age and, if unavailable, with data of adolescents and children (step by step, one batch of questionnaires at a time). The association analysis was thus

run on the joint exercise variable that was composed of adults' data (N= 7,349), adolescents' data (N= 997) and children's data (N= 422).

Genotyping and imputation

Genotype data were available from several projects within the NTR. Eight SNPs (rs265981, rs6275, rs1800497, rs6280, rs1800955, rs1611115, rs2519152 and rs4680) and three VNTRs (a 48-bp VNTR in exon III of DRD4, a dinucleotide repeat 18.5 kb upstream of DRD5 and a 40-bp VNTR in the 3' UTR of DAT1) were selected for this candidate gene study based on their known function in the dopaminergic reward system. For some individuals, genotype data were available from fingerprint sets that included 30-38 SNPs and 5-7 VNTRs in candidate genes (see van Beijsterveldt et al., 2013). For other individuals (partly overlapping with the fingerprint-sample), SNP data were available based on imputed genome-wide SNP arrays.

In the fingerprint set, samples were excluded based on low sample call rate, sex errors, inconsistencies between duplicate samples, Mendelian errors and erroneous IBS/IBD relationships. In the imputed dataset, samples were filtered on the same criteria, as well as on excessive heterozygosity. If samples were present in both the fingerprint and the imputed dataset, they were included only if they survived quality control (QC) in both sets.

In the fingerprint set, SNPs and VNTRs were tested for Hardy-Weinberg Equilibrium (HWE), Mendelian error rate, SNP/VNTR call rate, concordance rate for duplicate samples and allele frequency difference with a reference set (HapMap CEU). In the genome-wide SNP dataset, SNPs were filtered on the following criteria before imputation: HWE p-value >0.00001, minor allele frequencies (MAF) >0.01, Mendelian error rate <0.02, SNP call rate >0.95, SNP concordance rate >0.99 and allele frequency difference with the reference set <0.20.

Haplotype phasing and imputation of missing genotyped SNPs was done in MACH 1.0 and subsequent imputation of the missing SNPs was done with Minimach using 1000G as a reference set (March 2012 phase 3 release, all ethnicity panels). After imputation, SNPs were tested for HWE, Mendelian error rate, allele frequency difference with the reference set and imputation quality (R^2). For two SNPs (rs1611115 and rs1800955), we decided to use the fingerprint data only, since they showed a low R^2 and/or a high rate of Mendelian errors in the imputed set as well as a low concordance between the

fingerprint set and the imputed set (<95%). MAF and HWE for the final dataset are depicted in Table 2. Allele frequencies were similar to those in public data bases (e.g., HapMap CEU).

In individuals with genome-wide SNP data, information on ancestry was based on principal component analysis (Abdellaoui et al., 2013). For the remaining individuals, ancestry information was derived from questionnaire information on birth country of the parents. Individuals who were from non-Western European origin were excluded. The final sample consisted of 8,768 individuals with both phenotype data and genotype data on at least one variant. For the VNTRs and two SNPs (rs1611115 and rs1800955), data were derived from the fingerprint chip only. For two other SNPs (rs6275 and rs6280), data were derived from the imputed set only. For the remaining SNPs (rs265981, rs1800497, rs2519152 and rs4680), data were derived from the fingerprint chip for about 37% of the individuals and were complemented with data from the imputed set for 63% of the individuals. Concordance between genotyped and imputed SNP data in the individuals with both fingerprint chip and genome-wide data was higher than 95%.

Statistical analyses

The SNPs were coded based on the presence of one or two of each of the two alleles in the called genotype (0= allele 1 homozygote, 1= heterozygote and 2= allele 2 homozygote). For the SNPs, the exact combination of alleles corresponding to 0, 1 and 2 can be found in Table 2. VNTRs, particularly the ones in the DRD4 and DRD5 genes, are highly polymorphic. Based on the literature, we decided to focus on specific repeats and the coding was based on the presence or absence of those repeats. For the VNTR in the DRD4 gene, this resulted in the following coding: 0= no 7 allele, 1= one 7 allele and 2= two 7 alleles. For the DRD5 gene, it was: 0= no 148 allele, 1= one 148 allele and 2= two 148 alleles. For the DAT1 gene, it was: 0= no 480 allele, 1= one 480 allele and 2= two 480 alleles.

As a first step, the analyses were performed for each genetic variant separately. Mixed models were run in SPSS for Windows (version 20.0, SPSS Inc.) and were based on maximum likelihood estimation. The dependent variable was weekly MET hours. The following variables were included as fixed effects: sex (0= males, 1= females), age (z-score), sex x age interaction and the respective SNP/VNTR. We tested whether correction for a number of possible confounders had a significant effect on the results, namely, ancestry

differences within the Dutch population (3 principal components), ancestry differences based on the 1000 Genomes project (6 principal components), differences due to batch effects (1 principal component) and a dummy variable to correct for differences between genotyping platforms.

As the next steps, 1) multiple variants were included into a single mixed model to test their effects simultaneously and 2) mixed models were run with a polygenic risk score computed as the sum of the alleles that are hypothesized to increase dopamine level ("effect alleles") across multiple variants. As data were derived from family members (twins, siblings, parents and spouses of twins), we added genetic relatedness as a random effect to the models. The chosen alpha level was 0.05/11 (Bonferroni correction for 11 tests, alpha= 0.0045).

To get an indication of the power to detect genetic effects, simulated data were used, as this allows us to accommodate the large variation in family composition and the truncation of the phenotype distribution (R code available upon request). Due to differences in sample sizes and family structures between the "fingerprint data only" and the "(fingerprint data with additional) imputed data", the power was calculated for four genetic variants: 1) the SNP with the smallest sample size (rs1800955, N= 2,152), 2) the SNP with the largest sample size within the five variants that we had fingerprint data for only (rs1611115, N= 3,140), 3) the SNP with the smallest sample size within the six variants that included imputed data (rs6275, N= 7,734) and 4) the SNP with the largest sample size (rs1800497, N= 8,756). Thus, we approximated the upper and lower bounds of power within (a) five variants that were derived from the fingerprint set and (b) six variants that were derived from the imputed/combined set. The power calculations were based on 1000 replications and the chosen alpha level was 0.05/11. For the smaller dataset, the power ranged from 0.36 (95% confidence interval: 0.33-0.39) to 0.58 (0.55-0.61) to detect an effect explaining 0.5% of the phenotypic variance. The power to detect an effect explaining 1% of the variance ranged from 0.78 (0.75-0.80) to 0.91 (0.89-0.92). For the larger dataset, the power ranged from 0.69 (0.66-0.72) to 0.75 (0.72-0.77) to detect an effect explaining 0.25% of the variance. The power to detect an effect explaining 0.5% of the variance ranged from 0.96 (0.94-0.97) to 0.97 (0.96-0.98). These estimates are conservative as age and sex were not taken into account.

TABLE 2 Number of individuals with complete genotype and phenotype data, their mean age (SD), mean weekly MET hours for the three combinations of alleles (SD; the number of individuals across the three allele codings), minor allele frequencies (MAF), the p-value of the test for Hardy-Weinberg Equilibrium (HWE) and the p-value of the main effect of the variant on exercise behavior, for each SNP/VNTR separately.

Gene	Variant	N	Age μ(SD)	0 μ(SD;N)	1 μ(SD;N)	2 μ(SD;N)	MAF	HWE	p-value*
DRD1	**rs265981**	7873	33.28 (12.13)	*GG*: 12.51 (17.54; 3069)	*GA*: 12.39 (18.18; 3771)	*AA*: 12.57 (18.20; 1033)	.37	.02	.942
DRD2	**rs6275**	7734	33.23 (12.14)	*GG:* 12.41 (18.07; 3812)	*GA:* 12.44 (17.48; 3262)	*AA:* 13.40 (19.38; 660)	.30	.31	.672
	rs1800497	8756	32.46 (12.27)	*GG:* 12.92 (18.33; 5714)	*GA:* 13.18 (18.63; 2684)	*AA:* 14.52 (20.24; 358)	.19	.06	.357
DRD3	**rs6280**	7734	33.23 (12.14)	*CC*: 12.27 (18.30; 734)	*CT*:12.72 (18.65; 3272)	*TT*: 12.37 (17.23; 3728)	.31	.68	.878
DRD4	**rs1800955**	2152	23.94 (11.25)	*TT*: 17.34 (22.54; 680)	*TC*: 18.04 (22.30; 1103)	*CC*: 18.13 (21.29; 369)	.43	.03	.365
	7 allele	2476	23.34 (10.98)	18.29 (22.72; 1624)	19.69 (23.24; 756)	15.75 (17.88; 96)	.19	.49	.854

Gene	Variant	N	Age μ(SD)	0 μ(SD;N)	1 μ(SD;N)	2 μ(SD;N)	MAF	HWE	p-value*
DRD5	**148 allele**	2480	23.34 (10.98)	17.58 (23.02; 607)	19.17 (22.29; 1302)	18.33 (23.02; 571)	.49	.01	.477
DBH	**rs1611115**	3140	24.38 (11.21)	*TT*: 15.90 (19.34; 137)	*TC*: 18.23 (23.14; 1035)	*CC*: 17.96 (21.85; 1968)	.21	.95	.737
	rs2519152	8139	32.77 (12.28)	*CC*: 12.45 (16.91; 1752)	*CT*: 12.61 (17.76; 3948)	*TT*: 13.52 (20.08; 2439)	.46	.04	.028
DAT1	**480 allele**	2464	23.33 (10.98)	19.22 (21.11; 162)	18.20 (20.77; 925)	18.87 (24.19; 1377)	.25	.69	.882
COMT	**rs4680**	8755	32.46 (12.27)	*GG*: 13.79 (18.62; 1779)	*GA*: 13.16 (18.73; 4339)	*AA*: 12.40 (18.04; 2637)	.45	.94	.085

**Fixed effects: sex, age, sex x age interaction, SNP/VNTR; Random effect: latent genetic factor.*

RESULTS

Table 2 depicts - for each genetic variant - the number of individuals with complete genotype and phenotype data, their mean age (SD), the mean weekly MET hours across the three allele codings (SD; the number of individuals) and the p-value for the main effect of the respective SNP or VNTR. The table also includes the specific combinations of alleles for each SNP (not for the VNTRs). The sample size is lower for those variants that were collected with the fingerprint chip only (all VNTRs, rs1800955 and rs1611115) compared to the remaining variants that were derived from the fingerprint chip and complemented with imputed data or derived from the imputed data only. Also, the fingerprint data were derived from relatively young participants. The p-values in the table are based on the model that included sex, age, sex × age interaction and the respective variant as fixed effects and familial relatedness as a random effect. Main effects of sex and age were significant ($p<0.001$) with males and younger participants showing higher levels of exercise behavior and so was the sex x age interaction ($p<0.004$). Importantly, none of the SNPs or VNTRs had a significant effect on exercise behavior ($p>0.02$). In additional analyses, we 1) added possible confounders (differences in ancestry, batch effect and genotyping platforms) to the model and 2) reran the analyses on dosage scores (in which the uncertainty of imputation is taken into account). The effect of each SNP and VNTR remained non-significant.

Next, multiple variants were included into a single mixed model to investigate their joint effect. As the VNTRs and two SNPs (rs1800955, rs1611115) were derived from the fingerprint chip only, the number of individuals dropped to less than 2,000 individuals when including only individuals that had been genotyped on all variants. Therefore, a potential overall effect was tested in two steps. First, all variants were included, reducing the sample size to 1,954 individuals with full genotypic and phenotypic data. Second, only SNPs were included that we had imputed data for (mostly in addition to the fingerprint data; rs265981, rs6275, rs1800497, rs6280, rs2519152 and rs4680), resulting in 7,734 individuals with full genotypic and phenotypic data. In both cases, the joint effect of the variants was non-significant ($\chi^2= 15.65$, df= 11 and $\chi^2= 3.99$, df= 6, respectively).

Finally, the analyses on the polygenic risk scores also failed to show a significant association ($p>0.15$). Mixed models on the sum of the effect alleles across multiple variants were again run in two steps. First, the complete set of variants was included and second, only the variants that we had the larger

sample size for were included.

DISCUSSION

This study aimed to investigate the genetic basis of regular leisure time exercise behavior. Eight SNPs (rs265981, rs6275, rs1800497, rs6280, rs1800955, rs1611115, rs2519152 and rs4680) and three VNTRs (a 48-bp VNTR in exon III of DRD4, a dinucleotide repeat 18.5 kb upstream of DRD5 and a 40-bp VNTR in the 3' UTR of DAT1) with a known function in the dopaminergic reward system were investigated. None of them was significantly associated with exercise behavior.

It is well established from twin studies that exercise behavior is a heritable trait (de Geus & de Moor, 2011). Twin studies allow the decomposition of variance of any phenotype into variance due to genetic effects and variance due to environmental effects (genetic effects + environmental effects= 100% of the variance). In children, genetic effects have been shown to explain slightly more than 20% of the variance in exercise behavior (Huppertz et al., 2012). This heritability rises dramatically to 70-80% in adolescence (van der Aa et al., 2010) and stabilizes at about 50-60% in adulthood (Stubbe et al., 2006). However, it is not clear yet which genes contribute to individual differences in exercise behavior.

A priori, genetic variation in the dopaminergic signaling pathway provided a promising source for the biological basis of this phenotype. Dopaminergic neurotransmission is implicated in the experience of reward which in turn is likely to be a crucial agent in the take-up and maintenance of exercise behavior (Knab & Lightfoot, 2010). Engaging in exercise itself has been related to changes in dopaminergic transmission (Greenwood et al., 2011) and individual differences in the dopaminergic reward system, more specifically in genetic variants that affect the system, have previously been linked to differences in physical activity both in rodents (Knab et al., 2009) and in humans (Simonen, Rankinen, Perusse, Leon et al., 2003).

Admittedly, some of this previous evidence implicating dopaminergic genes looked at more general forms of physical activity (e.g., parts of Simonen, Rankinen, Perusse, Leon et al., 2003) instead of the trait of self-initiated exercise behavior used here (Kostrzewa & Kas, 2014). We focused on voluntary exercise behavior for two reasons. First, we hypothesized that the pleasure someone experiences when performing an exercise activity is a crucial

determinant of the voluntary take-up and maintenance of regular exercise habits (de Geus & de Moor, 2008). Secondly, excellent test-retest reliability has been established for assessing leisure time exercise behavior by survey (de Moor et al., 2008; Stubbe et al., 2007), probably because recall is relatively easy as those activities are not only self-initiated but often clearly defined in time. In contrast, general physical activity is harder to assess reliably by questionnaires or recall interviews. It has been shown that self-reported physical activity corresponds only poorly with actual physical activity (Prince et al., 2008). Reliability of self-reported physical activity may improve when focusing on activities that require moderate-to-vigorous effort, as these are more salient to the person. Nonetheless, even then recall will not be perfect. It may be hard, for instance, to recall the exact duration of non-voluntary physical activity at work (lifting and effortful manual labor) or activities like bicycling to work or effortful household activities (vacuum cleaning). Instead, more objective measurement instruments should be applied, such as accelerometers or doubly labeled water.

Our study was founded on the solid expectation that we would find an association between known functional allelic variations in the dopaminergic signaling pathway and the narrow but well-defined trait of regular leisure time exercise behavior. This expectation was clearly not borne out by the results. Do our findings rule out a role for the dopaminergic system in individual differences in regular leisure time exercise behavior? There are a number of reasons why this conclusion would be premature.

First, the selected SNPs and VNTRs might not have covered all genetic variation within the dopaminergic genes examined, especially in the case of low linkage disequilibrium between variants within a gene. We opted to choose alleles with known functional effects and/or previously reported effects on relevant phenotypes instead of examining the larger set of SNPs tagging the major haplotypes within dopaminergic genes (Xu, Rakovski, Xu & Laird, 2006). Also, by focusing on eight genes, we covered only a small portion of the total dopamine signaling pathway. Already there are many other proteins known to be involved in this signaling pathway (Beaulieu & Gainetdinov, 2011) and probably an even larger amount still eludes us. By definition, a candidate gene approach will miss these uncharted parts of the signaling cascade.

Second, one might argue that the effect sizes of the genetic variants measured here may have been too low to detect even with the substantial sample sizes available to us. Exercise behavior is a very complex phenotype and it is likely to

be affected by a lot of genes, each of which has only a small effect. These small effects might not be detectable in a sample of less than ten thousands of individuals. For six of the eleven variants, data of around 8,000 individuals were available and for the remaining five variants, data of around 2,500 individuals were available. A power analysis revealed that - for the larger samples - the power to detect an effect explaining 0.5% of the phenotypic variance was very good and the power to detect an effect explaining 0.25% of the variance was acceptable, taking into account multiple testing, family structures and the phenotypic distribution. For the smaller samples, power was more modest, but still the power to detect an effect explaining 1% of the phenotypic variance ranged between 0.78 and 0.91. Apart from increasing sample size, power could be increased by using intermediate phenotypes (Bryan et al., 2007). For instance, genetic association with reward sensitivity in the context of exercise activities or with exercise motivation could be investigated as intermediate biological precursors instead of the exercise behavior per se. These are potentially more directly related to the genetic mechanisms, thereby decreasing residual variance that might cover an effect. Replication of our study in large, independent cohorts would increase the confidence in our results.

Third, we should bear in mind that dopaminergic neurotransmission may mediate the effect of entirely different genetic variants on exercise behavior, in the absence of a direct effect of dopaminergic genes. For instance, there might be genetic variants that increase exercise ability, thereby triggering increased dopaminergic neurotransmission during exercise activities as it is rewarding to perform an activity that one is good at. In this case, genetic variants within the dopaminergic pathway may not be directly involved, but dopaminergic neurotransmission may still indirectly convey genetic effects on exercise behavior.

In sum, we did not confirm our hypothesis that allelic variants involved in dopaminergic function create individual differences in exercise behavior. This leads us to plea for a large-scale GWAS on leisure time exercise behavior involving more research groups, as the success of GWAS efforts clearly scales with sample size. Currently, leisure time exercise behavior is less frequently assessed than general physical activity, in spite of the potentially less favorable psychometric properties of the latter. We believe that a GWAS effort on leisure time exercise behavior is worth pursuing. In order to pick up effects, assessing intermediate phenotypes such as exercise motivation should be considered. An inactive lifestyle is one of the major public health burdens nowadays and

interventions that aim to tackle the problem are mostly unsuccessful. Given the substantial heritability of leisure time exercise behavior, it is of outmost importance to better understand its biological basis in order to improve interventions on this health-enhancing lifestyle.

Chapter 9

SUMMARY AND GENERAL DISCUSSION

This thesis used genetically informative designs to study the determinants and correlates of regular voluntary exercise behavior in children (ages 7, 10, 12), adolescents (ages 14 and 16) and adults (18+). In the first part of this thesis, the relative contribution of genes and the environment to individual differences in exercise behavior, including their contribution to stability over time and gene-by-environment interaction, was examined in twins aged approximately 7 to 18 years. The second part examined the nature of the association between exercise behavior and two commonly studied correlates, namely 1) the perceived benefits of and barriers towards exercising and 2) body mass index (BMI). The third part aimed to shed light on the molecular basis of the heritability of exercise behavior by means of a candidate gene study with genetic variants that play a role in the dopaminergic system. In this final chapter, the results of each study will be summarized in the order of appearance, followed by a discussion of their main implications and suggestions for future research. In addition, the fundamental assumptions of the classical twin design, exercise "omics", "deep" phenotyping of exercise behavior and the placement of exercise behavior into the broader context of "physical activity" are discussed. Finally, some overall conclusions will be drawn.

HERITABILITY OF REGULAR VOLUNTARY EXERCISE BEHAVIOR

Previous studies of the NTR have shown that both genes and the environment contribute to individual differences in exercise behavior both in adolescence (Boomsma et al., 1989; de Geus et al., 2003; de Moor et al., 2011; Koopmans et al., 1994; Stubbe et al., 2005; van der Aa et al., 2010) and adulthood (de Geus et al., 2003; de Moor & de Geus, 2013; de Moor et al., 2011; Stubbe et al., 2006; Stubbe & de Geus, 2009; Vink et al., 2011). Chapter 3 contains the first twin study on exercise behavior in childhood. Univariate ACE models were fitted to data of twins aged 7 (N= 3,966 individuals), 10 (3,562) and 12 years (8,687). With the exception of 10-year-old boys (A= 66%, C= 25%), most of the variance in exercise behavior could be explained by shared environmental factors (C= 50-72%). There were significant qualitative sex differences for the ages 7 and 12. At age 10, a drop in heritability was reported, which was likely a result of random fluctuation, as it had disappeared in a new and more powerful analysis of this age group in Chapter 4.

On several accounts, Chapter 4 is an extension of Chapter 3. It is based on a larger dataset and more age groups, namely on data of twins aged 7 (N= 7,331 individuals), 10 (8,007), 12 (14,629), 14 (9,030), 16 (6,019) and 18 years (2,759).

This allowed us to carry out longitudinal analyses on the same twins. As in Chapter 3, we found that the relative contribution of genes increased and the relative contribution of the shared environment decreased from childhood to adolescence. By also estimating the absolute variances, we noted that the absolute shared environmental variance was largely stable across ages, whereas the absolute genetic variance strongly increased. The phenotypic correlations across surveys were moderate to high with larger correlations for surveys that were in closer proximity to each other. In males, stability was mainly caused by transmission of the same genetic effects across ages with an increasing impact on the total variance. In females, genetic effects were transmitted from previous time points as well, but genetic innovation also contributed consistently to the increase in genetic variance of exercise behavior with age. Shared environmental effects were dominated by innovation in both males and females, meaning that different aspects of the shared environment played a role at different ages.

It is striking that the relative contribution of genes and the environment to exercise behavior changes vastly across childhood and youth. The generally large influence of the shared environment in childhood has also been found in previous small-scale studies on total physical activity measured with accelerometers (Fisher et al., 2010), respiratory gas exchange and doubly labeled water (Franks et al., 2005), and pedometers (Plomin & Foch, 1980). The relatively large heritability in adolescence has been found in data of the NTR (e.g., van der Aa et al., 2010) and other cohorts (Beunen & Thomis, 1999; Maia et al., 2002), especially in males. Most recently, Aaltonen et al. (2013) found heritability estimates of around 44-52% in approximately 16- to 18-year-old twins, with a shared environmental influence of 19-26%. It should be noted that in many studies (e.g., Beunen & Thomis, 1999; van der Aa et al., 2010), the shared environmental components were dropped when they were non-significant, meaning that any familial variance was modelled as genetic variance. The C-component is relatively hard to pick up with classical twin studies (Neale & Cardon, 1992; Posthuma & Boomsma, 2000; Visscher, Gordon & Neale, 2008), however, and non-significance of this component does not necessarily mean that it is absent. This is illustrated by Chapter 4 that reports somewhat lower heritability estimates compared to our earlier analyses of these age groups (e.g., van der Aa et al., 2010). This is because we decided, in contrast to our previous analyses, not to drop any components but to model the full ACE model.

Three overall conclusions can be drawn based on Chapter 3, Chapter 4 and

similar studies: 1) There are large individual differences in exercise behavior across the ages of 7 to 18 years, 2) these are mainly due to shared environmental factors in childhood and 3) mainly due to genetic factors in adolescence. In Chapter 3, we hypothesize that the most important shared environmental factor underlying exercise behavior in children might be parental support (which is for a part qualitatively different for boys and girls). This might gradually give way to the impact of peers and the school environment. De Moor et al. (2011) indeed show that generation specific environmental factors largely explain the shared environmental component in adolescence. The shared environmental component, however, is largely overwhelmed by genetic effects in this age group. The underlying factors driving heritability across childhood and adolescents are more stable than the shared environmental factors, but still there is innovation across ages, especially in girls, suggesting that there is no single set of genes that drives heritability in this age range. We suggested in Chapter 4 that exercise ability and trainability, the acute psychological response to exercise and a homeostatic need to be active or personality factors might underlie the high heritability of exercise behavior in adolescence (also see Rowland, 1998, and Eisenmann and Wickel, 2009). It is reasonable to assume that the contribution of these factors differs for younger versus older individuals. For instance, the homeostatic need to be active might be more relevant in children (Saudino, 2012), whereas exercise ability might play a major role in adolescents.

An important limitation of the twin studies on exercise behavior that have been conducted so far, is that they have not explicitly modeled gene-by-environment (GxE) interaction with exercise behavior as the outcome variable. Genes and environmental effects, however, may not act in splendid isolation and the expression of genetic variance may depend on the environment (Purcell, 2002). A more facilitating environment, for instance, might increase genetic variance, whereas a more restrictive environment might suppress genetic effects. In order to shed some light on potential effects of moderating variables, Chapter 5 assesses the effects of parental education on the means, total variances and variance components of children's exercise behavior in twins of the NTR and two Finnish cohorts. Consistent trends of a higher level of exercise behavior in children of high educated parents in both datasets point towards a role for parental education in offspring exercise behavior, which is in line with much previous research (Ferreira et al., 2006; Hanson & Chen, 2007; Singh et al., 2008). The tendency for lower total variances in this group has rarely been explored before (Johnson et al., 2010). In our study, we find only weak evidence for moderation of genetic or environmental variance of exercise

behavior by parental education. The lower genetic variance in exercise behavior in Dutch daughters of high educated parents suggests that genetic effects that would act against exercise behavior might be suppressed in this group.

Taken together, our studies highlight large individual differences in exercise behavior and a complex shift in the genetic and environmental determinants of exercise behavior from childhood to adolescence. This hopefully gives a better understanding of why there is no intervention that works for everyone. Interventions should be tailored to the specific needs of individuals and take into account causes of individual differences across ages and sex. One size surely does not fit all.

CAUSALITY TESTING USING TWIN DATA

Researchers have long been interested in correlates of exercise behavior, both as potential determinants and consequences of the behavior. Twin studies can shed more light on the nature of an association between two traits by assessing whether there could be a causal relationship or not. De Moor and colleagues (2008) have outlined and applied methods to test causality both in cross-sectional and longitudinal twin data. We have investigated the association of exercise behavior with a frequently studied potential determinant, namely the perceived benefits of and barriers towards exercise activities ("attitudes"), and a potential consequence, namely BMI.

Many social cognitive models of health behavior propose exercise attitudes to be important *determinants* of exercise behavior and highlight them as promising targets for interventions (Ajzen, 1985; Becker, 1974; Biddle & Nigg, 2000; Hagger et al., 2002; King et al., 1992; Schwarzer, 1992). In Chapter 6, we aimed 1) to unite this with the finding that exercise behavior is a heritable trait by showing - for the first time - that the perceived benefits of and barriers towards exercise behavior (summarized under the heading "exercise attitudes") are heritable themselves and 2) to test whether exercise attitudes and exercise behavior could be causally related. We ran a principal component analysis on questionnaire items assessing the perceived benefits of and barriers towards exercising in adult twins. Six components emerged, namely "Perceived benefits", "Lack of skills, support and/or resources", "Time constraints", "Lack of energy", "Lack of enjoyment" and "Embarrassment", and we showed that all of these were heritable, with heritability estimates ranging from 21% to 49%. Bivariate models revealed that the phenotypic correlations between the

attitude components and exercise behavior were all significant and ranged between -0.44 (“Lack of enjoyment”) and +0.32 (“Perceived benefits”), and together the attitude components explained 28% of the variance in exercise behavior. Moreover, the largest part of these correlations was due to overlapping genetic factors between the two traits.

It was tested whether the traits could be causally related by two means: bivariate genetic models and the monozygotic (MZ) twin intrapair differences model. The two approaches are fully described in Chapter 6 and will shortly be outlined in the following. It should be noted that both approaches make it possible to falsify, but not to prove, causality.

The rationale behind testing for causality with a bivariate genetic model is that if exercise attitudes causally influence exercise behavior, then everything that influences those attitudes will also, through the causal chain, influence exercise behavior (if A causes B, and B causes C, then A causes C). Therefore, if exercise attitudes are affected by both genes and the environment, then the genetic and environmental cross-trait correlations between the attitude components and exercise behavior need to be significant under the assumption of causality. Based on this rationale and the finding of significant genetic and environmental correlations in the bivariate models, a causal relationship could not be falsified for most attitude components in Chapter 6, with the exception of “Perceived benefits” and “Embarrassment” in males, where the unique environmental correlations were non-significant. However, the unique environmental factors contain both true environment, which is expected to be correlated between two causally linked traits, and measurement error, which is not expected to be correlated. The “true” unique environmental correlations might be very small and thus might have been non-significant due to a lack of power.

The results of the bivariate models were fully confirmed in a second approach to causality testing, the MZ twin intrapair differences model. If there is a causal association between attitudes and behavior, the twin with more positive attitudes should exercise more compared to his or her genetically identical co-twin with more negative attitudes. Therefore, within-pair differences in exercise attitudes should be associated with within-pair differences in exercise behavior. The within-pair difference scores were calculated for the attitude components and for exercise behavior in MZ twins. Next, the difference score of each attitude component was correlated with the difference score of exercise behavior. Significant correlations are compatible with a causal effect and these were found for 10 of the 12 correlations (6 attitude components in

both sexes). Non-significant correlations would imply that the phenotypic associations are caused by underlying genes influencing both phenotypes in the absence of causality ("genetic pleiotropy"). Again, this was found for "Perceived benefits" and "Embarrassment" in males only.

Overall, the results revealed that 1) exercise attitudes are heritable, 2) exercise attitudes are significantly related to exercise behavior and 3) it is likely that this relationship is causal. As this study was based on cross-sectional data, we could not draw any conclusions on possible directions of causation, however. The heritably of attitudes reveals that no one is born as tabula rasa when it comes to the future perceived benefits of and barriers towards exercise behavior. There are innate differences that make individuals more or less likely to have a positive attitude towards exercising. This makes intuitive sense. As stated above, personality factors, in part through a link with a homeostatic need to be active or the acute psychological response to exercise, as well as exercise ability and trainability are hypothesized to underlie the heritability of exercise behavior. Attitudes might be closely related to these. For instance, "Embarrassment" and "Lack of skills, support and/or resources" are probably related to exercise ability and trainability, "Lack of enjoyment" and "Perceived benefits" to the acute psychological response to exercise, "Lack of energy" to a homeostatic need to be active and "Time constraints" to personality factors such as neuroticism. Health promotion strategies often aim to change the populace's attitudes towards exercise behavior by educating people on the health benefits of regular exercise and ways to reduce barriers to engage in exercise activities. Although the largest part of variance in exercise attitudes was explained by the non-shared environment, such strategies should take into account innate differences between individuals. It does not make sense to try to convince someone that exercising will make him or her "feel energetic" when this effect does not apply to that specific person. This is all speculative, however, and replication in longitudinal studies is first needed to more firmly establish the direction of causality.

Next, we aimed to apply the same causality testing approach to investigate a very intensively studied potential *consequence* of exercise behavior, namely BMI. One of the main rationales for research on exercise behavior is that it has an effect on body weight and could therefore be a way to curb the obesity epidemic. A wealth of studies on the relationship between energy expenditure and body composition in childhood and youth has focused on daily physical activity, with rather mixed outcomes (Bleich et al., 2011; Jiménez-Pavón et al., 2010; Must & Tybor, 2005; Wareham et al., 2005; Wilks et al., 2011). The

majority of studies have methodological flaws such as a cross-sectional design that prohibits conclusions on cause-effect relationships, small sample sizes and suboptimal measurement instruments (Must & Tybor, 2005; Wareham et al., 2005). Systematic evidence for the often cited link between daily physical activity, let alone exercise behavior, and body composition in young people is still lacking.

In Chapter 7, we aimed 1) to test the widespread assumption of the presence of a significant negative association between exercise behavior and BMI and 2) to investigate in how far this association reflects a causal effect of exercise behavior on BMI. In contrast to many previous studies, our study was based on a large longitudinal dataset with detailed measures of exercise behavior. Based on the hypothesis that regular exercise behavior is a causal determinant of obesity, higher levels of exercise behavior in childhood and adolescence were expected to be associated with lower levels of BMI at all ages and changes in exercise behavior with time were expected to predict opposite changes in BMI.

Contrary to our expectations, we found no evidence for a cross-sectional or longitudinal association between exercise behavior and BMI at all in a (partly) longitudinal dataset of 7-, 10-, 12-, 14-, 16- and 18-year-old individuals. Alternative determinants of BMI such as basal metabolic rate, other aspects of daily physical activity and sedentary behavior, but prominently also energy intake are likely to be more important, meaning that weight loss programs that are based on increasing regular exercise only, i.e., without an accompanying dietary intervention, may not be successful. Importantly, this does not detract from the value of encouraging regular exercise behavior in childhood and youth, as this behavior has been shown to have many other favorable effects on health, even in the absence of an effect on body weight, and should thus still be promoted (Melanson et al., 2013). Claiming a primary role for exercise behavior in the variation of children's and adolescents' BMI, however, may foster false expectations. No one should be led to believe that every person can lose significant amounts of weight by exercising. It is better to stress the multiple potential health benefits of this behavior, such that even in the absence of a change in weight, advantageous changes may still take place in other risk factors.

More generally spoken, our null finding should remind us of the importance of understanding innate individual differences in the response to exercise before applying interventions (Bouchard et al., 2015; de Geus et al., 2014). The HERITAGE study and related work have brought about important findings on

the effects of training on a variety of fitness traits such as maximal oxygen uptake or skeletal muscle strength (Bouchard, 2012; Bouchard & Rankinen, 2001; Bouchard, Rankinen & Timmons, 2011). These kinds of studies will improve the ultimate effectiveness of interventions by making it possible to target so-called "responders" - individuals who will actually benefit. For instance, it does not make sense to motivate an overweight individual to exercise on a regular basis *in order to lose weight* if that individual's genetic makeup is not sensitive to such effects of exercise. Although currently not yet feasible, it would ultimately be more meaningful to focus on the health benefits that can be realistically expected given an individual's genetic makeup.

The increasing availability of DNA data for a large number of individuals makes it possible to not only test causality based on latent genetic variance components, but to directly test causality based on the effects of specific genes. The *Mendelian randomization technique* has gained popularity in the past years as a means to causality testing that is in principle similar to the approach that we have used (Davey Smith & Ebrahim, 2004; Davey Smith & Hemani, 2014; Lawlor, Harbord, Sterne, Timpson & Davey Smith, 2008). Instead of calculating the correlation between latent genetic and environmental factors that are thought to influence two traits, it is based on measured genetic variants. More specifically, a genetic variant that influences an exposure variable (such as exercise behavior) should also, through the causal chain, predict an outcome variable (such as BMI). The application of Mendelian randomization with exercise behavior as a predictor of any phenotype is challenging, as solid associations with genetic markers would first have to be identified, which is currently not the case (see p.190). However, the technique can already be applied to test potential reversed causal effects, for instance with BMI as the predictor. Large international consortia have yielded genetic risk scores for BMI that can be used as genetic instrumental variables. Richmond et al. (2014) found evidence that BMI causally influences physical activity in 4,296 children aged 11 years by regressing an allelic risk score for high BMI ("the genetic instrument") on physical activity as assessed by accelerometers. They also tried to test whether physical activity had a causal effect on BMI but found no evidence for this effect. In all fairness, the instrumentation of physical activity, namely a genome-wide prediction score based on their own study, provided a very weak genetic instrument and the authors indicate that their results should be interpreted with caution.

The big advantage of the Mendelian randomization technique is that it is based on measured genetic variants and can be applied to any large population-based

samples, whereas our methods for causality testing rely on latent (unmeasured) genetic and environmental factors and need large twin samples. A shortcoming with studies that are based on twins only is that the shared environmental variance (C) cannot be estimated simultaneously with the dominant genetic variance (D). Therefore, one of the two is usually selected and included into genetic models, based on twin correlations and/or fit statistics. When the dizygotic (DZ) twin correlations are larger than half the MZ correlations, shared environmental influence is assumed, whereas a lower DZ twin correlation compared to half the MZ correlation implies dominant genetic effects. However, in reality, both might be in place at the same time and it is even possible that they cancel each other out in the twin correlations. Therefore, a model estimating all four components (A, E, C and D) - which is only possible when including for instance data of parents - would be especially desirable in the context of causality testing using twin data.

In sum, we have shown that attitudes are heritable and that they might be causally related to exercise behavior, but we found no evidence for a (causal) association between exercise behavior and BMI. The genome-wide association era has yielded novel and powerful approaches to test for causality between traits and these should fully be exploited to better understand the nature of the relationship between exercise behavior and relevant correlates. Most importantly, researchers should not underestimate the relevance of studying individual differences in the response to exercise as targeting non-responders might not only lead to disappointment of the participants, but is actually not the optimal use of scarce public health resources.

ASSUMPTIONS OF THE CLASSICAL TWIN DESIGN

I hope that the outlined chapters have conveyed the beauty and versatility of twin studies to the reader. A number of critical assumptions have to be met, however, to obtain valid results. Most obviously, it is assumed that *MZ twins share all of their segregating genes, whereas DZ twin share on average 50% of their segregating genes identity-by-descent (IBD)*. Although there are MZ twins that are not entirely genetically identical, no systematic differences between the DNA sequences of MZ twins have been found so far (Baranzini et al., 2010; Veenma et al., 2012). The question of genetic similarity between DZ twins has been answered based on genome-wide marker data. In a sample of 11,214 sibling pairs, Visscher et al. (2007) have shown that the true proportion of IBD sharing lies within the range of 31% and 64%, with a mean of 50%. They calculated the heritability of height based on the empirical IBD sharing between

siblings and came to virtually the same heritability as previous twin studies. Thus, the assumption of the amount of genetic overlap between MZ and DZ twins is very likely met, at least under the *assumption of random mating*.

Non-random (or assortative) mating refers to spousal resemblance on a phenotype. Previous studies have found spousal correlations on exercise behavior-related traits ranging from 0.16 to 0.60 (Aarnio et al., 1997; Boomsma et al., 1989; Perusse et al., 1988; Perusse et al., 1989; Seabra et al., 2008). There are several possible mechanisms causing significant spousal correlations, including phenotypic assortment, social homogamy and social interaction (de Moor et al., 2011; Heath & Eaves, 1985). The most problematic of the three mechanisms for twin modelling is phenotypic assortment, meaning that initial partner selection is based on the phenotype under study (or a correlated phenotype), e.g., when exercisers are attracted to other exercisers. If genes are implicated in the phenotype, this would inflate the DZ twin correlation and thus result in an overestimation of shared environmental effects if assortment is not taken into account. Alternatively, spouses might resemble each other simply because individuals from similar social backgrounds are more likely to meet and might therefore start a relationship (social homogamy). Finally, spouses might not necessarily be alike in the first place, but might become more similar in the course of their relationship (social interaction). De Moor et al. (2011) were the first to explicitly investigate these three mechanisms in the context of exercise behavior using twin models and concluded that the observed spouse resemblance was best explained by phenotypic assortment. Unfortunately, a rather rough measure of exercise behavior (namely the dichotomy “regular exerciser” versus “non-exerciser”) was used. A replication of their results based on a more precise measure of exercise behavior would be desirable and would imply that future studies might want to correct for assortative mating - although if different (uncorrelated) genes determine exercise behavior at different points in life, this might not be necessary or even make any sense. Assortment would then only affect genes that influence exercise behavior in early adulthood (when most individuals select their mating partners). Applying any corrections was deemed to be premature in our studies, as the nature of assortative mating should first be solidly established.

The so-called *equal environments assumption* (EEA) has been subject to heated debates (e.g., Horwitz, Videon, Schmitz & Davis, 2003). It is well known that MZ twins tend to have more similar environments than DZ twins. For instance in childhood, MZ twins are more likely to share friends, share the same room and to dress alike. In adulthood, MZ twins often have a higher contact frequency

than DZ twins (Kendler, 1993). The EEA posits that these environmental differences are not related to the phenotype under study. Otherwise, a higher similarity of MZ twins compared to DZ twins could be due to genetic influences, environmental influences, or both, whereas the classical twin design ascribes a difference in similarity to genetic factors only. The EEA has been shown to be met for a wide range of phenotypes (Kendler, 1993).

One common test of the EEA is based on assessing indices of the amount of shared environment in twin pairs (such as perceived similarity of treatment by others, similarity of appearance or contact frequency) and to test their impact on heritability estimates. The first explicit test of the EEA for doing sports during leisure time and related physical activity phenotypes (Eriksson et al., 2006) investigated the effect of twins' contact frequency on their phenotypic similarity. Heritability was calculated in twins with high versus low contact frequency and higher estimates were found for the former group. This might be interpreted as a violation of the EEA. Eaves, Foley and Silberg (2003) challenge this interpretation, however, by proposing that genetic factors might drive niche selection such as the choice of with whom twins spend their time. Thus, so-called "environmental" differences might actually have a genetic origin and might thus not constitute a violation of the EEA. Based on simulated data, Eriksson et al. (2006) show indeed that the observed patterns could entirely be explained by niche selection.

A different approach to testing the EEA is based on the fact that there are twin pairs that misperceive their zygosity (e.g., Conley, Rauscher, Dawes, Magnusson & Siegal, 2013). Twins might think that they are monozygotic in the first place and then a closer examination (e.g., a DNA test) reveals that they actually are dizygotic or vice versa. Heritability can be estimated based on "real" versus "perceived" zygosity. If heritability estimates are higher in the latter case, this would imply that monozygotic twins are more similar to each other than what would be expected based on the difference in genetic relatedness with DZ twins alone, and that the EEA would thus not be met. To the best of my knowledge, this has not been tested for exercise behavior or other physical activity-related traits.

Thus, the EAA has been shown to be met for a wide range of phenotypes, but explicit tests in the context of physical activity are scarce. As suggested in Chapter 6, it would be particularly interesting to examine whether treatment of twins by others is more similar with a higher resemblance in athletic appearance and how this relates to heritability estimates in traits that are

associated with physical activity. One might argue that MZ twin correlations could be inflated as MZ twins are treated more equally due to how others perceive their athletic capabilities (based on their appearance), independent of their actual physical abilities.

Last but not least, twin research is based on the fundamental assumption that *twins are representative of the general population*. Testing this assumption is not only relevant to researchers, but also to the twins themselves. As twins are born in all strata of society, there is no reason to assume a systematic bias in the first place. Pregnancy and birth outcomes are often less favorable for twins than for singletons, however (Croft, Morgan, Read & Jablensky, 2010). Twins tend to be delivered preterm and they have lower birth weights (Croft et al., 2010; Estourgie-van Burk, Bartels, Boomsma & Delemarre-van de Waal, 2010). In a Dutch sample, Estourgie-van Burk et al. (2010) showed that compared to the general population, twins were smaller and weighted less on average at birth and around their first birthday. At the age of 4 years, they were not significantly different from the general population in height and weight, but they had somewhat smaller BMIs. Any differences in height, weight or BMI had disappeared at the age of 18 years (Estourgie-van Burk et al., 2010). Differences between twins and singletons in exercise behavior have - to the best of my knowledge - not been tested systematically before, although there is no reason

TABLE 1 Percentage of non-exercisers and mean weekly MET hours (variances; N) for multiples versus singletons.

		% non-exercisers*		Weekly MET hours		
Age	**Sex**	**Multiple**	**Singleton**	**Multiple**	**Singleton**	**p****
13	**M**	20.4%	17.6%	28.7 (24.9; 314)	29.7 (24.6; 119)	.77
	F	22.7%	19.1%	20.5 (23.1; 423)	19.2 (18.8; 131)	.82
14	**M**	19.1%	28.3%	29.7 (26.6; 1282)	26.7 (25.8; 60)	.36
	F	21.1%	19.3%	21.4 (22.8; 1688)	23.6 (24.0; 88)	.40
15	**M**	20.1%	22.3%	32.1 (28.5; 528)	27.8 (24.5; 103)	.28
	F	25.2%	23.7%	21.3 (23.3; 686)	24.2 (26.1; 131)	.39
16	**M**	24.5%	24.3%	31.1 (29.3; 912)	30.8 (30.4; 210)	.70
	F	28.3%	26.3%	21.2 (23.9; 1292)	21.3 (25.3; 262)	.84
17	**M**	25.0%	30.1%	31.5 (29.6; 520)	30.6 (29.4; 206)	.75
	F	32.4%	30.4%	19.4 (21.9; 720)	19.6 (23.7; 273)	.91
18	**M**	25.8%	28.8%	31.4 (32.5; 62)	27.0 (29.1; 208)	.44
	F	41.8%	37.6%	16.3 (21.7; 122)	15.6 (19.3; 340)	.79

**<4 weekly MET hours; **p-value of comparing weekly MET hours according to Mann-Whitney U test.*

to assume noteworthy differences in terms of physical capability or exercise motivation. Table 1 depicts a comparison of exercise behavior in multiples versus singletons based on data of the NTR (taken from Chapter 4). First-born multiples and siblings were selected in narrow age ranges (e.g., for “age 13”, they were >=13.0 and <14.0 years old) to compare the percentage of non-exercisers and the means and variances in weekly MET hours between these groups. No systematic differences were apparent.

To sum up, there is no reason to assume that the general assumptions of twin research are not met with regard to exercise behavior.

GENOMICS OF REGULAR VOLUNTARY EXERCISE BEHAVIOR

Chapter 8 contains the largest candidate gene study so far on dopaminergic variants and exercise behavior during leisure time both in terms of the number of genetic variants that were included and in terms of sample size. None of the variants were significantly associated with exercise behavior. Even when looking beyond dopaminergic genes, not a single genetic variant has been shown to affect regular exercise behavior at a level of “proof beyond reasonable doubt” in previous studies. Technological advancements make it possible to not only test a handful of genetic markers for their association with a phenotype, but to test the association with hundreds of thousands of markers simultaneously, covering genetic variation across the whole genome. As theory-based candidate gene studies have not been proven successful, such theory-free genome-wide association studies are the appropriate way forward as they provide the opportunity to discover entirely new pathways (Flint, 2013; Pearson & Manolio, 2008). Due to simultaneous testing of a very large number of genetic markers, many of these markers will be significantly associated with the phenotype merely by chance if not correcting for multiple testing, however (Sullivan, 2007). At the same time, exercise behavior is a quantitative trait that is influenced by many genes with very small effects. With a significance threshold that is corrected for multiple testing (e.g., $\alpha = 5x10^{-8}$), it needs a very large number of individuals to find any genome-wide significant associations at all and to confirm significant hits in independent samples. Although costs of genotyping are lower than ever before, genotyping DNA data of hundreds of thousands of individuals remains a very expensive undertaking, let alone the costs of (both genotypic and phenotypic) data collection and processing. No single research group has the necessary resources to acquire big enough sample sizes to push the field forward from where we are now. It is essential to establish an international consortium that pools data of cohorts with genome-

wide DNA data and corresponding data on exercise behavior (or, more generally, physical activity phenotypes). Such efforts are currently undertaken and the first large-scale GWAS by the GIANT consortium is underway.

MORE OMICS OF REGULAR VOLUNTARY EXERCISE BEHAVIOR

So far, I have investigated and discussed the starting point of the biological paths towards exercise behavior, namely variation in the genome. Although genes are a straightforward beginning to disentangle the biology of behavior, it would clearly be an oversimplification to ignore in this discussion the very complex array of processes from the genetic code to observable behavior that impact upon one another in a dynamic and hardly predictable fashion.

Every cell in our bodies contains the same genetic information. Differences between cells emerge as only genes that are relevant to each specific cell are transcribed into RNA. The basic process leading from genotype to phenotype is well-known. Simply put, DNA is first transcribed to RNA, which in turn is translated to or impacts upon proteins, the basic biological building blocks of phenotypes. Unfortunately, however, there is no one-to-one transformation from genes to proteins, let alone from proteins to behavior. Individual differences in these processes make the interpretation of genetic effects a very complex undertaking. Also, there are feedback mechanisms going from the phenotype back to RNA transcription. Knowing a person's genetic makeup is thus far from knowing the biological origin of that person's behavior. Therefore, epigenomes (Kaminsky et al., 2009; van Dongen et al., 2014), transcriptomes (Jansen et al., 2014; McRae et al., 2007; Tan et al., 2005), proteomes (Altelaar, Munoz & Heck, 2013) and metabolomes (Draisma et al., 2013; Draisma et al., 2015; Dunn et al., 2011; Gieger et al., 2008; Nicholson et al., 2011) are increasingly being studied.

Animal models are a popular means to unravel the underlying "omics" events that connect genes with behavior. Functional annotation studies are often conducted in animals and aim to confirm GWAS-derived results by assessing the effects of genetic variants on, for instance, proteins or metabolites that are relevant to the phenotype under study. Obviously, there are less complex ethical constraints in animal research compared to studies with human subjects, making it possible to study omics in all tissues (including, for instance, brain tissue) and to manipulate the suspected genotypes or the environment in order to elicit changes in the transcriptome, the proteome, the metabolome and/or the ultimate behavior.

Mice are especially suited to study the determinants and consequences of exercise behavior, mainly for three reasons. First, their genome is comparable to that of humans (Paigen, 2003). Second, voluntary wheel running serves as an elegant model for voluntary exercise behavior. And third, rodents have a relatively short lifespan which makes them well suited to study long term health consequences of exercise behavior and/or effects of ageing processes (de Geus et al., 2014). Mice models have confirmed the important role of genes in exercise behavior. In genetically well-characterized inbred strains of mice, larger between-strain differences compared to within-strain differences in running wheel activity became apparent (Lightfoot et al., 2010). In addition, mice can be selectively bred for high voluntary wheel running (Rezende, Gomes, Chappell & Garland, 2009). In line with our hypotheses, various studies in rodents have suggested that ability and motivation underlie differences in voluntary exercise behavior (Garland et al., 2011).

A CALL FOR "DEEP" PHENOTYPING OF EXERCISE BEHAVIOR

To improve the probability of finding significant associations with genetic markers, it is important to have a clearly defined phenotype. We have deliberately focused on regular exercise behavior during leisure time as it can be measured reliably by survey. However, this phenotype might still be defined too broadly as we collapse all kinds of exercise activities during leisure time. It is reasonable to assume, however, that the determinants of doing strength training are qualitatively different from those of playing basketball, for instance, which is likely to be reflected in different biological origins. A better understanding of individual differences as they relate to the choice for a *specific* exercise activity is therefore an important consideration. Relating these narrow and refined phenotypes (e.g., individual sports versus team sports, competitive exercise versus non-competitive exercise) to genes might delineate a more accurate picture than the somewhat broader approach that we have chosen. Lauderdale et al. (1997) have assessed and separately analyzed specific exercise activities (running, bicycling, swimming, racquet sports and other strenuous sports) in twin pairs of the Vietnam Era Twin Registry. Their results indeed show that heritability estimates differ between activity types. They also suggest that more strenuous activities might be more heritable than moderate activities.

Moreover, the motivational precursors of exercise behavior might be fundamentally different across individuals (e.g., performing an activity mainly for social reasons versus for health reasons, reward sensitivity, mood

responses), which again might be reflected in genetic effects on very different biological systems (Bryan et al., 2007). Most importantly, these precursors might be different across age and sex. For instance, competitive exercise ability might be more relevant to adolescents, whereas health benefits might be more relevant to adults. In addition, females might be more inclined to exercise for losing weight, whereas males might want to build up muscles.

PLACING EXERCISE BEHAVIOR IN THE BROADER CONTEXT OF "PHYSICAL ACTIVITY"

This thesis has not examined general physical activity or sedentary behavior ("any waking behavior characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture", Sedentary Behaviour Research Network (2012), p.540). This should not detract from the value of overall physical activity to increase health in the general population or from the detrimental effects of sedentary behavior. Exercise interventions are probably the most straightforward and efficient way to increase physical activity levels, but other means, such as increasing active transportation (Mueller et al., 2015) or decreasing sedentary time (van der Ploeg et al., 2012) are also promising intervention targets. Importantly, these behaviors are probably not independent of each other. For instance, Ridgers, Timperio, Cerin and Salmon (2014) showed that higher physical activity levels on one day were compensated with lower physical activity levels on the following day in 8- to 11-year-old children, supporting our hypothesis of a homeostatic need to be active that is rather fixed at a biological set point. The large problem when it comes to twin studies on general physical activity is that very large datasets are needed for genetic analyses, whereas objective assessment of these behaviors on a large scale is very expensive. This leaves subjective report of general physical activity using questionnaires, which may not be very reliable (Adamo et al., 2009; Prince et al., 2008). Other than salient (high intensity) voluntary activities, people are simply not very good at estimating their daily physical activity level.

A number of smaller-scaled twin studies and one larger study have estimated the heritability of physical activity and sedentary time based on accelerometer-derived twin data. Table 2 depicts studies that were (partly) conducted under free-living conditions in same-sex twin pairs aged older than 5 years and the heritability estimates of the accelerometer-derived measures outside the laboratory. Just as for exercise behavior, shared environmental factors seem to play a major role in childhood and genetic components affect physical activity

both in younger and older adults. We have recently collected accelerometer data in MZ twins aged 16-26 years and found a twin correlation of 0.58 for moderate-to-vigorous physical activity and a twin correlation of 0.50 for sedentary behavior (N= 38 pairs). These might be underestimations compared to the general MZ twin population as these twins were selected based on their discordance on exercise behavior. Still, the correlations show that there is clearly a familial factor involved in these behaviors. DZ twin data are now needed to decompose this familial factor into genetic effects and shared environmental effects and a large-scale study investigating the heritability of physical activity and sedentary behavior based on accelerometer data is underway in the NTR.

MAIN IMPLICATIONS AND FINAL CONCLUSION

I hope that this thesis has conveyed the enormous complexity of the factors influencing individual differences in exercise behavior, especially in childhood and youth, where we observed profound changes in the genetic architecture over time. Given these intricacies, it makes a lot of sense that applying "one-size-fits-all" interventions to largely differing subgroups of the population will not bring about satisfactory changes in behavior. Although we have roughly revealed the underlying causes of variation in exercise behavior between the ages of 7 and 18 years by decomposing total variance into genetic, shared environmental and non-shared environmental effects, the specific factors that underlie those variance components have not been identified, which is the most important challenge for future research in order to develop successful "personalized" interventions. The genetic component in regular exercise behavior tells us that it will be harder to engage some people in physical exercise than others - but "harder" does not mean "undoable". On the contrary, accepting innate human variation can help us increase the net yield of intervention efforts.

Based on the findings of this thesis, I have suggested that we are best served by family-based interventions for children and individual-based interventions for adolescents. Family-based interventions for children 1) should explicitly include parents, 2) should be tailored to different needs of boys versus girls and 3) they may want to primarily target at risk families (e.g., low parental education). Individual-based interventions for adolescents should aim to target genetically caused differences in exercise behavior. If it would appear, as we speculated, that exercise ability strongly impacts on adolescent exercise behavior, sports clubs entrusted with our 12- to 18-year-olds might want to emphasize

TABLE 2 Overview of studies on the heritability of physical activity and sedentary time as assessed by accelerometry in free-living conditions (twins aged >5 years).

Reference	Sample	Phenotype	ACDE (percentages)
Joosen, Gielen, Vlietinck & Westerterp (2005)	N=18 twin pairs (12 MZ, 6 DZ); 2 sibling pairs; Aged 18-39 years	Total PA	A=78, E=22
Fisher et al. (2010)	234 individuals (57 MZ twin pairs, 60 DZ); Aged 9-12 years	(1) Total PA (2) MVPA (3) Sedentary time	(1) C=73, E=27 (2) C=61, E=39 (3) C=55, E=45
den Hoed et al. (2013)	1654 twins (420 MZ pairs, 352 DZ); Mean age 56.3 years; Mostly women	(1) Acceleration of the trunk (2) PA energy expenditure (3) MVPA (4) Sedentary time As derived from a combined heart rate and movement sensor.	(1) A=35, C=2, E=63 (2) A=47, C=2, E=52 (3) A=47, E=53 (4) A=31, C=15, E=55
Gielen et al. (2014)	51 twin pairs, 1 male triplet (29 MZ, 23 DZ); Mean age 22 years	(1) Total PA (2) Low-intensity PA (3) Moderate-intensity PA (4) high-intensity PA	(1) A=57, E=43 (2) A=38, E=62 (males); A=72, E=28 (females) (3) ACE, AE, CE and ADE equally parsimonious; AE: A=55, E=45 (4) AE: A=47, E=53 or ADE: D=55, E=45

PA=physical activity, MVPA=moderate-to-vigorous physical activity, MZ=monozygotic, DZ=dizygotic, A=additive genetic effects, C=shared environmental effects, D=dominant genetic effects, E=non-shared environmental effects.

participation and pleasure rather than performance to increase exercise behavior in this group by 1) not selectively favoring resources (availability of trainers, coaches, fields and equipment) to the best teams/players, 2) de-emphasizing the competitive aspect and 3) offering a larger selection of exercise activities and intensities to suit different levels and forms of physical ability.

For understandable reasons, exercise interventions are often the domain of those who have a strong personal interest in and affiliation with exercise: there are very few exercise interventionists who are not themselves (ardent and proficient) exercisers. Notwithstanding the many benefits of such positive role models ("believers"), there are also dangers in strong personal beliefs and in propagated folk wisdom about exercise benefits. The studies on attitudes and BMI are an illustration of these dangers. Instead of repeating over and over again that exercise behavior will lead to weight loss or "feeling energetic", a more nuanced and realistic message would be wiser. Namely that for some, it will lead to weight loss and for others, it does not, and that for some, it will lead to feeling energetic and for others, it does not. It does not make any sense to convince individuals of the benefits of exercise behavior if these benefits simply do not match their genotypes. In fact, the initial motivation to exercise may be irreparably damaged when these promised effects do not occur. Up until today, exercise intervention programs have been mainly informed by the genome of regular exercisers. To increase their impact on the overall population, however, future intervention programs should also carefully heed the genome of those who are currently non-exercisers.

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Nederlandse samenvatting

Waarom sporten sommige mensen en anderen niet? Is sportgedrag erfelijk? Zo ja, wat zijn dan de onderliggende biologische mechanismen? En leidt sporten tot een lager lichaamsgewicht? Dit proefschrift probeert een antwoord te vinden op deze en andere vragen. De belangrijkste resultaten worden hieronder samengevat, na een korte toelichting op het klassieke tweelingonderzoek.

HET KLASSIEKE TWEELINGONDERZOEK

Tweelingen vormen voor de wetenschap een bijzondere groep, want met hun hulp kan worden bepaald in hoeverre genen en de omgeving leiden tot gedragsverschillen tussen mensen. Dit is mogelijk omdat er twee soorten tweelingen zijn: eeneiige tweelingen ontstaan als een eicel kort na de bevruchting splitst waardoor zij dus (bijna) 100% van hun genetisch materiaal delen, terwijl dit voor twee-eiige tweelingen gemiddeld maar 50% is, net als bij gewone broers en zussen. Voor eeneiige en twee-eiige tweelingen geldt echter dat zij onder vergelijkbare omstandigheden opgroeien. Als nu blijkt dat eeneiige tweelingen meer op elkaar lijken wat betreft hun gedrag dan twee-eiige tweelingen, dan is er sprake van erfelijkheid. Daarnaast is het ook mogelijk om een onderscheid te maken tussen gedeelde en unieke omgevingsinvloeden. Gedeelde omgevingsinvloeden worden gedeeld door twee personen van een tweelingpaar, zoals opgroeien in hetzelfde gezin of in dezelfde buurt. Daarentegen worden unieke omgevingsinvloeden niet gedeeld - het zou bijvoorbeeld kunnen dat de ene helft van een tweeling heel andere vrienden heeft dan de andere helft. Er is sprake van gedeelde omgevingsinvloeden als het gedrag van twee-eiige tweelingen meer op elkaar lijkt dan zou worden verwacht op basis van hun genetische overeenkomsten. Verschillen tussen eeneiige tweelingen wijzen op unieke omgevingsinvloeden.

DE ERFELIJKHEID VAN SPORTGEDRAG

Het eerste deel van dit proefschrift richt zich op de erfelijkheid van sportgedrag tijdens de kindertijd en de adolescentie. Met data van 7-, 10-, 12-, 14-, 16- en 18-jarige tweelingen die staan ingeschreven bij het Nederlands Tweelingen Register (NTR) hebben we in kaart gebracht hoe groot de invloed van genen en van de omgeving is op verschillen in sportgedrag binnen iedere leeftijdscategorie, en hoe deze invloeden bijdragen aan stabiliteit en verandering in sportgedrag naarmate kinderen ouder worden. Het viel op dat de genetische architectuur van sportgedrag sterk verandert met de leeftijd. *Absoluut gezien* verklaart de gedeelde omgeving ongeveer evenveel van de

verschillen tussen kinderen in sportgedrag over de leeftijden heen, maar nemen de verschillen die door genetische aanleg komen toe. Daardoor speelt *relatief gezien* de gedeelde omgeving bij kinderen een belangrijkere rol dan de genetische aanleg, maar krijgt de genetische aanleg een steeds grotere invloed met toenemende leeftijd, met name bij jongens.

In hoofdstuk 4 hebben we gebruik gemaakt van herhaalde metingen. Uit onze analyses bleek dat sportgedrag matig stabiel is tijdens de kindertijd, maar dat de stabiliteit toeneemt naarmate kinderen ouder worden. Een kind dat bijvoorbeeld op 7-jarige leeftijd sport, heeft een gematigde kans om nog steeds te sporten als het 10 jaar oud is. De kans dat een adolescent die op zijn 14^{e} sport dit twee jaar later nog steeds doet, is daarentegen groter. We zagen ook dat de kans om te blijven sporten daalde naarmate de tijd tussen twee metingen toenam. Als volgende stap hebben we onderzocht welke rol genen en de gedeelde omgeving bij deze stabiliteit van sportgedrag spelen. Genetische invloeden en gedeelde omgevingsinvloeden kunnen namelijk worden doorgegeven naar een volgende leeftijd (transmissie), of zij kunnen specifiek zijn voor een bepaalde leeftijd (innovatie). Uit onze analyses bleek dat er vooral bij jongens sprake was van genetische transmissie. Dit betekent dat er een set genen is die invloed heeft op meerdere leeftijden. Vooral bij meisjes speelden leeftijdsspecifieke genen ook een belangrijke rol. Dit verklaart waarom de erfelijkheid van sportgedrag bij jongens sterker toeneemt naarmate ze ouder worden dan bij meisjes. De gedeelde omgeving werd zowel bij jongens als bij meisjes vooral gekenmerkt door innovatie. Dit betekent dat verschillende elementen van de gedeelde omgeving op verschillende leeftijden een rol spelen.

De erfelijkheid van een eigenschap wordt vaak onterecht beschouwd als een vaststaand gegeven. De expressie van genen kan echter afhangen van de omgeving. Een bepaalde omgeving kan er bijvoorbeeld voor zorgen dat mensen zich meer volgens hun genetische aanleg gaan gedragen, waardoor er meer door genen veroorzaakte verschillen ontstaan in gedrag. Of deze verschillen kunnen juist worden onderdrukt. De omgeving waarin kinderen opgroeien, hangt onder andere af van het opleidingsniveau van hun ouders. In hoofdstuk 5 hebben we daarom onderzocht in hoeverre de bijdrage van genen en de omgeving verschilt tussen kinderen met minstens één hoog opgeleide ouder en kinderen waarvan beide ouders laag zijn opgeleid. We hebben ook gekeken of er een verschil is in het gemiddelde sportgedrag van deze kinderen. De studie is gebaseerd op data van het NTR en op data van twee Finse tweelingcohorten (FinnTwin12 en FinnTwin16). Bij het NTR werd sportgedrag gemeten rond de

leeftijden van 7, 10, 12, 14, 16 en 18 jaar; bij het FinnTwin12 cohort werden vragenlijsten verzameld rond de leeftijden van 12, 14 en 17 jaar; en bij het FinnTwin16 cohort werden zij verzameld rond de leeftijden van 16, 17 en 18 jaar. We hebben de data binnen iedere leeftijdsgroep van ieder cohort apart geanalyseerd.

We vonden dat kinderen met minstens één hoog opgeleide ouder meer sportten dan kinderen met twee laag opgeleide ouders. Bovendien waren de verschillen in sportgedrag tussen kinderen in de eerste groep kleiner, dus er was minder spreiding rond de gemiddeldes. Er was maar weinig bewijs voor een interactie tussen opleiding van de ouders en genetische invloeden. Alleen bij Nederlandse meisjes met minstens een hoog opgeleide ouder vonden we minder invloed van genetische effecten dan bij meisjes met twee laag opgeleide ouders. Omdat meisjes met minstens een hoog opgeleide ouder meer sportten, suggereert dit dat hoog opgeleide ouders de genetische invloeden die sportgedrag zouden belemmeren voor een deel kunnen onderdrukken.

Op basis van hoofdstukken 3 en 4 en eerder onderzoek concluderen we dat 1) er grote individuele verschillen zijn in sportgedrag bij kinderen en jongeren, 2) deze verschillen in de kindertijd vooral worden verklaard door gedeelde omgevingsinvloeden en 3) de verschillen in de adolescentie vooral worden verklaard door genetische invloeden. Mogelijk is bij kinderen vooral de invloed van ouders belangrijk en zijn individuele eigenschappen, zoals vaardigheid en trainbaarheid, de onmiddellijke psychologische reactie op sporten, een homeostatische behoefte om actief te zijn en persoonlijkheidskenmerken belangrijker bij adolescenten. Deze mogelijke invloeden worden nader toegelicht in de desbetreffende hoofdstukken. Deel 1 van dit proefschrift maakt inzichtelijk waarom er niet één interventie is die het sportgedrag van iedereen kan verhogen. Het is belangrijk dat interventies goed aansluiten bij de specifieke behoeftes van een individu. Dit houdt in dat rekening moet worden gehouden met verschillen in geslacht, leeftijd, sportvaardigheid, sportbeleving, maar ook - dat blijkt uit hoofdstuk 5 - omgevingsfactoren zoals ouderlijk opleidingsniveau.

HET VERBAND TUSSEN SPORTGEDRAG EN ANDERE EIGENSCHAPPEN

In deel 2 van dit proefschrift komt het verband tussen sportgedrag en andere eigenschappen aan bod. Als twee eigenschappen tegelijk optreden, dan wordt vaak onterecht aangenomen dat de ene eigenschap veroorzaakt wordt door de

andere. Er zijn twee methoden om de zogenaamde “causale hypothese” met behulp van tweelingdata te toetsen, dat wil zeggen de hypothese dat een eigenschap A de oorzaak is van een eigenschap B. Deze methoden worden in hoofdstuk 6 uitgelegd en toegepast.

Het achterliggende principe van de eerste methode luidt als volgt: Als A de oorzaak is van B, dan moeten alle factoren die A beïnvloeden, uiteindelijk ook B beïnvloeden. In het geval van tweelingonderzoek betekent dit dat de genen en de omgeving die eigenschap A beïnvloeden dus ook eigenschap B moeten beïnvloeden. Dit kan worden onderzocht met behulp van een bivariaat tweelingmodel.

De tweede methode is gebaseerd op verschillen bij een bepaalde eigenschap binnen eeneiige tweelingparen. Als een hogere score op eigenschap A bijvoorbeeld leidt tot een hogere score op eigenschap B, dan zou men ook bij eeneiige tweelingen verwachten dat een verschil in scores op eigenschap A (bijvoorbeeld tweeling 1 scoort hoger dan tweeling 2) samengaat met een verschil in scores op eigenschap B (tweeling 1 moet weer hoger scoren dan tweeling 2). Indien dit niet wordt gevonden, pleit dit voor een onderliggende set genen die een effect heeft op beide eigenschappen zonder dat er sprake is van causaliteit. De samenhang die op populatieniveau wordt gevonden verdwijnt dan namelijk bij eeneiige tweelingen omdat deze vrijwel identieke genen hebben.

In hoofdstuk 6 wordt de rol van iemands houding ten opzichte van sportgedrag nader onderzocht. Eerder onderzoek heeft aangetoond dat deze houding een voorspeller kan zijn van sportgedrag. Tegelijk weten we dat sportgedrag niet alleen bij kinderen, maar ook bij volwassenen, voor een deel erfelijk is. Om deze twee bevindingen met elkaar te verenigen, hebben we in eerste instantie onderzocht of de houding ten opzichte van sporten zelf erfelijk is. Met behulp van vragenlijsten die zijn verzameld bij volwassen tweelingen van het NTR en hun broers en zussen hebben we aangetoond dat het ervaren van de voor- en nadelen van sportgedrag inderdaad voor een deel kan worden verklaard door de genetische aanleg van een persoon, maar dat het grootste deel van de verschillen tussen mensen wordt verklaard door de unieke omgeving.

Vervolgens hebben we het verband onderzocht tussen iemands houding ten opzichte van sporten en zijn of haar sportgedrag. We vonden een samenhang tussen deze twee eigenschappen en deze samenhang lijkt te komen door een causaal effect, ook al konden we geen uitspraak doen over de richting van het

effect. Interventies die iemands houding beïnvloeden zouden dus een effect kunnen hebben op het sportgedrag van deze persoon. Aangezien deze houding voor een deel erfelijk is, is het echter belangrijk dat er rekening wordt gehouden met genetische verschillen tussen mensen. Sommige mensen zullen de voordelen van sportgedrag eerder inzien dan anderen.

In hoofdstuk 7 hebben we de samenhang onderzocht tussen sporten en body-mass index (BMI). BMI geeft de verhouding weer tussen lengte en gewicht bij een persoon. Mensen gaan er vaak van uit dat sporten leidt tot een lager lichaamsgewicht en dus een lager BMI. Opvallend genoeg vonden wij in een grote groep kinderen en jongeren van het NTR in de leeftijd van 7 tot 18 jaar geen samenhang tussen sportgedrag en BMI. Eerder onderzoek heeft deze samenhang ook niet duidelijk kunnen bevestigen. De bevindingen gaan namelijk vele kanten op, wat onder andere komt omdat eerdere studies heel verschillend waren opgezet en belangrijke beperkingen hadden. Onze studie was gebaseerd op herhaalde metingen, we hadden een grote steekproef en we hebben sportgedrag en BMI betrouwbaar gemeten - toch vonden we geen samenhang.

Hiervoor zijn er een aantal mogelijke verklaringen. Het is duidelijk dat sporten leidt tot een hoger energieverbruik, wat kan leiden tot minder vetopslag en dus een lager lichaamsgewicht. Er zijn echter een aantal andere factoren die een invloed hebben op lichaamsgewicht en die het verband kunnen laten verdwijnen, zoals de energie die het lichaam gebruikt voor de meest basale processen zoals zuurstofvoorziening en spijsvertering, dagelijkse fysieke lichaamsactiviteit zoals ergens naartoe fietsen of lopen, de tijd die iemand zittend doorbrengt, maar vooral ook voeding. Als mensen meer gaan eten zodra zij beginnen met sporten, zal het lichaamsgewicht nauwelijks veranderen.

Het feit dat er op het niveau van de totale bevolking geen sterke samenhang is tussen sporten en BMI betekent natuurlijk niet dat sporten geen andere positieve effecten kan hebben. Zelfs zonder enig effect op lichaamsgewicht, blijven er belangrijke effecten op de gezondheid en moeten we er naar streven om iedereen regelmatig te laten bewegen. Het helpt daarbij echter niet effecten te beloven die zich uiteindelijk niet voordoen, zoals het vaak gehoopte gewichtsverlies. Idealiter is het ooit mogelijk om mensen die het meest profiteren van sporten te identificeren aan de hand van hun genetisch profiel. Dan kunnen we teleurstelling voorkomen bij personen die minder profiteren van sporten en kunnen we schaarse middelen voor de volksgezondheid

optimaal inzetten.

SPECIFIEKE GENEN VOOR SPORTGEDRAG

Het is inmiddels duidelijk dat verschillen in sportgedrag tussen mensen voor een deel worden verklaard door verschillen in genetische aanleg. In het derde deel van dit proefschrift hebben we de moleculair genetische basis van deze verschillen nader onderzocht. We hebben daarbij gebruik gemaakt van kinderen, adolescenten en volwassen deelnemers van het NTR die op een vragenlijst hebben aangegeven hoeveel zij sporten en hun DNA hebben afgestaan. Dit maakt het mogelijk om bepaalde genen te relateren aan sportgedrag.

We hebben ervoor gekozen om een set genen te onderzoeken die te maken heeft met het ervaren van beloning. We gaan er namelijk van uit dat een deel van de erfelijkheid van sportgedrag komt door genen die de psychologische reactie op sporten beïnvloeden. Mensen herhalen gedrag dat hen een positief gevoel geeft - en zij vermijden gedrag dat niet goed aanvoelt. Het ervaren van beloning wordt onder andere beïnvloed door de neurotransmitter dopamine. Neurotransmitters zijn stofjes in de hersenen die informatie overdragen tussen zenuwcellen. Eerder dieronderzoek heeft aangetoond dat dopamine gerelateerd is aan beweeggedrag. Met de grootste steekproef tot nu toe wilden we aantonen dat verschillen tussen mensen in genen die betrokken zijn bij dopamine leiden tot verschillen in sportgedrag. We vonden echter dat er geen samenhang was.

Ook in eerder onderzoek werd niet één genetische variant gevonden die duidelijk gerelateerd is aan sportgedrag. Het is dus heel moeilijk om de genetische basis van sportgedrag te begrijpen. Dit komt omdat er niet één gen is met een sterk effect op sportgedrag. In plaats daarvan zijn er heel veel genen die allemaal een klein effect hebben. Deze kleine effecten kunnen alleen met enorm grote steekproeven worden opgepikt. Het is ontzettend kostbaar om deze steekproeven te verzamelen en de financiële middelen daarvoor kan een enkel onderzoeksinstituut niet alleen opbrengen. Daarom is er nu een groot samenwerkingsproject gaande tussen een aantal instituten, waaronder het NTR, met het doel om de grootst mogelijke dataset met zowel informatie over sportgedrag als over DNA samen te voegen en te analyseren. Dit zal hopelijk meer inzicht leveren in de moleculaire basis van de erfelijkheid van sportgedrag.

TOEKOMSTIG ONDERZOEK

Dit proefschrift biedt inzichten in de complexiteit van factoren die verschillen tussen mensen in sportgedrag bepalen. Vooral tijdens de kindertijd en adolescentie zien we grote veranderingen van de genetische architectuur over de tijd. Het is nu vrij duidelijk, hoeveel van de verschillen tussen mensen in sportgedrag wordt verklaard door genen, de gedeelde omgeving en de unieke omgeving. Toekomstig onderzoek zal moeten achterhalen welke specifieke factoren hieraan ten grondslag liggen.

Het feit dat sportgedrag voor een deel erfelijk is, betekent dat het voor sommigen makkelijker is dan voor anderen om regelmatig te gaan sporten. Het betekent *niet* dat iemand met een ongunstige genetische achtergrond nooit zal sporten! Als we aangeboren verschillen tussen mensen beter begrijpen en accepteren, dan kunnen we uiteindelijk succesvollere interventies ontwerpen die zijn toegesneden op de specifieke behoeftes en vermogens van een individu. Hierbij moet er ook rekening worden houden met genetische verschillen in de *effecten* van sportgedrag voor een realistische inschatting van de beoogde uitkomst.

Education and training

COURSES

Bachelor's courses

Behavior genetics (6 ECTS)
Molecular genetics (6 ECTS)

Master's courses

Behavioral genetics (6 ECTS)
Quantitative genetics (5 ECTS)
Statistical genetics for gene finding (5 ECTS)
LISREL structural equation modelling (6 ECTS)
Presenting in English (3 ECTS)
Ethics in the life sciences (3 ECTS)

PhD courses or higher

Programming in R (2 ECTS)
"The 2012 International Workshop on Statistical Genetics and Methodology of Twin and Family Studies: The Introductory Course" in Boulder, Colorado, U.S. (2.5 ECTS)
"The 2015 International Workshop on Statistical Genetic Methods for Human Complex Traits" in Boulder, Colorado, U.S. (2.5 ECTS)
Supervising and grading theses and internships (1 ECTS)
Data management
PhD success and personal efficacy (2 ECTS)

GUEST LECTURES

November 2011	Loughborough University, England
June 2014	Pennington Biomedical Research Center, Louisiana, U.S.
October 2014	University of Helsinki, Finland
October 2014	UKK Institute, Tampere, Finland
October 2014	University of Jyväskylä, Finland

TEACHING

March 2014	Teaching assistant for the course "Methodology III"
2014-2015	Supervision of two Bachelor theses
May 25-29, 2015	Faculty member at the workshop "Genetic Modeling using Twin and Family Data" for doctoral students and post-doctoral researchers, University of Helsinki

List of publications

ARTICLES

Huppertz C, Bartels M, de Geus EJC, van Beijsterveldt CEM, Rose RJ, Kaprio J & Silventoinen K (under review). The effects of parental education on exercise behavior in childhood and youth: A study in Dutch and Finnish twins.

Huppertz C, Bartels M, de Zeeuw EL, van Beijsterveldt CEM, Hudziak JJ, Willemsen G, Boomsma DI & de Geus EJC (under review). Individual differences in exercise behavior: Stability and change in genetic and environmental determinants from age 7 to 18.

Huppertz C, Bartels M, van Beijsterveldt CEM, Willemsen G, Hudziak JJ & de Geus EJC (2015). Regular exercise behavior in youth is not related to current body mass index or body mass index at 7-year follow-up. *Obesity Science & Practice, 1*(1), 1-11.

Huppertz C, Bartels M, Groen-Blokhuis M, de Moor MHM, van der Aa N, Abdellaoui A, van Beijsterveldt CEM, Ehli E, Hottenga JJ, Willemsen G, Xiao X, Scheet P, Davies G, Boomsma DI, Hudziak J & de Geus EJC (2014). The dopaminergic reward system and leisure time exercise behavior: A candidate allele study. *BioMed Research International,* doi: 10.1155/2014/591717.

Huppertz C, Bartels M, Jansen IE, Boomsma DI, Willemsen G, de Moor MHM & de Geus EJC (2014). A twin-sibling study on the relationship between exercise attitudes and exercise behavior. *Behavior Genetics, 44*(1), 45-55.

Huppertz C, Bartels M, van Beijsterveldt CEM, Boomsma DI, Hudziak JJ & de Geus EJC (2012). Effect of shared environmental factors on exercise behavior from age 7 to 12. *Medicine & Science in Sports & Exercise, 44*(10), 2025-2032.

Minica CC, Dolan CV, Hottenga JJ, Pool R, The Genome of the Netherlands Consortium, Fedko IO, Mbarek H, **Huppertz C**, Bartels M, Boomsma DI & Vink JM (2015). Heritability, SNP- and gene-based analyses of cannabis use initiation and age at onset. *Behavior Genetics, 45*(5), 503-513.

Vink JM, van Beijsterveldt CEM, **Huppertz C**, Bartels M & Boomsma DI (2015). Heritability of compulsive internet use in adolescents. *Addiction Biology, 21*(2), 460-468.

Treur JL, Willemsen G, Bartels M, Geels LM, van Beek JH, **Huppertz C**, van Beijsterveldt CEM, Boomsma DI & Vink JM (2014). Smoking during adolescence as a risk factor for attention problems. *Biological*

Psychiatry, 78(9), 656-663.

van Beijsterveldt CE, Groen-Blokhuis M, Hottenga JJ, Franić S, Hudziak JJ, Lamb D, **Huppertz C**, (…) & Boomsma DI (2013). The Young Netherlands Twin Register (YNTR): Longitudinal twin and family studies in over 70,000 children. *Twin Research and Human Genetics, 16*(1), 252-267.

PUBLISHED ABSTRACTS

Huppertz C, Bartels M, van Beijsterveldt CEM, Willemsen G, Boomsma DI, Hudziak JJ & de Geus EJC (2015, June). Exercise behavior in childhood and adolescence: Age-moderation and temporal stability. Oral presentation at the **45rd Annual Meeting of the Behavior Genetics Association (BGA)**, San Diego, California.

Huppertz C, Bartels M, Groen-Blokhuis M, de Moor MHM, van der Aa N, Abdellaoui A, van Beijsterveldt CEM, Ehli E, Hottenga JJ, Willemsen G, Xiao X, Scheet P, Davies G, Boomsma DI, Hudziak J & de Geus EJC (2014, May). The dopaminergic reward system and leisure time exercise behavior: A candidate allele study. Oral presentation at the **61rst Annual Meeting of the American College of Sports Medicine (ACSM)**, Orlando, Florida.

Huppertz C, Bartels M, van Beijsterveldt CEM, Willemsen G, Boomsma DI, Hudziak JJ & de Geus EJC (2014, May). The association between exercise behavior and body mass index throughout childhood and adolescence. Poster presentation at the **5th World Congress on Exercise is Medicine (EIM)**, Orlando, Florida.

Huppertz C, Bartels M, Groen-Blokhuis M, de Moor MHM, van der Aa N, Abdellaoui A, van Beijsterveldt CEM, Ehli E, Hottenga JJ, Willemsen G, Xiao X, Scheet P, Davies G, Boomsma DI, Hudziak J & de Geus EJC (2014, March). The dopaminergic reward system and leisure time exercise behavior: A candidate allele study. Poster presentation at the **VU University Medical Center Science Exchange Day**, Amsterdam.

Huppertz C, Bartels M, van Beijsterveldt CEM, Willemsen G, Boomsma DI, Hudziak JJ & de Geus EJC (2013, July). The longitudinal relationship between leisure time exercise behavior and body mass index in 7- to 18-year old twins. Oral presentation at the **43rd Annual Meeting of the Behavior Genetics Association (BGA)**, Marseille, France.

Huppertz C, Bartels M, Jansen IE, Boomsma DI, Willemsen G, de Moor MHM & de Geus, EJC (2013, March). Do exercise attitudes cause exercise behavior? A genetic perspective. Poster presentation at the **VU University Medical Center Science Exchange Day**, Amsterdam.

Huppertz C, Bartels M, Jansen IE, Boomsma DI, Willemsen G, de Moor MHM & de Geus EJC (2012, July). The association between exercise attitudes and exercise behavior partly reflects genetic effects underlying both attitudes and behavior. Oral presentation at the **17th Annual Congress of the European College of Sport Science (ECSS)**, Bruges, Belgium.

Huppertz C, Bartels M, van Beijsterveldt CEM, Boomsma DI, Hudziak JJ & de Geus EJC (2012, July). The shared environment explains individual differences in children's exercise behavior: A twin study. Poster presentation at the **17th Annual Congress of the European College of Sport Science (ECSS)**, Bruges, Belgium.

Huppertz C, Bartels M, van Beijsterveldt CEM, Boomsma DI, Hudziak JJ & de Geus EJC (2012, March). The shared environment explains individual differences in children's exercise behavior: A twin study. Poster presentation at the **VU University Medical Center Science Exchange Day**, Amsterdam.

Huppertz C, Bartels M, van Beijsterveldt CEM, Boomsma DI, Hudziak JJ & de Geus EJC (2011, October). Children's exercise behavior in the Netherlands: Prevalence, heritability, and tracking over time. Oral presentation at the **7th Annual Meeting of HEPA Europe, the European Network for the Promotion of Health-Enhancing Physical Activity (HEPA)**, Amsterdam.

Appendices

APPENDIX I (p.239-254):
Items assessing exercise behavior in children, parents and adolescents

APPENDIX II (p.255-271):
Items assessing traits related to exercise behavior and physical activity in children and adolescents

APPENDIX III (p.272-274):
Example of personalized feedback to the participants

APPENDIX I:
Items assessing exercise behavior in children, parents and adolescents

Parental report (Surveys 7-12)

Survey 7 and survey 10 were identical for the versions 1-3 and slightly different from each other later on. Versions 1 and 2 did not contain items on exercise behavior. These items were also not included in versions 1 and 2 of survey 12. The following data on exercise behavior are available for each survey:

Survey(s) and version	Children*	Parents*
Survey 7 and survey 10, version 3, collected between 2005 and 2008 (Lijst7-10_versie3_ouders_oranje-grijs Boekje_2005.pdf)	YES (1)	YES (1)
Survey 7, version 4, collected between 2010 and 2012 (Lijst7_versie1_ouders_druk1_2010.pdf)	YES (2)	YES (2)
Survey 7, version 5, collected between 2012 and 2013 (Lijst7_versie2_ouders_druk1_2012.pdf)	YES (2)	YES (2)
Survey 10, version 4, collected between 2009 and 2013 (Lijst9_versie1_Ouders1lijst_2009.pdf)	YES (2)	YES (2)
Survey 12, version 3, collected between 1999 and 2004 (Lijst12_versie3_ouders_paarsBoekje_2000.pdf)	YES (3)	NO
Survey 12, version 4, collected between 2003 and 2005 (Lijst12_versie4a_ouders_geelBoekje_2003.pdf)	YES (4)	NO
Survey 12, version 5, collected between 2004 and 2006 (Lijst12_versie4b_ouders_geelBoekje_verbeteringSportvraag.pdf)	YES (5)	NO
Survey 12, version 6, collected between 2006 and 2009 (Lijst12_versie5_ouders_geel-grijsBoekje_2006.pdf)	YES (6)	YES (1)
Survey 12, version 7, collected between 2008 and 2013 (Lijst12_versie6_ouders1Lijst_druk2_2009.pdf)	YES (2)	YES (3)

**Different wordings of the questions/ response options are indicated in brackets, see following pages.*

Children's exercise behavior

(1)

Omcirkel hieronder het cijfer bij de sport(en) die de oudste en de jongste van de tweeling **op dit moment** beoefent. Geef aan hoeveel jaar, hoeveel maanden per jaar, hoeveel keer per week en hoe lang ze per keer deze sport(en) beoefenen.

	sport		**aantal jaar**		**aantal maanden per jaar**		**aantal keren per week**		**gemiddelde tijd per keer in minuten**	
	o	**j**	**o**	**j**	**o**	**j**	**o**	**j**	**o**	**j**
schoolgym	**1**	**1**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schoolzwemmen	**2**	**2**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
atletiek	**3**	**3**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
badminton	**4**	**4**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
ballet/ dansen	**5**	**5**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
basketbal	**6**	**6**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
conditietraining/ fitness	**7**	**7**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
gymnastiek/ turnen	**8**	**8**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
handbal	**9**	**9**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hardlopen/ joggen	**10**	**10**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hockey	**11**	**11**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
korfbal	**12**	**12**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
paardrijden	**13**	**13**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schaatsen/ skaten	**14**	**14**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
tennis	**15**	**15**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
vechtsport	**16**	**16**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
voetbal	**17**	**17**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
zwemmen	**18**	**18**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
volleybal	**19**	**19**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl:	**20**	**20**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min

**o=oudste, j=jongste.*

(2)

Wilt u hieronder invullen welke sport(en) het kind **op dit moment** beoefent? Geef aan hoeveel jaar, hoeveel maanden per jaar, hoeveel keer per week en hoe lang ze per keer deze sport(en) beoefenen.

	sport		**aantal jaar**		**aantal maanden per jaar**		**aantal keren per week**		**gemiddelde tijd per keer in minuten**	
	j	**o**	**j**	**o**	**j**	**o**	**j**	**o**	**j**	**o**
ballet	**1**	**1**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hockey	**2**	**2**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
paardrijden	**3**	**3**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
tennis	**4**	**4**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
voetbal	**5**	**5**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
volleybal	**6**	**6**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schoolgym	**7**	**7**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
gym/ turnen	**8**	**8**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schoolzwemmen	**9**	**9**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
zwemmen buiten school	**10**	**10**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl:	**11**	**11**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl:	**12**	**12**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min

**o=oudste, j=jongste.*

(3)

Welke sporten beoefent de tweeling? Hoeveel uur per week beoefenen ze die sporten?

OUDSTE:	.....	..... uur per week
	.....	..... uur per week
	.....	..... uur per week
JONGSTE:	.....	..... uur per week
	.....	..... uur per week
	.....	..... uur per week

(4)

Omcirkel hieronder het cijfer bij de sport(en) die de oudste en de jongste van de tweeling beoefent. Geef aan hoeveel jaar, hoeveel maanden per jaar, hoe vaak per maand en hoe lang ze gemiddeld per week deze sport(en) beoefenen. Tel de tijd van de trainingen en wedstrijden bij elkaar op.

	sport		**aantal jaar**		**aantal maanden per jaar**		**aantal keer per maand**		**gemiddelde tijd per keer in minuten**	
	o	**j**	**o**	**j**	**o**	**j**	**o**	**j**	**o**	**j**
schoolgym	**1**	**1**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schoolzwemmen	**2**	**2**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
atletiek	**3**	**3**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
badminton	**4**	**4**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
ballet/ dansen	**5**	**5**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
basketbal	**6**	**6**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
conditietraining/ fitness	**7**	**7**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
gymnastiek/ turnen	**8**	**8**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
handbal	**9**	**9**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hardlopen/ joggen	**10**	**10**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hockey	**11**	**11**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
korfbal	**12**	**12**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
paardrijden	**13**	**13**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schaatsen/ skaten	**14**	**14**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
tennis	**15**	**15**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
vechtsport	**16**	**16**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
voetbal	**17**	**17**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
zwemmen	**18**	**18**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl: …….	**19**	**19**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl: …….	**20**	**20**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min

**o=oudste, j=jongste.*

(5)

Omcirkel hieronder het cijfer bij de sport(en) die de oudste en de jongste van de tweeling **op dit moment** beoefent. Geef aan hoeveel jaar, hoeveel maanden per jaar, hoeveel keer per maand en hoe lang ze per keer deze sport(en) beoefenen.

	sport		**aantal jaar**		**aantal maanden per jaar**		**aantal keer per maand**		**gemiddelde tijd per keer in minuten**	
	o	**j**	**o**	**j**	**o**	**j**	**o**	**j**	**o**	**j**
schoolgym	**1**	**1**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schoolzwemmen	**2**	**2**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
atletiek	**3**	**3**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
badminton	**4**	**4**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
ballet/ dansen	**5**	**5**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
basketbal	**6**	**6**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
conditietraining/ fitness	**7**	**7**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
gymnastiek/ turnen	**8**	**8**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
handbal	**9**	**9**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hardlopen/ joggen	**10**	**10**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hockey	**11**	**11**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
korfbal	**12**	**12**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
paardrijden	**13**	**13**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schaatsen/ skaten	**14**	**14**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
tennis	**15**	**15**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
vechtsport	**16**	**16**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
voetbal	**17**	**17**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
zwemmen	**18**	**18**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl: …….	**19**	**19**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl: …….	**20**	**20**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min

**o=oudste, j=jongste.*

(6)

Kruis hieronder het vakje aan bij de sport(en) die de oudste en de jongste van de tweeling **op dit moment** beoefent. Geef aan hoeveel jaar, hoeveel maanden per jaar, hoeveel keer per week en hoe lang ze per keer deze sport(en) beoefenen.

	sport		**aantal jaar**		**aantal maanden per jaar**		**aantal keren per week**		**gemiddelde tijd per keer in minuten**	
	o	**j**	**o**	**j**	**o**	**j**	**o**	**j**	**o**	**j**
schoolgym	**1**	**1**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schoolzwemmen	**2**	**2**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
atletiek	**3**	**3**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
badminton	**4**	**4**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
ballet/ dansen	**5**	**5**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
basketbal	**6**	**6**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
conditietraining/ fitness	**7**	**7**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
gymnastiek/ turnen (geen schoolgym)	**8**	**8**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
handbal	**9**	**9**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hardlopen/ joggen	**10**	**10**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
hockey	**11**	**11**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
korfbal	**12**	**12**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
paardrijden	**13**	**13**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
schaatsen/ skaten	**14**	**14**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
tennis	**15**	**15**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
vechtsport	**16**	**16**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
voetbal	**17**	**17**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
zwemmen (geen schoolzwemmen)	**18**	**18**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
volleybal	**19**	**19**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min
anders, nl:	**20**	**20**	..jaar	..jaar	..mnd	..mnd	..keer	..keer	..min	..min

**o=oudste, j=jongste.*

Parents' exercise behavior

(1)

Doet u regelmatig aan sport?

1 nee
2 ja

Wilt u hieronder invullen welke sport(en) u beoefent? Geef per sport aan hoeveel jaren u deze al beoefent, hoeveel maanden per jaar, hoeveel keer per week u de sport beoefent en hoelang u gemiddeld per keer deze sport(en) beoefent.

	naam van de sport	**aantal jaren**	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer**
a.	.....	..... jaren	..... maanden	..... keer	..... minuten
b.	.....	..... jaren	..... maanden	..... keer	..... minuten
c.	.....	..... jaren	..... maanden	..... keer	..... minuten
d.	.....	..... jaren	..... maanden	..... keer	..... minuten
e.	.....	..... jaren	..... maanden	..... keer	..... minuten

(2)

Doet u regelmatig aan sport?

1 nee
2 ja

Wilt u hieronder invullen welke sport(en) u beoefent? Geef per sport aan hoeveel jaren u deze al beoefent, hoeveel maanden per jaar, hoeveel keer per week u de sport beoefent en hoe lang u gemiddeld per keer deze sport beoefent.

	naam van de sport	**aantal jaren**	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer**
a.	.....	..... jaren	..... maanden	..... keer	..... minuten
b.	.....	..... jaren	..... maanden	..... keer	..... minuten
c.	.....	..... jaren	..... maanden	..... keer	..... minuten

(3)

Doet u regelmatig aan sport?

1 nee
2 ja

Wilt u hieronder invullen welke sport(en) u beoefent? Geef per sport aan hoeveel jaren u deze al beoefent, hoeveel maanden per jaar, hoeveel keer per week u de sport beoefent en hoelang u gemiddeld per keer deze sport(en) beoefent.

	naam van de sport	**aantal jaren**	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer**
a.	.....	..... jaren	..... maanden	..... keer	..... minuten
b.	.....	..... jaren	..... maanden	..... keer	..... minuten
c.	.....	..... jaren	..... maanden	..... keer	..... minuten

Self-report (DHBQ14-18)

DHBQ14, 16 and 18 were identical for the pilot study and versions 1 and 2. DHBQ14 and 16 were slightly different from each other later on. DHBQ18 was not collected after version 2. The following data are available for each survey:

Survey(s) and version	**Exercise behavior***
DHBQ 14, 16, and 18, pilot, collected in 2004 (DHBQ_14-16-18_pilot_2004.pdf)	YES (1)
DHBQ 14, 16, and 18, version 1, collected between 2004 and 2006 (DHBQ_14-16-18_versie1_rood-grijsBoekje.pdf)	YES (2)
DHBQ 14, 16, and 18, version 2, collected between 2005 and 2009 (DHBQ_14-16-18_versie2_blauwBoekje_druk1.pdf)	YES (2)
DHBQ 14, version 3, collected between 2008 and 2009 (DHBQ14_versie3_ONLINE_2009.pdf)	YES (3)
DHBQ 16, version 3, collected between 2009 and 2010 (DHBQ16_versie3_ONLINE_2009.pdf)	YES (4)
DHBQ 14 & 16, version 4, collected in 2011 (DHBQ14_versie4_ONLINE_2011.pdf; DHBQ16_versie4_ONLINE_2011.pdf)	YES (5)
DHBQ 14 & 16, version 5, collected between 2011 and 2012 (DHBQ14_versie5_ONLINE_2012.pdf; DHBQ16_versie5_ONLINE_2012.pdf)	YES (6)
DHBQ 14 & 16, version 6, collected in 2013 (DHBQ14_versie6_ONLINE_2013.pdf; DHBQ16_versie6_ONLINE_2013.pdf)	YES (7)

**Different wordings of the questions/ response options are indicated in brackets, see following pages.*

(1)

Doe je regelmatig aan sport?

1 nee
2 ja

Kruis hieronder aan welke sport(en) je **tegenwoordig** beoefent.
Geef aan hoeveel jaren, hoeveel maanden per jaar, hoe vaak per maand en hoelang je gemiddeld per week deze sport(en) beoefent. Tel de tijd van de trainingen en wedstrijden bij elkaar op.

naam van de sport		**aantal jaren**	**aantal maanden per jaar**	**aantal keren per maand**	**gemiddelde tijd per week in minuten**
ik doe op dit moment niet aan sport	**0**				
schoolgym	**1**	.. jaren	.. mnd	.. keer	.. min
schoolzwemmen	**2**	.. jaren	.. mnd	.. keer	.. min
atletiek	**3**	.. jaren	.. mnd	.. keer	.. min
badminton	**4**	.. jaren	.. mnd	.. keer	.. min
ballet/ dansen	**5**	.. jaren	.. mnd	.. keer	.. min
basketbal	**6**	.. jaren	.. mnd	.. keer	.. min
conditietraining/ fitness	**7**	.. jaren	.. mnd	.. keer	.. min
gymnastiek/turnen	**8**	.. jaren	.. mnd	.. keer	.. min
handbal	**9**	.. jaren	.. mnd	.. keer	.. min
hardlopen/ joggen	**10**	.. jaren	.. mnd	.. keer	.. min
hockey	**11**	.. jaren	.. mnd	.. keer	.. min
korfbal	**12**	.. jaren	.. mnd	.. keer	.. min
paardrijden	**13**	.. jaren	.. mnd	.. keer	.. min
schaatsen/ skaten	**14**	.. jaren	.. mnd	.. keer	.. min
tennis	**15**	.. jaren	.. mnd	.. keer	.. min
vechtsport	**16**	.. jaren	.. mnd	.. keer	.. min
voetbal	**17**	.. jaren	.. mnd	.. keer	.. min
zwemmen	**18**	.. jaren	.. mnd	.. keer	.. min
anders, nl …….	**19**	.. jaren	.. mnd	.. keer	.. min

(2)

Doe je regelmatig aan sport?

1 nee

2 ja

Kruis hieronder aan welke sport(en) je **op dit moment** beoefent. Geef aan hoeveel jaren, hoeveel maanden per jaar, hoe vaak per week en hoelang je per keer deze sport(en) beoefent.

naam van de sport		**aantal jaren**	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer**
ik doe op dit moment niet aan sport	**0**				
schoolgym	**1**	.. jaren	.. mnd	.. keer	.. min
schoolzwemmen	**2**	.. jaren	.. mnd	.. keer	.. min
atletiek	**3**	.. jaren	.. mnd	.. keer	.. min
badminton	**4**	.. jaren	.. mnd	.. keer	.. min
ballet/ dansen	**5**	.. jaren	.. mnd	.. keer	.. min
basketbal	**6**	.. jaren	.. mnd	.. keer	.. min
conditietraining/ fitness	**7**	.. jaren	.. mnd	.. keer	.. min
Gymnastiek (geen schoolgym)	**8**	.. jaren	.. mnd	.. keer	.. min
handbal	**9**	.. jaren	.. mnd	.. keer	.. min
hardlopen/ joggen	**10**	.. jaren	.. mnd	.. keer	.. min
hockey	**11**	.. jaren	.. mnd	.. keer	.. min
judo	**12**	.. jaren	.. mnd	.. keer	.. min
korfbal	**13**	.. jaren	.. mnd	.. keer	.. min
paardrijden	**14**	.. jaren	.. mnd	.. keer	.. min
schaatsen/ skaten	**15**	.. jaren	.. mnd	.. keer	.. min
tennis	**16**	.. jaren	.. mnd	.. keer	.. min
turnen	**17**	.. jaren	.. mnd	.. keer	.. min
vechtsport	**18**	.. jaren	.. mnd	.. keer	.. min
voetbal	**19**	.. jaren	.. mnd	.. keer	.. min
volleybal	**20**	.. jaren	.. mnd	.. keer	.. min
zwemmen (geen schoolzwemmen)	**21**	.. jaren	.. mnd	.. keer	.. min
anders, nl …….	**22**	.. jaren	.. mnd	.. keer	.. min

(3)

Doe je regelmatig aan sport?

1 nee
2 ja

Klik hieronder aan welke sport(en) je **op dit moment** regelmatig beoefent buiten de gymnastieklessen op school.

Bij eerste vervolgvraag
Hieronder staan de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Geef aan hoeveel jaren, hoeveel maanden per jaar, hoe vaak per week en hoelang je per keer deze sport(en) beoefent.

Bij tweede vervolgvraag
Hieronder staan nogmaals de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Klik hieronder het niveau aan waarop je deze sport beoefent in vergelijking met je leeftijdgenoten: **plezier**= ik sport alleen voor mijn plezier, **competitie**= ik speel competitie bij een club of sportschool, **selectie**= ik zit in de hoogste groep of het selectieteam van mijn club of sportschool, **regionaal**= ik zit in de regionale selectie, **nationaal**= ik zit in de nationale selectie (bijv. (Jong) Oranje).

naam van de sport		**aantal jaren**	**aantal maan-den per jaar**	**aantal keren per week**	**gemiddel-de tijd per keer**	plezier	competitie	selectie	regionaal	nationaal
fitness/ conditie-training	**1**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hardlopen/ joggen	**2**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hockey	**3**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
paardrijden	**4**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
tennis	**5**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
voetbal	**6**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
volleybal	**7**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
zwemmen	**8**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
ballet	**9**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**10**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**11**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**12**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**13**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**14**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**

(4)

Doe je regelmatig aan sport?

1 nee

2 ja

Vul hieronder in welke sport(en) je **op dit moment** regelmatig beoefent buiten de gymnastieklessen op school. Geef aan hoeveel jaren, hoeveel maanden per jaar, hoe vaak per week en hoelang je per keer deze sport(en) beoefent. Kruis ook het niveau aan waarop je deze sport beoefent in vergelijking met je leeftijdgenoten: **plezier**= ik sport alleen voor mijn plezier, **competitie**= ik speel competitie bij een club of sportschool, **selectie**= ik zit in de hoogste groep of het selectieteam van mijn club of sportschool, **regionaal**= ik zit in de regionale selectie, **nationaal**= ik zit in de nationale selectie (bijv. (Jong) Oranje).

naam van de sport		**aantal jaren**	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer**	plezier	competitie	selectie	regionaal	nationaal
fitness/ conditie-training	**1**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hardlopen/ joggen	**2**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hockey	**3**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
paardrijden	**4**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
tennis	**5**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
voetbal	**6**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
volleybal	**7**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
zwemmen	**8**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
ballet	**9**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**10**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**11**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**12**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**13**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**14**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**

(5)

Doe je regelmatig aan sport?

1 nee

2 ja

Klik hieronder aan welke sport(en) je **op dit moment** regelmatig beoefent buiten de gymnastieklessen op school.

Bij eerste vervolgvraag

Hieronder staan de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Geef aan hoeveel jaren, hoeveel maanden per jaar, hoe vaak per week en hoelang je per keer deze sport(en) beoefent.

Bij tweede vervolgvraag

Hieronder staan nogmaals de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Klik hieronder het hoogste niveau aan waarop je deze sport beoefent: **plezier**= ik sport alleen voor mijn plezier, **competitie**= ik speel competitie bij een club of sportschool, **selectie**= ik zit in de hoogste groep of het selectieteam van mijn club of sportschool, **regionaal**= ik zit in de regionale selectie, **nationaal**= ik zit in de nationale selectie (bijv. (Jong) Oranje).

naam van de sport		**aantal jaren**	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer**	plezier	competitie	selectie	regionaal	nationaal
fitness/ conditie-training	**1**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hardlopen/ joggen	**2**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hockey	**3**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
paardrijden	**4**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
tennis	**5**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
voetbal	**6**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
volleybal	**7**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
zwemmen	**8**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
ballet	**9**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**10**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**11**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**12**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**13**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**14**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**

(6)

Doe je regelmatig aan sport?

1 nee

2 ja

Klik hieronder aan welke sport(en) je **op dit moment** regelmatig beoefent buiten de gymnastieklessen op school.

Bij eerste vervolgvraag

Hieronder staan de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Geef aan hoeveel jaren, hoeveel maanden per jaar, hoe vaak per week en hoelang je per keer deze sport(en) beoefent.

Bij tweede vervolgvraag

Hieronder staan nogmaals de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Klik hieronder het hoogste niveau aan waarop je deze sport op dit moment beoefent: **plezier**= ik sport alleen voor mijn plezier, **competitie**= ik speel competitie bij een club of sportschool, **selectie**= ik zit in de hoogste groep of het selectieteam van mijn club of sportschool, **regionaal**= ik zit in de regionale selectie, **nationaal**= ik zit in de nationale selectie (bijv. (Jong) Oranje).

		aantal jaren	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer**	plezier	competitie	selectie	regionaal	nationaal
fitness/ conditie-training	**1**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hardlopen/ joggen	**2**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hockey	**3**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
paardrijden	**4**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
tennis	**5**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
voetbal	**6**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
volleybal	**7**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
zwemmen	**8**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
ballet	**9**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**10**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**11**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**12**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**13**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**14**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**

(7)

Doe je regelmatig aan sport?

1 nee

2 ja

Klik hieronder aan welke sport(en) je **op dit moment** regelmatig beoefent buiten de gymnastieklessen op school. Je kunt meerdere sporten aanklikken.

Bij eerste vervolgvraag

Hieronder staan de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Geef aan hoeveel jaren, hoeveel maanden per jaar, hoe vaak per week en hoelang je per keer deze sport(en) beoefent.

Bij tweede vervolgvraag

Hieronder staan nogmaals de sporten waarvan jij hebt aangegeven dat je ze op dit moment beoefent. Klik hieronder het hoogste niveau aan waarop je deze sport op dit moment beoefent: **plezier**= ik sport alleen voor mijn plezier, **competitie**= ik speel competitie bij een club of sportschool, **selectie**= ik zit in de hoogste groep of het selectieteam van mijn club of sportschool, **regionaal**= ik zit in de regionale selectie, **nationaal**= ik zit in de nationale selectie (bijv. (Jong) Oranje).

		aantal jaren	**aantal maanden per jaar**	**aantal keren per week**	**gemiddelde tijd per keer in minuten**	plezier	competitie	selectie	regionaal	nationaal
fitness/ conditie-training	**1**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hardlopen/ joggen	**2**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
hockey	**3**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
paardrijden	**4**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
tennis	**5**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
voetbal	**6**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
volleybal	**7**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
zwemmen	**8**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
ballet	**9**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**10**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**11**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**12**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**13**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**
anders, nl ..	**14**	.. jaren	.. mnd	.. keer	.. min	**1**	**2**	**3**	**4**	**5**

APPENDIX II:
Items assessing traits related to exercise behavior and physical activity in children and adolescents

Parental report (Survey 7-12)

Survey 7 and survey 10 were identical for the version 1-3 and slightly different from each other later on. Versions 1 and 2 did not contain items on exercise-related traits. Versions 1, 2 and 3 of survey 12 did not contain those items either.

The following data are available for each survey:

Survey(s) and version	Active * transport	Perceived ability *	Level *
Survey 7 and survey 10, version 3, collected between 2005 and 2008 (Lijst7-10_versie3_ouders_oranje-grijs Boekje_2005.pdf)	YES (1)	NO	NO
Survey 7, version 4, collected between 2010 and 2012 (Lijst7_versie1_ouders_druk1_2010.pdf)	YES (2)	YES (1)	YES (1)
Survey 7, version 5, collected between 2012 and 2013 (Lijst7_versie2_ouders_druk1_2012.pdf)	YES (2)	YES (1)	YES (1)
Survey 10, version 4, collected between 2009 and 2013 (Lijst9_versie1_Ouders1lijst_2009.pdf)	YES (2)	YES (2)	YES (2)
Survey 12, version 4, collected between 2003 and 2005 (Lijst12_versie4a_ouders_geelBoekje_2003.pdf)	YES (3)	NO	NO
Survey 12, version 5, collected between 2004 and 2006 (Lijst12_versie4b_ouders_geelBoekje_verbeteringSportvraag.pdf)	YES (3)	NO	NO
Survey 12, version 6, collected between 2006 and 2009 (Lijst12_versie5_ouders_geel-grijsBoekje_2006.pdf)	YES (1)	NO	NO
Survey 12, version 7, collected between 2008 and 2013 (Lijst12_versie6_ouders1Lijst_druk2_2009.pdf)	YES (2)	YES (2)	YES (2)

**Different wordings of the questions/ response options are indicated in brackets, see following pages.*

English translation of the corresponding questions:

Active transport:
How does the twin usually go to school?
→ walking, cycling, public transport, car...
How long does it take the twin to go to school?

Perceived ability:
How good is the twin at sports and physical exercise compared to peers?
How good is the twin's physical endurance compared to peers?
How good is the twin's muscle strength compared to peers?

Level:
At what level does the twin perform his or her main exercise activity/-ies?
→ "for fun" up to "national selection"

Active transport

(1)

Hoe gaat de tweeling **meestal** naar school?		**OUDSTE**	**JONGSTE**
a.	lopend	**1**	**1**
b.	op de fiets	**2**	**2**
c.	met het openbaar vervoer (bus, trein, tram, enz.)	**3**	**3**
d.	met de auto	**4**	**4**
e.	een combinatie van het bovenstaande, nl: …….	**5**	**5**
f.	anders, namelijk: …….	**6**	**6**

Hoe lang reist de tweeling **meestal** naar school? *(Tel heen- en terugreis bij elkaar op.)*		**OUDSTE**	**JONGSTE**
a.	minder dan 5 minuten	**1**	**1**
b.	tussen de 5 en 15 minuten	**2**	**2**
c.	tussen de 15 en de 30 minuten	**3**	**3**
d.	méér dan 30 minuten	**4**	**4**

(2)

Hoe gaat de tweeling **meestal** naar school? (meerdere antwoorden mogelijk)		**OUDSTE**	**JONGSTE**
a.	lopend	**1**	**1**
b.	op de fiets	**2**	**2**
c.	met het openbaar vervoer (bus, trein, tram, enz.)	**3**	**3**
d.	met de auto	**4**	**4**
e.	anders, namelijk: …….	**5**	**5**

Hoe lang reist de tweeling **meestal** naar school? *(Tel heen- en terugreis bij elkaar op.)*		**OUDSTE**	**JONGSTE**
a.	minder dan 5 minuten	**1**	**1**
b.	tussen de 5 en 15 minuten	**2**	**2**
c.	tussen de 15 en de 30 minuten	**3**	**3**
d.	méér dan 30 minuten	**4**	**4**

(3)

Hoe gaat de tweeling meestal naar school?		**OUDSTE**	**JONGSTE**
a.	lopend	**1**	**1**
b.	op de fiets	**2**	**2**
c.	met het openbaar vervoer (bus, trein, tram, enz.)	**3**	**3**
d.	met de auto	**4**	**4**
e.	anders	**5**	**5**

Hoe lang reist de tweeling naar school?		OUDSTE	JONGSTE
a.	minder dan 5 minuten	**1**	**1**
b.	tussen de 5 en 15 minuten	**2**	**2**
c.	tussen de 15 en de 30 minuten	**3**	**3**
d.	méér dan 30 minuten	**4**	**4**

Perceived ability

(1)

Hoe goed is de tweeling in sport ten opzichte van leeftijdsgenoten?		OUDSTE	JONGSTE
a.	veel minder goed dan leeftijdsgenoten	**1**	**1**
b.	minder goed dan leeftijdsgenoten	**2**	**2**
c.	ongeveer even goed als leeftijdsgenoten	**3**	**3**
d.	beter dan leeftijdsgenoten	**4**	**4**
e.	veel beter dan leeftijdsgenoten	**5**	**5**

Hoe goed is het uithoudingsvermogen van de tweeling ten opzichte van leeftijdsgenoten?		OUDSTE	JONGSTE
a.	veel minder goed dan leeftijdsgenoten	**1**	**1**
b.	minder goed dan leeftijdsgenoten	**2**	**2**
c.	ongeveer even goed als leeftijdsgenoten	**3**	**3**
d.	beter dan leeftijdsgenoten	**4**	**4**
e.	veel beter dan leeftijdsgenoten	**5**	**5**

Hoe goed is de spierkracht van de tweeling ten opzichte van leeftijdsgenoten?		OUDSTE	JONGSTE
a.	veel minder goed dan leeftijdsgenoten	**1**	**1**
b.	minder goed dan leeftijdsgenoten	**2**	**2**
c.	ongeveer even goed als leeftijdsgenoten	**3**	**3**
d.	beter dan leeftijdsgenoten	**4**	**4**
e.	veel beter dan leeftijdsgenoten	**5**	**5**

(2)

Hoe goed is de tweeling in sport ten opzichte van leeftijdsgenoten?		OUDSTE	JONGSTE
a.	veel minder goed dan leeftijdsgenoten	**1**	**1**
b.	minder goed dan leeftijdsgenoten	**2**	**2**
c.	ongeveer even goed als leeftijdsgenoten	**3**	**3**
d.	beter dan leeftijdsgenoten	**4**	**4**
e.	veel beter dan leeftijdsgenoten	**5**	**5**

Level

(1)

Kunt u aangeven op welk niveau de tweeling de sport beoefent waarin hij of zij het beste is?		**OUDSTE**	**JONGSTE**
a.	alleen voor het plezier	**1**	**1**
b.	in competitieverband bij club of sportschool	**2**	**2**
c.	hoogste groep of selectieteam van club of sportschool	**3**	**3**
d.	regionale selectie	**4**	**4**
e.	nationale selectie	**5**	**5**

(2)

Kunt u aangeven op welk niveau de **OUDSTE** van de tweeling sport beoefent?		**naam sport:**	**naam 2e sport:** .....
a.	alleen voor het plezier	**1**	**1**
b.	in competitieverband bij club of sportschool	**2**	**2**
c.	hoogste groep of selectieteam van club of sportschool	**3**	**3**
d.	regionale selectie	**4**	**4**
e.	nationale selectie	**5**	**5**

Kunt u aangeven op welk niveau de **JONGSTE** van de tweeling sport beoefent?		**naam sport:**	**naam 2e sport:** .....
a.	alleen voor het plezier	**1**	**1**
b.	in competitieverband bij club of sportschool	**2**	**2**
c.	hoogste groep of selectieteam van club of sportschool	**3**	**3**
d.	regionale selectie	**4**	**4**
e.	nationale selectie	**5**	**5**

Self-report (DHBQ 14-18)

DHBQ14, 16 and 18 were identical for the pilot study and versions 1 and 2. DHBQ14 and 16 were slightly different from each other later on. DHBQ18 was not collected after version 2.

Nr.	Survey(s) and version
1	DHBQ 14, 16, and 18, pilot, collected in 2004 (DHBQ_14-16-18_pilot_2004.pdf)
2	DHBQ 14, 16, and 18, version 1, collected between 2004 and 2006 (DHBQ_14-16-18_versie1_rood-grijsBoekje.pdf)
3	DHBQ 14, 16, and 18, version 2, collected between 2005 and 2009 (DHBQ_14-16-18_versie2_blauwBoekje_druk1.pdf)
4	DHBQ 14, version 3, collected between 2008 and 2009 (DHBQ14_versie3_ONLINE_2009.pdf)
5	DHBQ 16, version 3, collected between 2009 and 2010 (DHBQ16_versie3_ONLINE_2009.pdf)
6	DHBQ 14, version 4, collected in 2011 (DHBQ14_versie4_ONLINE_2011.pdf)
7	DHBQ 16, version 4, collected in 2011 (DHBQ16_versie4_ONLINE_2011.pdf)
8	DHBQ 14, version 5, collected between 2011 and 2012 (DHBQ14_versie5_ONLINE_2012.pdf)
9	DHBQ 16, version 5, collected between 2011 and 2012 (DHBQ16_versie5_ONLINE_2012.pdf)
10	DHBQ 14 & 16, version 6, collected in 2013 (DHBQ14_versie6_ONLINE_2013.pdf; DHBQ16_versie6_ONLINE_2013.pdf)

The following data are available for each survey:

Nr.	Active transport	Cycling/ dancing	Perceived ability	Ability index
1	YES (1)	YES (1)	NO	NO
2	YES (2)	YES (2)	NO	NO
3	YES (2)	YES (2)	NO	NO
4	YES (3)	YES (3)	YES (1)	NO
5	NO	YES (3)	YES (1)	NO
6	YES (3)	YES (3)	YES (2)	YES (1)
7	NO	YES (3)	YES (2)	YES (1)
8	YES (3)	YES (4)	YES (3)	YES (1)
9	NO	YES (4)	YES (3)	YES (1)
10	NO	YES (4)	YES (3)	YES (1)

Nr.	Condition	Benefits	Barriers	Sweat index
1	YES (1)	NO	NO	YES (1)
2	YES (1)	NO	NO	YES (2)
3	YES (1)	NO	NO	YES (2)
4	NO	NO	NO	NO
5	NO	NO	NO	NO
6	NO	YES (1)	YES (1)	NO
7	NO	YES (1)	YES (1)	NO
8	NO	YES (1)	YES (1)	NO
9	NO	YES (1)	YES (1)	NO
10	NO	YES (1)	YES (2)	NO

**Different wordings of the questions/ response options are indicated in brackets, see following pages.*

English translation of the corresponding questions:

Active transport:
How do you usually go to school?
→ walking, cycling, public transport, car...
How long does it take you to go to school?

Cycling/ dancing:
How many days a week and how many minutes each day do you spend cycling/ dancing/ being active outdoors (e.g., skateboarding)? (weekday versus weekend)

Perceived ability:
How good are you at sports and physical exercise compared to your peers?
How good is your physical endurance compared to your peers?
How good is your muscle strength compared to your peers?

Ability index:
How good are you at sports and physical exercise on a scale from 1 to 10?

Condition:
How good is your physical condition when you are exercising?

Benefits:
Please indicate in how far you agree or disagree with the statements below on the benefits of exercise behavior.

Barriers:
Please indicate in how far you agree or disagree with the statements below on the barriers towards exercise behavior.

Sweat index:
Are you at least once a week physically active in your leisure time on a level that makes you sweat?

Active transport

(1)

Hoe ga je meestal naar school en/of werk?
a. lopend
b. op de fiets
c. met het openbaar vervoer (bus, trein, tram, enz)
d. op de brommer/ scooter
e. anders, nl …….

Hoe lang reis je naar school en/of werk?
a. minder dan 5 minuten
b. tussen de 5 en 15 minuten
c. tussen de 15 en de 30 minuten
d. meer dan 30 minuten, namelijk: ……. minuten

(2)

Hoe ga je meestal naar school? (als je niet meer naar school gaat, maar werkt deze vraag beantwoorden voor de reis naar je werk)
a. lopend
b. op de fiets
c. met het openbaar vervoer (bus, trein, tram, enz)
d. op de brommer/ scooter
e. een combinatie van het bovenstaande, nl …….
f. anders, nl …….

Hoe lang reis je naar school? (tel heen-en terugreis bij elkaar op) (als je niet meer naar school gaat, maar werkt deze vraag beantwoorden voor de reis naar je werk)
a. minder dan 5 minuten
b. tussen de 5 en 15 minuten
c. tussen de 15 en de 30 minuten
d. tussen de 30 en de 45 minuten
e. meer dan 45 minuten, namelijk: ……. minuten

(3)

Hoe ga je meestal naar school/werk? **Let op:** meerdere antwoorden mogelijk
a. lopend
b. op de fiets
c. met het openbaar vervoer (bus, trein, tram, enz)
d. op de brommer/ scooter
e. met de auto
f. anders, namelijk …….

Hoe lang reis je van en naar school/werk? (tel heen- en terugreis bij elkaar op)
a. minder dan 5 minuten
b. tussen de 5 en 15 minuten
c. tussen de 15 en de 30 minuten
d. tussen de 30 en de 45 minuten
e. meer dan 45 minuten, namelijk: ……. minuten

Cycling/ dancing

(1)

Neem een normale week van de afgelopen maand in je gedachten. Wil je aangeven hoeveel dagen per week je de onderstaande activiteiten verrichtte en hoelang je daar dan op zo'n dag mee bezig was? Indien je een bepaalde activiteit niet hebt gedaan kun je deze activiteit overslaan.

	aantal dagen per week	**gemiddelde tijd per dag**
Fietsen		
a. Doordeweeks (maandag tot en met vrijdag)	……. dagen	……. minuten
b. In het weekend (zaterdag en zondag)	……. dagen	……. minuten
Dansen tijdens het uitgaan / Disco dansen		
a. Doordeweeks (maandag tot en met vrijdag)	……. dagen	……. minuten
b. In het weekend (zaterdag en zondag)	……. dagen	……. minuten
Sportieve activiteiten buitenshuis (bv. Skateboarden)		
a. Doordeweeks (maandag tot en met vrijdag)	……. dagen	……. minuten
b. In het weekend (zaterdag en zondag)	……. dagen	……. minuten

(2)

Neem een normale school- /werkweek van de afgelopen maand in je gedachten. Wil je aangeven hoeveel dagen per week je de onderstaande activiteiten verrichtte en hoelang je daar dan op zo'n dag mee bezig was? Indien je een bepaalde activiteit niet hebt gedaan kun je deze activiteit overslaan. (Hierbij de "sport" die je bij vraag 9 hebt ingevuld niet meetellen)

	aantal dagen per week	**gemiddelde tijd per dag**
Fietsen		
a. Doordeweeks (maandag tot en met vrijdag)	……. dagen	……. minuten
b. In het weekend (zaterdag en zondag)	……. dagen	……. minuten
Dansen tijdens het uitgaan / Disco dansen		
a. Doordeweeks (maandag tot en met vrijdag)	……. dagen	……. minuten
b. In het weekend (zaterdag en zondag)	……. dagen	……. minuten
Sportieve activiteiten buitenshuis		
a. Doordeweeks (maandag tot en met vrijdag)	……. dagen	……. minuten
b. In het weekend (zaterdag en zondag)	……. dagen	……. minuten

(3)

Neem een normale school- /werkweek van **de afgelopen maand** in je gedachten.

Wil je aangeven op hoeveel doordeweekse dagen (maandag t/m vrijdag) je hebt gefietst?
Hoe lang was je gemiddeld op een doordeweekse dag (maandag t/m vrijdag) bezig met fietsen?
Wil je aangeven op hoeveel dagen in het weekend (zaterdag en zondag) je hebt gefietst?
Hoe lang was je gemiddeld op een dag in het weekend (zaterdag en zondag) bezig met fietsen?

Wil je aangeven op hoeveel doordeweekse dagen (maandag t/m vrijdag) je hebt gedanst tijdens het uitgaan?
Hoe lang was je gemiddeld op een doordeweekse dag (maandag t/m vrijdag) bezig met dansen tijdens het uitgaan?
Wil je aangeven op hoeveel dagen in het weekend (zaterdag en zondag) je hebt gedanst tijdens het uitgaan?
Hoe lang was je gemiddeld op een dag in het weekend (zaterdag en zondag) bezig met dansen tijdens het uitgaan?

Fietsen		**aantal dagen per week**		**gemiddelde tijd per dag**	
a.	Doordeweeks (maandag tot en met vrijdag)	**0**	0 → *Fietsen weekend*	**1**	minder dan 30 min.
		1	1	**2**	31 - 60 min.
		2	2	**3**	61 - 120 min.
		3	3	**4**	121 - 180 min.
		4	4	**5**	meer dan 180 min.
		5	5		
b.	In het weekend (zaterdag en zondag)	**0**	0 → *Dansen doordeweeks*	**1**	minder dan 30 min.
		1	1	**2**	31 - 60 min.
		2	2	**3**	61 - 120 min.
				4	121 - 180 min.
				5	meer dan 180 min.
Dansen tijdens het uitgaan		**aantal dagen per week**		**gemiddelde tijd per dag**	
a.	Doordeweeks (maandag tot en met vrijdag)	**0**	0 → *Dansen weekend*	**1**	minder dan 30 min.
		1	1	**2**	31 - 60 min.
		2	2	**3**	61 - 120 min.
		3	3	**4**	121 - 180 min.
		4	4	**5**	meer dan 180 min.
		5	5		
b.	In het weekend (zaterdag en zondag)	**0**	0 → *Door naar vraag X*	**1**	minder dan 30 min.
		1	1	**2**	31 - 60 min.
		2	2	**3**	61 - 120 min.
				4	121 - 180 min.
				5	meer dan 180 min.

(4)

Neem een normale school- /werkweek van **de afgelopen maand** in je gedachten.

Wil je aangeven op hoeveel doordeweekse dagen (maandag t/m vrijdag) je hebt gefietst?
Hoe lang was je gemiddeld op een doordeweekse dag (maandag t/m vrijdag) bezig met fietsen?
Wil je aangeven op hoeveel dagen in het weekend (zaterdag en zondag) je hebt gefietst?
Hoe lang was je gemiddeld op een dag in het weekend (zaterdag en zondag) bezig met fietsen?

Wil je aangeven op hoeveel doordeweekse dagen (maandag t/m vrijdag) je hebt gedanst tijdens het uitgaan?
Hoe lang was je gemiddeld op een doordeweekse dag (maandag t/m vrijdag) bezig met dansen tijdens het uitgaan?
Wil je aangeven op hoeveel dagen in het weekend (zaterdag en zondag) je hebt gedanst tijdens het uitgaan?
Hoe lang was je gemiddeld op een dag in het weekend (zaterdag en zondag) bezig met dansen tijdens het uitgaan?

Fietsen					
a.	Doordeweeks (maandag tot en met vrijdag)	**0**	0 → *Fietsen weekend*	**0**	niet
		1	1	**1**	minder dan 30 min.
		2	2	**2**	31 - 60 min.
		3	3	**3**	61 - 120 min.
		4	4	**4**	121 - 180 min.
		5	5	**5**	meer dan 180 min.
b.	In het weekend (zaterdag en zondag)	**0**	0 dagen → *Dansen doordeweeks*	**0**	niet
		1	1 dag	**1**	minder dan 30 min.
		2	2 dagen	**2**	31 - 60 min.
				3	61 - 120 min.
				4	121 - 180 min.
				5	meer dan 180 min.
Dansen tijdens het uitgaan					
a.	Doordeweeks (maandag tot en met vrijdag)	**0**	0 → *Dansen weekend*	**0**	niet
		1	1	**1**	minder dan 30 min.
		2	2	**2**	31 - 60 min.
		3	3	**3**	61 - 120 min.
		4	4	**4**	121 - 180 min.
		5	5	**5**	meer dan 180 min.
b.	In het weekend (zaterdag en zondag)	**0**	0 dagen → *Door naar vraag X*	**0**	niet
		1	1 dag	**1**	minder dan 30 min.
		2	2 dagen	**2**	31 - 60 min.
				3	61 - 120 min.
				4	121 - 180 min.
				5	meer dan 180 min.

Perceived ability

(1)

Hoe goed ben je in je sport ten opzichte van je leeftijdsgenoten?	
a.	ik ben veel minder goed dan mijn leeftijdsgenoten
b.	ik ben minder goed dan mijn leeftijdsgenoten
c.	ik ben ongeveer even goed als mijn leeftijdsgenoten
d.	ik ben beter dan mijn leeftijdsgenoten
e.	ik ben veel beter dan mijn leeftijdsgenoten

(2)

		veel minder goed	minder goed	ongeveer even goed	beter	veel beter
a.	Hoe goed ben je in sport ten opzichte van je leeftijdsgenoten?	**1**	**2**	**3**	**4**	**5**
b.	Hoe goed is je uithoudingsvermogen ten opzichte van je leeftijdsgenoten?	**1**	**2**	**3**	**4**	**5**
c.	Hoe goed is je spierkracht ten opzichte van je leeftijdsgenoten?	**1**	**2**	**3**	**4**	**5**

(3)

Zou je met behulp van vijf antwoordmogelijkheden antwoord willen geven op onderstaande vragen?		veel minder goed	minder goed	ongeveer even goed	beter	veel beter
a.	Hoe goed ben je in sport ten opzichte van je leeftijdsgenoten?	**1**	**2**	**3**	**4**	**5**
b.	Hoe goed is je uithoudingsvermogen ten opzichte van je leeftijdsgenoten?	**1**	**2**	**3**	**4**	**5**
c.	Hoe goed is je spierkracht ten opzichte van je leeftijdsgenoten?	**1**	**2**	**3**	**4**	**5**

Ability index

(1)

Hoe goed ben je in sport?											
helemaal niet goed	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	heel erg goed

Condition

(1)

Welke van de uitspraken beschrijft het beste jouw conditie tijdens het sporten?	
0	ik doe niet aan sport
1	ik ben erg buiten adem en/of zweet veel
2	ik ben een beetje buiten adem en/of zweet een beetje
3	ik ben nauwelijks buiten adem en/of zweet bijna niet
4	ik ben niet buiten adem en/of zweet niet
5	ik ben buiten adem, maar zweet niet

Benefits

(1)

De onderstaande stellingen gaan over mogelijke effecten van regelmatig sporten. Wil je aangeven of je het eens of oneens bent met deze stellingen?		sterk mee oneens	mee oneens	mee eens	sterk mee eens
a.	Door te sporten ziet je lichaam er beter uit	**1**	**2**	**3**	**4**
b.	Je voelt je beter als je regelmatig sport	**1**	**2**	**3**	**4**
c.	Sporten geeft je meer energie	**1**	**2**	**3**	**4**
d.	Sporten geeft je een gevoel dat je iets bereikt	**1**	**2**	**3**	**4**
e.	Sporten houdt de geest actief	**1**	**2**	**3**	**4**
f.	Sporten is goed voor je hart	**1**	**2**	**3**	**4**
g.	Sporten is goed voor je gemoedstoestand	**1**	**2**	**3**	**4**
h.	Mensen sporten om gezond te blijven	**1**	**2**	**3**	**4**
i.	Door te sporten voel je je minder gestrest en gespannen	**1**	**2**	**3**	**4**
j.	Door te sporten kom je in contact met anderen	**1**	**2**	**3**	**4**

Barriers

(1)

Hoe vaak word je door het volgende gehinderd om lichamelijk actief te worden of te gaan sporten?		nooit	zelden	af en toe	vaak	heel vaak
a.	Ik ben onzeker over mijn uiterlijk als ik actief ben	**1**	**2**	**3**	**4**	**5**
b.	Ik heb geen interesse in lichamelijke activiteit	**1**	**2**	**3**	**4**	**5**
c.	Ik heb geen zelfdiscipline of wilskracht	**1**	**2**	**3**	**4**	**5**
d.	Ik heb er geen tijd voor	**1**	**2**	**3**	**4**	**5**
e.	Ik heb er de energie niet voor	**1**	**2**	**3**	**4**	**5**
f.	Ik heb niemand om samen mee te sporten	**1**	**2**	**3**	**4**	**5**
g.	Ik beleef geen plezier aan sport of lichamelijke activiteit	**1**	**2**	**3**	**4**	**5**
h.	Ik wil niet falen, dus ik probeer het niet	**1**	**2**	**3**	**4**	**5**
i.	Ik heb niet de vereiste sportbenodigdheden	**1**	**2**	**3**	**4**	**5**
j.	Ik vind het weer vaak te slecht	**1**	**2**	**3**	**4**	**5**
k.	Ik heb te weinig sportieve vaardigheden	**1**	**2**	**3**	**4**	**5**
l.	Ik ben te moe om te sporten	**1**	**2**	**3**	**4**	**5**
m.	Ik heb te weinig kennis over hoe ik moet sporten	**1**	**2**	**3**	**4**	**5**
n.	Ik heb een slechte gezondheid	**1**	**2**	**3**	**4**	**5**
o.	Ik ben bang voor blessures	**1**	**2**	**3**	**4**	**5**
p.	Ik vind bewegen zwaar	**1**	**2**	**3**	**4**	**5**

q.	Ik heb geen goed bereikbare sportfaciliteiten in de buurt	**1**	**2**	**3**	**4**	**5**
r.	Ik ben te dik	**1**	**2**	**3**	**4**	**5**
s.	Ik vind sporten saai	**1**	**2**	**3**	**4**	**5**
t.	Ik heb werkverplichtingen	**1**	**2**	**3**	**4**	**5**
u.	Ik heb sociale verplichtingen	**1**	**2**	**3**	**4**	**5**
v.	Ik heb familieverplichtingen	**1**	**2**	**3**	**4**	**5**
w.	Ik vind sporten te duur	**1**	**2**	**3**	**4**	**5**

(2)

Hoe vaak word je door het volgende gehinderd om lichamelijk actief te worden of te gaan sporten?		nooit	zelden	af en toe	vaak	heel vaak
a.	Ik ben onzeker over mijn uiterlijk als ik actief ben	**1**	**2**	**3**	**4**	**5**
b.	Ik heb geen interesse in lichamelijke activiteit	**1**	**2**	**3**	**4**	**5**
c.	Ik heb geen zelfdiscipline of wilskracht	**1**	**2**	**3**	**4**	**5**
d.	Ik heb er geen tijd voor	**1**	**2**	**3**	**4**	**5**
e.	Ik heb er de energie niet voor	**1**	**2**	**3**	**4**	**5**
f.	Ik heb niemand om samen mee te sporten	**1**	**2**	**3**	**4**	**5**
g.	Ik beleef geen plezier aan sport of lichamelijke activiteit	**1**	**2**	**3**	**4**	**5**
h.	Ik wil niet falen, dus ik probeer het niet	**1**	**2**	**3**	**4**	**5**
i.	Ik heb niet de vereiste sportbenodigdheden	**1**	**2**	**3**	**4**	**5**
j.	Ik vind het weer vaak te slecht	**1**	**2**	**3**	**4**	**5**
k.	Ik heb te weinig sportieve vaardigheden	**1**	**2**	**3**	**4**	**5**
l.	Ik ben te moe om te sporten	**1**	**2**	**3**	**4**	**5**
m.	Ik heb te weinig kennis over hoe ik moet sporten	**1**	**2**	**3**	**4**	**5**
n.	Ik heb een slechte gezondheid	**1**	**2**	**3**	**4**	**5**
o.	Ik ben bang voor blessures	**1**	**2**	**3**	**4**	**5**
p.	Ik vind bewegen zwaar	**1**	**2**	**3**	**4**	**5**
q.	Ik heb geen goed bereikbare sportfaciliteiten in de buurt	**1**	**2**	**3**	**4**	**5**
r.	Ik ben te zwaar	**1**	**2**	**3**	**4**	**5**
s.	Ik vind sporten saai	**1**	**2**	**3**	**4**	**5**
t.	Ik heb werkverplichtingen	**1**	**2**	**3**	**4**	**5**
u.	Ik heb sociale verplichtingen	**1**	**2**	**3**	**4**	**5**
v.	Ik heb familieverplichtingen	**1**	**2**	**3**	**4**	**5**
w.	Ik vind sporten te duur	**1**	**2**	**3**	**4**	**5**

Sweat index

(1)

Ben je tenminste één keer per week in je vrije tijd zo lichamelijk actief dat je ervan gaat zweten?					
1	nee	**2**	ja, namelijk →	**1**	één keer per week
				2	twee keer per week
				3	drie keer per week
				4	meer dan drie keer per week, namelijk ……. keer

(2)

Ben je tenminste één keer per week in je vrije tijd zo lichamelijk actief dat je ervan gaat zweten? (inclusief sporten)					
1	nee	**2**	ja, namelijk →	**1**	één keer per week
				2	twee keer per week
				3	drie keer per week
				4	meer dan drie keer per week, namelijk ……. keer

APPENDIX III:
Example of personalized feedback to the participants

Beste NTR-deelnemer,

Hartelijk bedankt voor het invullen van de vragenlijst van het Nederlands Tweelingen Register! Jouw medewerking is van groot belang voor het medische en wetenschappelijke onderzoek naar geestelijke en lichamelijke gezondheid. Op onze website staan steeds de laatste resultaten. Hieronder vind je jouw resultaten gebaseerd op wat je hebt ingevuld in de vragenlijst.

Jouw persoonlijke uitslag op de persoonlijkheidsvragenlijst
Wetenschappers zijn tot de conclusie gekomen, dat je de persoonlijkheid van een mens het beste kunt beschrijven op basis van vijf dimensies. Deze dimensies zijn 1. “emotionele stabiliteit - neuroticisme”, 2. “introversie - extraversie”, 3. “geslotenheid voor (nieuwe) ervaring - openheid voor (nieuwe) ervaring”, 4. “kwaadaardigheid - goedaardigheid” en 5. “laksheid - zorgvuldigheid”. Hieronder vind je jouw scores op deze persoonlijkheidsdimensies.

Extraversie: 7 (hoog)
Mensen die laag scoren op extraversie (en dus hoog op introversie), zijn vaak serieus en graag alleen (of samen met een beperkt aantal goede vrienden), terwijl mensen die hoog scoren op extraversie liefst samen zijn met andere mensen en de neiging hebben om heel actief te zijn.

Openheid voor (nieuwe) ervaring: 9 (zeer hoog)
Mensen die laag scoren op openheid voor (nieuwe) ervaring (en dus hoog op geslotenheid) zijn meestal behoudend en praktisch, terwijl mensen die hoog scoren op openheid steeds weer nieuwe dingen willen beleven en veel interesses hebben.

Neuroticisme: 4 (gemiddeld)
Mensen die laag scoren op neuroticisme (en dus hoog op emotionele stabiliteit) blijven meestal rustig en ontspannen, zelfs als ze in moeilijke situaties terecht komen, terwijl mensen die hoog scoren op neuroticisme best wel sensibel zijn en heel emotioneel op gebeurtenissen kunnen reageren.

Goedaardigheid: 6 (gemiddeld)
Mensen die laag scoren op goedaardigheid (en dus hoog op kwaadaardigheid) zijn best wel sceptisch, competitief en ze gaan ruzie niet uit de weg, terwijl mensen die hoog scoren op goedaardigheid snel medelijden voelen en

conflicten uit de weg gaan.

Zorgvuldigheid: 6 (gemiddeld)
Mensen die laag scoren op zorgvuldigheid (en dus hoog op laksheid) zijn vaak niet zo goed in tijdsplanning en organiseren, terwijl mensen die hoog scoren op zorgvuldigheid precies zijn en altijd nauwkeurig te werk zullen gaan om hun doelen te bereiken.

Meer informatie over de vijf persoonlijkheidsdimensies vind je hier of hier.

Sportgedrag
Je hebt aangegeven dat je wel regelmatig sport en dat je een goede sporter bent. Ongeveer 82% van de jongens en 78% van de meisjes die deze vragenlijst hebben ingevuld, doet regelmatig aan sport. Zij doen vooral aan voetbal (35,8% van de jongens en 8,8% van de meisjes), hardlopen/joggen (9,6% van de jongens en 10,9% van de meisjes) en conditietraining/fitness (15% van de jongens en 12,2% van de meisjes).

Verder bleek uit je resultaten dat je geen duidelijke mening erover hebt of sporten wel of niet goed voor je is. Je hebt het gevoel dat je er wel de tijd voor hebt en dat je de vaardigheden ervoor (over het algemeen) ook hebt. Je hebt mensen om samen mee te sporten. De belangrijkste reden die deelnemers van dit onderzoek geven wanneer ze niet sporten, is een gebrek aan tijd - met name door sociale verplichtingen.

Je fietst vaak. Nederland behoort tot de landen waar het meeste wordt gefietst, het wordt ook wel de “fietshoofdstad van de wereld” genoemd. In andere landen is fietsen vaak lastig, bijvoorbeeld omdat je bergen hebt en/of geen fietspaden. Dan wordt het (te) vermoeiend en soms zelfs gevaarlijk.

Je body mass index (BMI) is prima. De BMI is de verhouding tussen je lengte en je gewicht. Als je je gewicht door (je lengte x je lengte) deelt, kom je uit bij je BMI. Kijk hier voor meer informatie over wat BMI betekent en wat een gezond BMI is.

Leefgewoontes & tevredenheid
Uit onze vragenlijsten bleek dat iets meer dan 20% van de jongens en meisjes met 14 jaar al ooit hebben gerookt in hun leven, terwijl dit met 16 jaar dubbel zo veel zijn. Voor alcohol zijn het op 14-jarige leeftijd ongeveer 56% en twee jaar later 92%. Uit onderzoek bleek dat het percentage jongeren wat regelmatig rookt enorm is afgenomen sinds begin jaren negentig, terwijl het aantal jongeren wat drinkt juist is toegenomen.

Jij hebt aangegeven dat je de afgelopen vier weken gemiddeld 7 uur per nacht hebt geslapen. 65% van de jongeren die deze vragenlijst hebben ingevuld slaapt gewoonlijk 8-9 uur, terwijl 27% minder dan 9 uur per nacht slaapt. Voor jongeren wordt vaak aanbevolen 9 tot 10 uur per nacht te slapen. Maar er zijn mensen die meer of minder slaap nodig hebben. Sommige mensen hebben genoeg aan 5 uur per nacht en anderen slapen meer dan 10 uur en zijn dan nog steeds niet uitgerust. Je kunt heel makkelijk testen, hoeveel slaap je nodig hebt. Let op het aantal uren dat je 's nachts slaapt en kijk dan of je je de volgende dag uitgerust voelt. Als dit zo is, heb je voldoende slaap gehad. Klik hier voor meer informatie.

Je hebt ook aangegeven dat je tevreden bent met je leven. Verder bleek dat je vooral positieve verwachtingen hebt met betrekking tot je toekomst. Wist jij dat tevredenheid niet afhangt van bijvoorbeeld rijkdom? Uit onderzoek bleek dat mensen ongeveer even tevreden zijn als ze veel of weinig geld verdienen. Je gelukkig te voelen blijkt wel erfelijk te zijn en ook samen te hangen met je sociale omgeving, zoals familie en vrienden.

Tenslotte gaf je nog aan dat je soms problemen hebt om de aandacht ergens bij te houden. Jongens hebben vaker concentratieproblemen dan meisjes. Dat kan lastig zijn op school, want daar moet je je aandacht natuurlijk bij de les houden.

Vrijetijdsbestedingen
Je hebt aangegeven dat je niet vaak of nooit muziek maakt, regelmatig een boek leest en niet vaak of nooit tekent of schildert. 23% van de deelnemers van dit onderzoek maakt regelmatig muziek of is lid van een koor, 54% leest regelmatig boeken en 26% tekent en/of schildert regelmatig. Er wordt ook graag uitgegaan met vrienden of vriendinnen. De meeste meisjes gaven aan, 1-4 vrienden en 3-6 vriendinnen te hebben, terwijl jongens over het algemeen 3-6 vrienden en 1-4 vriendinnen hebben. Uiteindelijk maakt het aantal vrienden niet zo veel uit voor hoe je je voelt, de kwaliteit van vriendschappen is belangrijker. Meisjes en jongens gaan vaak anders om met hun vrienden. Terwijl meisjes vooral willen praten met hun vriendinnen en geheimen willen delen, doen jongens graag samen heel praktische en/of actieve dingen, zoals auto's repareren of voetballen.

Namens alle medewerkers van het NTR: bedankt!

Met vriendelijke groet,
Dorret Boomsma

Dankwoord

Graag wil ik iedereen bedanken die heeft bijgedragen aan het tot stand komen van mijn proefschrift.

Als eerste wil ik alle meerlingen en hun familieleden bedanken die belangeloos deelnemen aan onderzoek van het Nederlands Tweelingen Register.

Dan wil ik mijn promotoren bedanken, Eco en Meike. Bedankt voor jullie gegeven vertrouwen en de energie die jullie in mij hebben gestoken! Ik heb veel geleerd - niet alleen over genetisch onderzoek - door jullie expertise en ervaring. Eco, jouw oprechte enthousiasme en passie voor het vak werken aanstekelijk. Ook vind ik het indrukwekkend hoe snel en grondig jij feedback gaf (geeft) op mijn stukken, terwijl je het zo druk hebt met je twee banen bij het NTR en Emgo^{+}. Meike, je relativeringsvermogen heeft vaak geholpen de dingen niet ingewikkelder te maken dan zij waren. Ook was het leuk om je benoeming tot professor van dichtbij mee te maken.

I would like to thank the members of the reading committee for investing their time in evaluating my thesis and for agreeing to be my opponents on the day of my defense. Willem, het zou best kunnen dat ik nooit was begonnen bij het NTR zonder jouw hulp, want ik had in eerste instantie jou benaderd voor een PhD project bij EMGO^{+}. Ik vind het daarom bijzonder leuk dat jij in mijn commissie zit! Conor, bedankt voor je hulp bij al mijn statistische vragen en voor de chocola die jij vaak voor onze kamer langs komt brengen (omdat je toch een reep voor jezelf ging kopen), wij zijn er altijd blij mee!

Karri, many thanks for being my host during my visit to the University of Helsinki, for getting me involved with other projects in the department and for inviting me to teach at the twin workshop in Helsinki. Those were great experiences! Jaakko, thank you for allowing me to be part of your department! My thanks also go to Sari, Maarit, Aileen, Leonie, Jadwiga, Anu, Antti, Kauko, Sara, Eero and other colleagues in Helsinki for your hospitality. I hope that our collaboration will continue!

Mijn collega's van BioPsy - wat is het toch een gezellige, betrokken en inspirerende afdeling! De lunches met soms uitgebreide discussies, de vrijdagmiddagborrels en de vele gesprekken tussendoor hebben mijn promotietraject tot meer gemaakt dan alleen werk. Het was mooi om het 25-jarig bestaan van het NTR te vieren met als hoogtepunt een groot feest in Burgers' Zoo. Dorret, jij hebt dit allemaal opgericht en nu leid je het tweelingenregister met een oneindige passie voor de wetenschap! Natascha,

wat zouden we zonder jou moeten? Het lijkt bijna alsof alles wat jij regelt vanzelf gaat en wat hebben we vaak met je gelachen! Heel erg bedankt voor je hulp bij alles wat geregeld moest worden rond mijn promotietraject. Dank aan Michiel, Ellen en Stephanie voor alle hulp die ik heb gekregen bij de praktische uitvoering van mijn onderzoek. Michelle, Cyrina en Corina: Bedankt voor jullie hulp met Panter, brieven en stickers! Toos, bedankt voor het snelle aanleveren van databestanden en voor je inzet als co-auteur op bijna alle papers in dit proefschrift. Je was altijd beschikbaar voor vragen, hoe druk je ook was.

Ik ben blij dat er op onze afdeling een hoop Aio's rondlopen, waardoor je mensen hebt die in een vergelijkbare situatie zitten en waarmee je ervaringen kan delen. Bedankt voor jullie gezelligheid bij alle congressen, borrels, workshops en de Aio-club! Michel, bedankt voor je statistisch advies! Maria, bedankt voor je hulp met het dopamine-paper en voor je oneindige interesse - je bent veel te lief! Bochao, thanks for doing touristy things with me! Charles, thanks for your gezelligheid during your stay in Amsterdam! Fiona, bedankt voor onze boulder- en klimavonden!

En dan wil ik mijn kamergenootjes bedanken: Eveline, Nienke en Ineke - en Suzanne, die er bij hoort ook al was haar kamer veel te ver weg! Jullie hebben op dit promotietraject een eigen stempel gedrukt. Ik ben heel dankbaar dat ik jullie heb ontmoet en dat we het ook buiten werk gezellig hebben! We hebben samen door Amerika gereisd, zijn gaan zingen en pilatessen, hebben elkaars sportwedstrijden bezocht, we waren weekendjes weg en hebben ons klimbewijs gehaald. Het was fijn om zo veel luisterende oren te hebben! Eveline, ik ben drie maanden na jou begonnen en dus hebben we elkaars promotietrajecten van dichtbij mogen meemaken. Ik ben blij dat jij nu mijn paranimf wilt zijn. Je stond altijd voor me klaar en je hebt me door de laatste fase van mijn Aio-tijd heen gesleept. Dankjewel!

I am honored that many good friends will travel all the way to Amsterdam for my defense, thank you so much!

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