A growing number of twin and family studies provide evidence that a substantial part of the variation in voluntary exercise behavior is determined by genetic predisposition. Starting in adolescence, an increase is observed in heritability estimates, which are consistently moderate to high at the ages of 16 to 18, continuing into young adulthood. The aim of this thesis was to identify the mechanisms that give rise to this heritability of voluntary exercise behavior in adolescents and young adults.

Unraveling the genetic components of voluntary exercise behavior in adolescents and young adults

Nienke M Schutte

UNRAVELING THE GENETIC COMPONENTS OF VOLUNTARY EXERCISE BEHAVIOR IN ADOLESCENTS AND YOUNG ADULTS

NIENKE M SCHUTTE

Reading Committee

prof.dr. W. van Mechelen	VU University Medical Center Amsterdam
prof.dr. H. Schnieder	University Medical Center Groningen
prof.dr. M.T.E. Hopman	Radboud University Medical Center Nijmegen
dr. M. den Hoed	Uppsala University
dr. J. de Koning	Vrije Universiteit Amsterdam

Paranymphs

Ineke Nederend

Charlotte Huppertz

Acknowledgements

We thank the members of the twin families registered with the Netherlands Twin Register for their continued support of scientific research.

This research was supported by the National Institute of Diabetes and Digestive and Kidney Diseases (RO1DK-092127) and is part of the Graduate School Program, which is financed by the Netherlands Organization for Scientific Research (NWO, 022.003.010).

ISBN: 978-94-6332-212-6

Printing: GVO drukkers & vormgevers B.V.

VRIJE UNIVERSITEIT

Unraveling the genetic components of voluntary exercise behavior in adolescents and young adults

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. V. Subramaniam,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Gedrags- en Bewegingswetenschappen
op vrijdag 22 september 2017 om 13.45 uur
in de aula van de universiteit,
De Boelelaan 1105

door

Nienke Maria Schutte

geboren te Apeldoorn

promotoren: prof.dr. J.C.N. de Geus
prof.dr. M. Bartels

CONTENTS

Chapter 1

General Introduction

Humans have been physically active since the beginning of their existence to provide for themselves and their offspring. Contributing to activities such as hunting and gathering was not so much a choice as it was essential for the survival of the group. Although there might be individual differences in how they spent their evenings; relaxing by the fire, dancing, or chewing on mind-altering substances, in the morning every individual had to pick up their daily, physically demanding activities. As centuries passed, humans invented many tricks and tools to make their lives easier and less labor intensive. In addition, more spare time became available to focus on other interests beyond mere survival and pursue other activities (i.e. hobbies) in order to fuel feelings of enjoyment. These developments made being physically active not a requisite for survival of the group, but an individual choice.

Nowadays, large individual differences are observed in the population at large regarding the amount of time spent on physical activity. These individual differences are not without consequence. In industrial countries physical *in*activity is an important contributor to non-communicable diseases (Lee et al., 2012), while moderate to vigorous intensity physical activity has been shown to have a large protective effect on mortality (Samitz et al., 2011). Public health authorities worldwide have launched interventions aimed at physical activity during work/school time and transportation to work and school, and at physical activity in leisure time (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), and the Physical Activity Guidelines for Americans by the U.S. Department of Health and Human Services (2008)). In view of the obvious advantage of a physically active lifestyle, the question arises why some individuals choose to regularly engage in physical activity, while others do not, consequently referred to as 'couch potatoes'.

Correlates and determinants of a physically active lifestyle have been studied intensively over the past three decades as mapping the determinants of health behaviors is crucial for defining targets of intervention. Over 30 years ago, Dishman and colleagues stated that *'one barrier to developing effective methods to encourage physical activity (…) is the lack of knowledge of the determinants of regular physical activity'* (Dishman et al., 1985). In the following years, researchers have identified numerous potential determinants in cross-sectional or longitudinal studies that to a greater or lesser extent contribute to the maintenance of physical activity. Although physical activity encompasses a broad domain of activities at work,

at home and during transportation, the emphasis of many studies has been on voluntary exercise behavior in leisure time. Exercise behavior is rapidly becoming the major source of moderate-to-vigorous activity in many industrialized countries. A large body of studies has addressed various environmental and social factors as determinants of exercise behavior, for example low socioeconomic status, high job strain, health beliefs, access to sport facilities, and support by peers and family (Bergstrom et al., 1996; Dishman et al., 1985; Drenowatz et al., 2010; Haase et al., 2004; Matson-Koffman et al., 2005; Payne et al., 2005; Sallis et al., 2000; Varo et al., 2003). However, when environmental circumstances are identical for a specific population, individuals still differ in exercise status. Starting in the 1980s, twin and family studies have provided evidence that a substantial part of the variation in exercise behavior is determined by genetic predisposition.

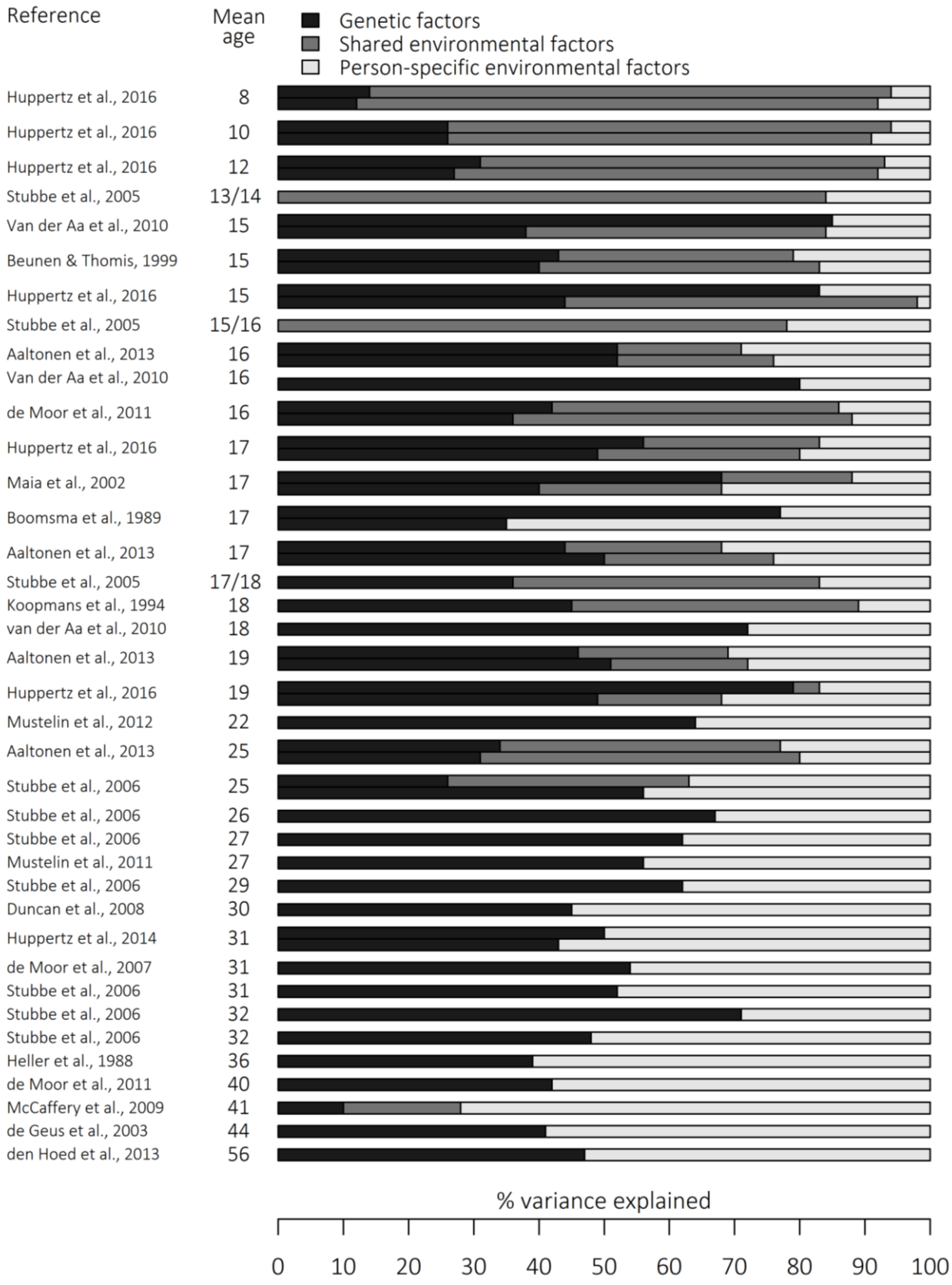
TWIN STUDIES

Twin and family studies have been paramount in understanding the genetic architecture of complex traits (Polderman et al., 2015). Children often resemble their parents regarding exercise status (de Moor et al., 2011; Seabra et al., 2014). To separate genetic effects (the heritability) from other factors that are shared by family members (i.e. upbringing, neighborhood), the classical twin design exploits the known differences in genetic similarity in identical and non-identical twins (or siblings). Genetically identical twins or monozygotic (MZ) twins are the result of the division of a single fertilized egg during an early stage in embryonic development, and non-identical twins or dizygotic (DZ) twins result from two separate fertilized eggs. Consequently, MZ twins are genetically identical and the observed difference between the twins is due to person-specific environmental factors: experiences that one of the twins has and the co-twin does not. Dizygotic twins share on average 50% of their genetic make-up. Consequently, the observed differences between the twins are a result of genetic differences and person-specific environmental factors. If MZ-resemblance for the trait of interest is higher than DZ-resemblance, it constitutes evidence for genetic influences on the trait (the classical twin model).

Figure 1.1 shows the relative importance of genetic factors on exercise behavior (measured using surveys) across the lifespan. These heritability estimates vary widely (from 0% up to 85%). A large part of this variation is due to the age of the subjects; the genetic architecture

of exercise behavior is different over age. Up to 14 years of age, the heritability estimates are moderate (Huppertz et al., 2016; Stubbe et al., 2005). The notion that environmental factors play a greater role in childhood than in adolescence can be explained by the important role of the parents; they provide children with the opportunity to become active by means of transportation to exercise activities, give exercise activities priority over other leisure time activities, and provide motivation and encouragement to exercise (Huppertz et al., 2016; Stubbe et al., 2005). After the age of 14, an increase is observed in heritability estimates, with consistently moderate to high estimates at the ages of 16 and 17 (Aaltonen et al., 2013; Boomsma et al., 1989; de Moor et al., 2011; Huppertz et al., 2016; Maia et al., 2002; Stubbe et al., 2005; van der Aa et al., 2010), continuing into young adulthood (Aaltonen et al., 2013; de Geus et al., 2003; de Moor et al., 2007; den Hoed et al., 2013; Duncan et al., 2008; Heller et al., 1988; Huppertz et al., 2014b; Huppertz et al., 2016; Koopmans et al., 1994; McCaffery et al., 2009; Mustelin et al., 2011; Mustelin et al., 2012; Stubbe et al., 2006; van der Aa et al., 2010). To provide a comprehensive overview of current literature, Chapter 2 reviews published studies on the heritability of exercise behavior (and physical activity) and shows in a meta-analysis in different age groups the sample size weighted heritability estimate for exercise behavior.

Figure 1.1 Summary of previous published studies on the relative influence of genetic factors, shared environmental influences and person-specific environmental influences on voluntary exercise behavior in across the lifespan. When two bars per studies are displayed, the first bar represents the results for males; the second bar represents the results for females.


Reference
Mean age
Genetic factors
Shared environmental factors
Person-specific environmental factors
Huppertz et al., 2016 8
Huppertz et al., 2016 10
Huppertz et al., 2016 12
Stubbe et al., 2005 13/14
Van der Aa et al., 2010 15
Beunen & Thomis, 1999 15
Huppertz et al., 2016 15
Stubbe et al., 2005 15/16
Aaltonen et al., 2013 16
Van der Aa et al., 2010 16
de Moor et al., 2011 16
Huppertz et al., 2016 17
Maia et al., 2002 17
Boomsma et al., 1989 17
Aaltonen et al., 2013 17
Stubbe et al., 2005 17/18
Koopmans et al., 1994 18
van der Aa et al., 2010 18
Aaltonen et al., 2013 19
Huppertz et al., 2016 19
Mustelin et al., 2012 22
Aaltonen et al., 2013 25
Stubbe et al., 2006 25
Stubbe et al., 2006 26
Stubbe et al., 2006 27
Mustelin et al., 2011 27
Stubbe et al., 2006 29
Duncan et al., 2008 30
Huppertz et al., 2014 31
de Moor et al., 2007 31
Stubbe et al., 2006 31
Stubbe et al., 2006 32
Stubbe et al., 2006 32
Heller et al., 1988 36
de Moor et al., 2011 40
McCaffery et al., 2009 41
de Geus et al., 2003 44
den Hoed et al., 2013 56
% variance explained
0 10 20 30 40 50 60 70 80 90 100

A MODEL TO EXPLAIN DIFFERENCES IN VOLUNTARY EXERCISE BEHAVIOR

In order to identify the mechanisms that give rise to the heritability of exercise behavior, De Geus & de Moor (2008) proposed a model in which the maintenance of exercise behavior is, based on the principles of instrumental conditioning, determined by the positive reinforcement or feelings of punishment (Figure 1.2). This model focuses on the genetic modulation of acute affective responses to exercise and longer-term effects on self-esteem through genetic effects on exercise ability.

The aim of this thesis is to put this model to use in an effort to explain the heritability of exercise behavior in adolescents and young adults. The likelihood of engaging in or maintaining exercise behavior might increase by the presence of genetic variants that amplify the feelings of pleasure, performance, or sense of accomplishment.

To this end, a laboratory study was set up. Over 200 adolescent twin pairs and their siblings were selected from the Netherlands Twin Register (van Beijsterveldt et al., 2013) and invited to participate. The experimental design included assessment of their exercise ability (aerobic fitness and muscle strength) and the affective response to various types of exercise on a treadmill and cycle ergometer. At the end of the session a maximal exercise test was performed. Details on the experimental protocol can be found in Chapter 3.

ZOOMING IN ON THE MODEL

In accordance with the Hedonic theory, which suggests that individuals repeat behavior regularly when it makes them feel good; a positive affective response to exercise will make a person more likely to repeat this activity, whereas repeated negative affective responses will lead to discontinuation of the behavior. Individuals for whom the net rewarding effects are dominant will repeat the behavior and become regular lifetime exercisers, whereas individuals that experience aversive effects of exercise might drop out of an exercise program (upper part Figure 1.2). Exercise induced positive affective responses ('feel good' experiences during or shortly after an exercise bout) may be an important contributor to appetitive effects of exercise. In contrast to the persistent general belief that exercise is enjoyable for everyone, strong individual differences are found in the affective responses during and after exercise. Some individuals report an increase in pleasure or no change and others report reduced

pleasure (Ekkekakis et al., 2005; Ekkekakis et al., 2011; Van Landluyt et al., 2000; Welch et al., 2007). De Geus & de Moor (2008) have hypothesized that these individual differences in part reflect differences in genetic sensitivity to the psychological effects of exercise. In **Chapter 4** the heritability of the affective responses during and after an exercise bout was estimated for the first time.

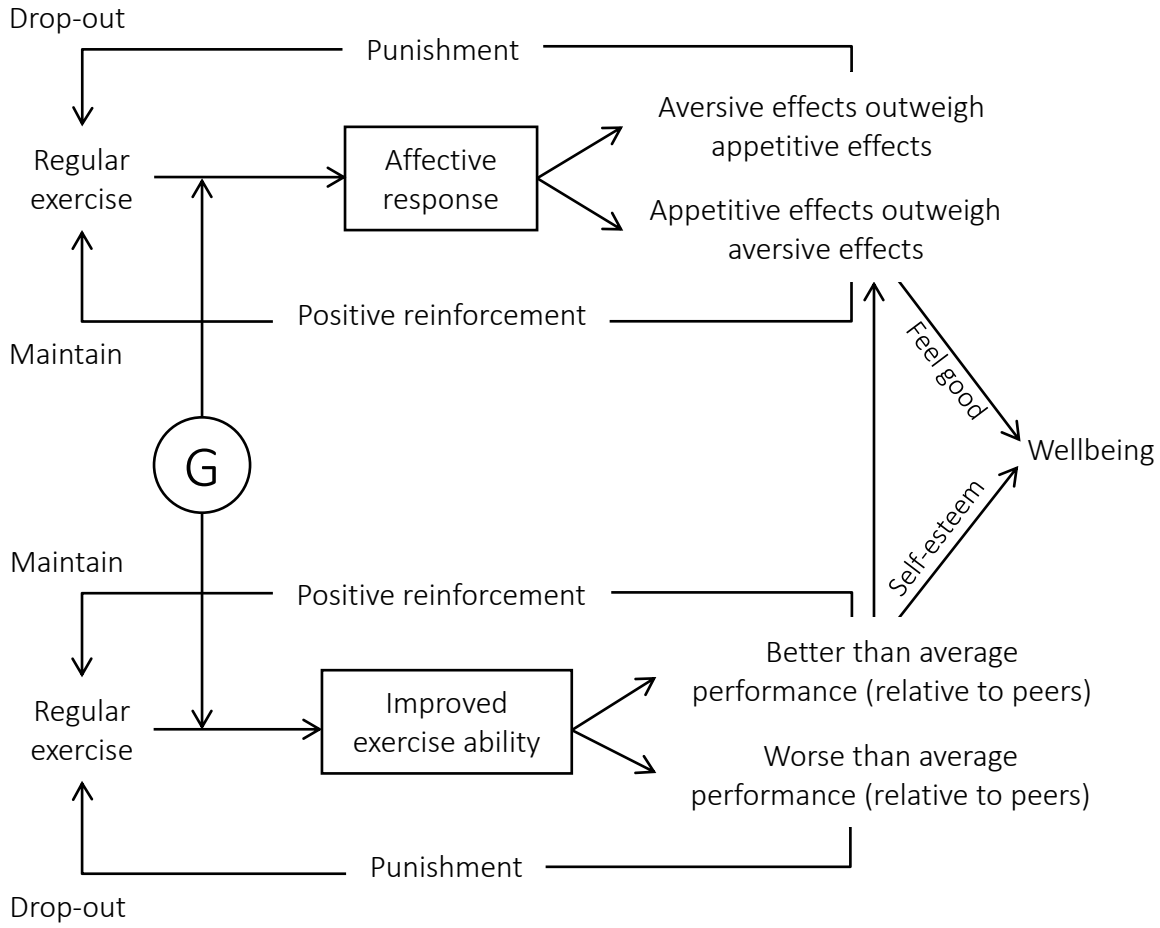


Figure 1.2 Model on the heritability of exercise behavior (de Geus & de Moor, 2008). G = Genes.

A second contributor to the net appetitive effects of exercise might be the improvement of exercise ability induced by regular exercise (lower part Figure 1.2). These improvements will not be the same for everyone and depend on trainability. This may impact on the appetitive effect of regular exercise. When an individual finds himself outperforming others, or gaining more rapidly than peers when exposed to comparable training regimes, this will lead to feelings of competence. Lower levels of performance and trainability might lead to disappointment or shame (particularly when the exercise is performed in a competitive context). This may be a strong factor in adolescence, when the sensitivity to one's own relative ranking among peers may be largest. Evidence for genetic influences on exercise ability and trainability is provided by many studies, for example the HERITAGE study (Bouchard et al., 1995). This and other studies provided evidence for genetic influences on cardiorespiratory fitness, muscle strength, balance, and flexibility. However, heritability estimates vary across samples and estimates for adolescents are not always available. To replicate and expand the literature on the genetic architecture of physical fitness components, we estimated the heritability of muscle strength measures (vertical jump and handgrip strength), balance, and flexibility in a large sample of adolescent twins and their siblings in Chapter 5. These estimates were incorporated in a meta-analysis on the heritability of muscle strength, flexibility and balance. In addition, Chapter 6 reports on the heritability of $\dot{V}O_{2max}$ in adolescents and to arrive at a robust estimate for the heritability of $\dot{V}O_{2max}$ in children to young adults, a sample size weighted meta-analysis was performed on all extant twin and sibling studies in adolescents and young adults.

Regular exercise is argued to be effective in reducing anxious and depressive symptoms and several meta-analyses indicate that exercise has an antidepressant effect in clinical populations. However, it is difficult to rule out that these findings can be explained by underlying (genetic) factors influencing both exercise behavior at one time point and influence symptoms of anxiety and depression at a later time point (a phenomenon also known as genetic pleiotropy). Earlier, Bartels et al. (2008) and De Moor et al. (2011) showed that in population-based twin studies the nature of the association between exercise and anxious-depressive symptoms is best explained by correlated genetic effects on these two traits. Chapter 7 shows that the model by De Moor & De Geus (2008) accommodates this

genetic correlation while still allowing exercise to causally increase wellbeing in specific subgroups of the population.

EXPANDING THE MODEL

The sports psychology literature has provided us with other factors that are also robustly correlated with regular exercise behavior, such as personality (Allender et al., 2006; de Moor et al., 2006; Hoyt et al., 2009; Rhodes & Smith, 2006) and perceived benefits and barriers (Allender et al., 2006; Hagger et al., 2002; Rhodes et al., 2009). Personality might influence the complex balance of appetitive and aversive effects induced by exercising. Regular exercisers score lower on neuroticism and higher on extraversion, conscientiousness, and sensation seeking. Furthermore, a positive attitude towards exercise and, consequently, the likelihood of maintaining exercise behavior increases when an individual perceives that the benefits of exercise outweigh the disadvantages. As these factors are proven to be heritable as well, they might all contribute to the heritability of voluntary exercise behavior in adolescents and young adults. Chapter 8 incorporates these factors in the model of De Geus & de Moor (2008).

Finally, Chapter 9 provides a summary of the main findings. In addition, we discuss these findings in a broader context. Attention to innate characteristics and biological mechanisms in the research on determinants of exercise behavior will provide new insights into how to best shape interventions. The results could allow for more stratified or personalized approaches that exploit genetic variation influencing exercise behavior in interventional strategies.

CHAPTER 2

GENETICS OF PHYSICAL ACTIVITY & EXERCISE BEHAVIOR: REVIEW AND META-ANALYSIS

BASED ON

Schutte NM, Bartels M & de Geus EJC (2017). Genetics of physical activity and physical fitness

In: Oxford Textbook of Children's Exercise Science and Medicine

Edited by Armstrong N & van Mechelen W. Oxford University Press UK

ABSTRACT

Because regular physical activity and exercise behavior are key contributors to children's health, it is important to understand the sources of variation in these phenotypes seen among children and adolescents. Twin and family studies provide the ability to calculate the relative importance of genetic and environmental factors to the observed individual differences. Heritability estimates of physical activity and exercise behavior vary, depending on sample size and measurement instrument, but the overall importance of environmental factors on exercise behavior seems to decrease in adolescence, whereas genetic effects become more prominent in explaining individual differences. A sample size weighted meta-analysis in children, adolescents and late-adolescents showed increasing meta-analytic heritability estimates of 20% (95% CI: 13, 27), 35% (95% CI: 17, 52), and 53% (95% CI: 47, 59) respectively. Some evidence is found for specific genes coding for physical activity and exercise behavior, but in children and adolescents these studies are limited. This should be a priority for future research because knowledge on the source of individual differences in physical activity at different time points during childhood and adolescence can optimize the choice and timing of exercise intervention.

INTRODUCTION

Regular physical activity is key contributors to children's health (Janssen & Leblanc, 2010). However, the majority of the youth does not engage in regular exercise at the recommended level (Martinez-Gonzalez et al., 1999; Troiano et al., 2008). Traditionally, the individual differences in an active lifestyle of children and adolescents have been explained by environmental and social factors, such as socioeconomic status (of the parents), health beliefs and support by peers and family (Bergstrom et al., 1996; Dishman et al., 1985; Drenowatz et al., 2010; Sallis et al., 2000). However, as is the case for many human (behavioral) traits, (Polderman et al., 2015) another major source of variation in physical activity is innate biological differences.

The contribution of genes to the differences in physical activity is under study for many decades, since the first heritability study by Kaprio et al. (1981) published in 1981 in a large sample of adult male twins. Twin and family studies provide the ability to calculate the relative importance of genetic and environmental factors to the observed individual differences. Evidence of familial aggregation of a behavioral trait can be found when this specific trait occurs more in members of a family than can be readily accounted for by chance. A twin design exploits the known differences in genetic similarity in monozygotic and dizygotic twins (or siblings) to separate the genetic effects (the heritability) from other factors that are shared by the family members (e.g. family environment, school).

Studying the heritable components of a trait such as physical activity is referred to as quantitative genetics. Whereas these family and twin studies provide a starting point in exploring the effects of genetic and environmental variance on a phenotype, molecular genetic studies aim to detect the genes underlying the heritability. Studies in animals are used to identify the genetic mechanisms underlying physical activity by means of selective breeding and (fine) mapping of genomic regions. The progress in molecular genetics makes it feasible to collect and analyze DNA on a large scale also in humans.

In this chapter the principles of family, twin, animal and molecular genetic studies are shortly introduced followed by an overview of published studies on the quantitative genetics and molecular genetic findings for physical activity and exercise behavior.

THE PRINCIPLES OF FAMILY, TWIN, ANIMAL AND MOLECULAR GENETIC STUDIES

Family studies

Familial aggregation is seen when the occurrence of the trait among relatives is substantially higher than that among non-relatives.(Liang & Beaty, 2000) For quantitative traits (i.e. continuous traits: the trait has a quantitative value), such as amount of physical activity, familial aggregation can be investigated by computing correlations among relatives such as siblings, parents and their offspring, grandparents and grandchildren, nieces, et cetera, depending on the extent of the pedigrees from which data are available. Most family studies use sibling and parent-offspring correlations. Siblings among each other and parents and their offspring share on average half of their genes in common. They also share a household, the neighborhood, and various other aspects of belonging to the same family (the so-called shared environment). Therefore, evidence of familial aggregation may be due to shared exposure to a risk factor, due to genetic factors, or result from a mixture of both. Thus, this familial resemblance includes both genetic and shared environmental sources of covariance. If the effect of shared environment can be assumed zero, the familial resemblance in the trait can be ascribed to genetic factors and can be used to estimate its heritability. When familial environmental factors influence the trait of interest as well, familial resemblance only provides us with an indication of the upper value of the traits' heritability.

Twin Studies

A more powerful design to disentangle the relative importance of environmental and genetic influences on a trait or behavior is the classical twin design. This design compares the intrapair resemblance between two types of sibling relationships; genetically identical twins or monozygotic (MZ), a result of division of a single fertilized egg during an early stage in embryonic development, and non-identical twins or dizygotic (DZ), resulting from two separate fertilized eggs. Consequently, MZ twins are genetically identical and the differences between the twins are due to person-specific environmental factors: experiences that one of the twins has and the co-twin does not. DZ twins share on average 50% of their genetic make-up. If MZ-resemblance for the trait of interest is higher than DZ-resemblance, this constitutes evidence for genetic influences (referred to as 'A') on the trait.

Twin studies decompose all phenotypic variance of the trait of interest in sources of genetic influences (A), shared environmental influences (influences shared with other family members e.g. upbringing; referred to as 'C') and person-specific influences (influences that are unique to the individual; referred to as 'E'). An important assumption is that the shared environmental effects are independent of zygosity (and thus equal for both MZ and DZ twins). Thus: the correlation between MZ twins (r_{MZ}) comprises A+C, whereas the DZ twin correlation (r_{DZ}) is an estimate of ½A+C. Following from this, a simple formula by Falconer (1960) computes the relative contribution of genetic influences (A) to the total variance, as twice the difference in MZ/DZ resemblance:

$$\text{Heritability} = 2(r_{MZ} - r_{DZ})$$

An alternative to this simple formula is the use of structural equation modelling to obtain a more precise estimate of heritability (Neale & Cardon, 1992). In contrast to familial aggregation studies that cannot separate genetic and familial environmental sources of variance, the classic twin design can separate how much of the variance in a trait is due to genetic effects (the heritability; A) and how much appears to be due to shared environmental effects (the shared environment; C).

Animal Studies

Artificial selection practices in animals have long provided proof of genetic influences on phenotypes. For example farmers selectively interbreed cattle that produce the most milk to increase production in offspring generations. The increased milk production in the offspring provides evidence of this trait being influenced by genetic factors and can be used to estimate its heritability. With regard to physical activity, Swallow *et al.* (Swallow et al., 1998) used selective breeding to create four lines of mice with high activity levels: 10 generations of selective breeding of voluntary wheel-running behavior resulted in an increase of approximately 75% in activity level compared with mice from control lines (Swallow et al., 1998).

Another way to show heritability of a trait is measuring this trait in different strains of inbred mice, growing up in identical environments. Systematically mating brother and sisters for 20 consecutive generations will results in isogenic (genetically identical strains) groups of mice

allowing the mean of the trait to be compared across different strains. With regard to daily wheel running Lightfoot et al. (2004) detected significant interstrain differences in 13 strains of inbred mice, which suggests that genetic background indeed plays a role in determining spontaneous daily wheel running activity in mouse strains.

Molecular Genetic Studies

After establishing heritability of a trait, the next step is to identify the genomic regions that contribute to the heritable trait variation. For quantitative traits it is likely that heritability reflects the additive effects of thousands of genetic variants in a manifold of different genes. Molecular genetic studies such as linkage analysis and association studies provide an opportunity to localize genetic variants and confirm its association with the trait of interest.

Linkage analysis examines whether specific genetic markers, positioned strategically across the entire genome, segregate jointly with traits in clusters of related individuals. The markers that are linked to the genomic region that influences the trait will be seen to segregate more frequently with the occurrence of this trait. The genomic region carrying such markers is likely to harbor causative genetic variants for the trait. Genetic linkage can easily demonstrated in breeding experiments in mice, when two mice that differ genetically for a trait of interest are crossed (parental line) and the segregation of genetic markers, along with phenotypic characteristics, can be followed in each of the offspring.

Genes of interest found in these animal studies can provide the first clues for conducting association studies in both animals and humans. Alternatively candidate genes can be selected based on known or inferred biological function that makes it plausible that they may predispose to the trait of interest. Association studies are similar to traditional epidemiological approaches in which an a priori hypothesis between exposure to a given factor, in this case, a genotype at a given locus, and trait is formulated: Candidate gene studies test the association of quantitative traits with the frequency of specific genetic variants, or compare the frequency of such variants in selected groups of low-scoring (unaffected controls) and high-scoring (affected cases) individuals.

QUANTITATIVE GENETICS OF PHYSICAL ACTIVITY & EXERCISE BEHAVIOR

Since the first twin study by Kaprio et al. (1981) on the heritability of physical activity, several studies provided evidence for genetic influences on physical activity. A number of studies measured total physical activity objectively with accelerometers, in a respiration chamber or with the double labelled water method. However, most twin and family studies used physical activity questionnaires (self-report) to quantify total physical activity. Surveys are a more convenient tool for epidemiological-scaled research, even though the correlation with accelerometry or doubly labelled water varies to a great extent (Chinapaw et al., 2010). The phenotypes used in these survey studies often captured rather different constructs: some measured sport participation specifically, with questionnaires including items such as: 'Do you participate in (moderate to vigorous) sports regularly?'. Others used questionnaires such as an activity record, in which subjects are asked to note the energy expenditure of the dominant activity of every 15 minutes using a list of categorized activities.

When discussing twin and family studies on physical activity, a distinction will be made between physical activity due to all possible sources (total physical activity) and physical activity due to sports participation in leisure time (voluntary exercise behavior). The distinction is not always clear. A large part of total physical activity is classified as light to moderate and will be due to transportation (walking, biking, standing) or many light work or household activities. This will typically not contain sports activities. Moderate to vigorous intensity (MVPA) activities are more ambiguous and may often include voluntary sports activities in leisure time. Therefore, studies reporting on moderate to vigorous physical activities will be discussed together with studies on voluntary exercise behavior.

All twin and family studies on the heritability of total physical activity in childhood or adolescent samples are summarized in Table 2.1, ordered by age. All twin and family studies on the heritability of MVPA or voluntary exercise behavior are summarized in Table 2.2.

Total physical activity

The heritability estimates found in family studies include both genetic and familial environmental sources of variance, and are therefore listed in a separate column in Table 2.1. Especially in younger children (up until the age of 11), accelerometers or doubly labelled

water were used to quantify physical activity (Butte et al., 2006; Cai et al., 2006; Fisher et al., 2010; Franks et al., 2005; Saudino & Zapfe, 2008; Wood et al., 2008). The family studies by Cai et al. (2006) and Butte et al. (2006) were largest in sample size and reported moderate to high estimates of familial aggregation. In addition, Saudino and Zapfe (Saudino & Zapfe, 2008) showed that 32% of the variation in total physical activity in 2 year old twins could be explained by genetic factors. The twin studies by Franks et al. (2005) and Fisher et al. (2010) did not find significant genetic factors, perhaps because the sample size was modest and the study could be underpowered to find small genetic influences. Indeed the MZ correlations in the study by Fisher et al. (2010) were slightly higher than DZ correlations. A more robust finding is the substantial part (35% to 73%) of the variance in physical activity in these twins that could be attributed to shared environmental factors: 35% to 73%.

Four studies reported heritability estimates of physical activity measured by surveys (de Chaves et al., 2014; Maia et al., 2002; Perusse et al., 1989; Seabra et al., 2014). The mean age in these studies was 13 to 17 years old; indicating that self-report of physical activity is feasible in adolescence. The heritability estimates range from 6% for work-related physical activity (Seabra et al., 2014) to 63% for leisure time physical activity (Maia et al., 2002).

Table 2.1 Heritability of physical activity: an overview of twin and family studies. *Note.* A = variance explained by genetic factors; C = variance explained by shared environmental factors; TPA = Total Physical Activity; PAEE = Physical Activity Energy Expenditure; PAL = Physical Activity Level; LPA = Low Physical Activity; MPA = Moderate Physical Activity; WPA = Work Physical Activity; LTPA = Leisure Time Physical Activity; [a] Adjusted for age; [b] Adjusted for sex; [c] Adjusted for body weight; [d] Adjusted for SES; [e] boys/girls.

Age Mean (±SD)	Reference	Sample	Instrument	Pheno-type	Familial aggregation	A	C
2.1 (±0.1)	Saudino & Zapfe, 2008	144 MZ pairs / 168 DZ pairs	Accelerometer	TPA		31%	55%
6.8 (±1.4)	Franks et al., 2005	62 MZ pairs / 38 DZ pairs	Doubly labeled water	PAEE[ab]		41%	35%
			Doubly labeled water	PAEE[abc]		0%	69%
			Doubly labeled water	PAL[ab]		0%	65%
8.5 (±0.4)	Wood et al., 2008	150 MZ pairs / 113 DZ pairs	Accelerometer	TPA		35%	40%
10.9 (±0.2)	Butte et al., 2006	319 families	Accelerometer	TPA[ab]	60%		
11.0 (±3.9)	Cai et al., 2006	319 families	Accelerometer	TPA[ab]	55%		
				LPA[ab]	46%		
				MPA[ab]	49%		
11.2 (±0.5)	Fisher et al., 2010	57 MZ pairs / 60 DZ pairs	Accelerometer	TPA		0%	73%
12.7 (±3.5)	Chaves et al., 2014	260 families	Baecke Questionnaire	TPA[abd]	24%		
14.6 (±3.3)	Perusse et al., 1989	375 families; 55 MZ pairs / 56 DZ pairs	3-day activity record	TPA[abd]		29%	0%
14.5 (±2.8)	Santos et al., 2014	339 families	Baecke Questionnaire	TPA[ab]	23%		
16.1 (±4.0)	Seabra et al., 2008	2375 families	Baecke Questionnaire	TPA[abd]	23%		
				WPA[abd]	6%		
				LTPA[abd]	25%		
16.9 (±5.6)	Maia et al., 2002	203 MZ pairs / 208 DZ pairs	Baecke Questionnaire	LTPA		63%/32%[f]	0%/38%[f]

Voluntary exercise behavior

Fifteen twin and/or family studies reported heritability estimates for voluntary exercise behavior or moderate to vigorous physical activity. Heritability estimates vary widely, ranging from 0% to 85%. Possible sources of this variation are differences in the age, differences in measurement instrument and sample size. The age of all studies shown in Table 2.2 ranges from 4 (Cai et al., 2006) to 25 (Maia et al., 2002). Up to 12 years of age, heritability estimates are low to moderate (Cai et al., 2006; Fisher et al., 2010; Huppertz et al., 2016). In adolescence, heritability estimates of voluntary exercise behavior are moderate to high with the exception of two studies in which heritability estimates are low or zero (Stubbe et al., 2005). Nevertheless, the importance of shared environmental factors seems to decrease in adolescence, whereas genetic effects become more prominent in explaining individual differences in voluntary exercise behavior. In adults, heritability of voluntary exercise behavior levels off to about 40% (de Geus et al., 2003; de Moor et al., 2011). The changing genetic architecture of voluntary exercise behavior across the life span has been described before (Huppertz et al., 2016; Stubbe et al., 2005; Stubbe & de Geus, 2009). The notion that shared environmental factors play a greater role in childhood than adolescence can be explained by the important role of the parents; they provide the children with the opportunity to become active, by means of transportation to exercise activities, give exercise activities the priority over other leisure time activities and motivation and encouragement to exercise.

Two studies employed accelerometers (Actiwatch or Actigraph) to quantify moderate to vigorous physical activity in children (~11 year olds) (Cai et al., 2006; Fisher et al., 2010). The heritability estimates were low, but Fisher et al. (2010) demonstrated significant influences of shared environmental factors (61%). Two studies using prospective 3-day activity recording, which may be more accurate then retrospective surveys, reported no significant influence of genetic factors (Perusse et al., 1989; White et al., 2014) However, the majority of the studies specifically measured voluntary exercise behavior by starting their surveys with items similar to “Do you participate in sports regularly?” These studies generally found evidence of significant genetic influences. By comparing the heritability estimates of voluntary exercise behavior (Table 2.2) with the estimates for total physical activity (Table 2.1) one can conclude that the part of the variation in adolescents that can be attributed to genes appears higher in

voluntary exercise behavior than in total physical activity. It is important to note that such a finding could be driven in part by higher measurement error in self-reported total physical activity compared to in self-reported voluntary exercise behavior. Consciously planning of exercise activities is easier to recall than how much energy is spent on activities at school or commuting. This might introduce more measurement error in self-reported total physical activity surveys, which will inflate the environmental contribution (E) to the variance in this trait. Consequently, the relative contribution of genetic contribution to the total variance, i.e. the heritability decreases.

A meta-analysis on exercise behavior

All heritability estimates in Table 2.3, based on a twin sample, were included in three meta-analyses: 7 to 12 year olds (childhood), 13 to 15 year olds (adolescence) and 16 to 18 year olds (late-adolescence). By weighing these heritability estimates from all studies by the number of subjects, the weighted average heritability can be computed using Microsoft Excel (2010) (Li et al., 2003; Neyeloff et al., 2012). When the standard errors (SEs) or confidence intervals (CIs) of the heritability estimates were not reported, these were calculated using the standard errors (SEs) or CIs from studies who did report these statistics (Li et al., 2003). Some studies reported one (equated) heritability estimate for boys and girls; others estimated the heritability of exercise behavior for boys and girls separately. These heritability estimates for boys and girls were treated as if these were independent samples. The I^2 statistic was used to assess heterogeneity and was calculated as (Q – df)/Q, where Q is Cochran's heterogeneity statistic and df the degrees of freedom (Higgins & Thompson, 2002).

Results of the meta-analyses are presented in Table 2.3. In childhood, the meta-analytic weighted average heritability was 20% (95% CI: 13, 27). Table 2.3 shows that the confidence intervals for the results of individual studies do show overlap with the confidence interval for the meta-analytic average, indicating the presence of statistical homogeneity. This meta-analytic average increased to a heritability estimate of 35% (95% CI: 17, 52) in adolescence, with an I^2 value of 22, which suggests that a percentage of the variability in the heritability estimates in adolescents is due to heterogeneity rather than sampling error (chance). Table 2.3 shows higher heritability estimates for boys than for girls in the studies by van der Aa et al (2010) and Beunen & Thomis (1999), which may explain this heterogeneity. In late-

adolescence, a meta-analytic weighted average heritability of 53% (95% CI: 47, 59) was found, and the studies included in this meta-analysis were less heterogeneous (I^2 = 10). The results from these meta-analyses confirm increasing influence of genetic factors with age on exercise behavior.

Table 2.2 Heritability of exercise behavior: an overview of twin and family studies. *Note.* A = variance explained by genetic factors; C = variance explained by shared environmental factors; EB = Voluntary Exercise Behavior; VPA = Vigorous Physical Activity; MVPA = Moderate to Vigorous Physical Activity; [a] Adjusted for age; [b] Adjusted for sex; [c] Adjusted for SES; [d] boys/girls.

Age Mean (±SD)	Reference	Sample	Instrument	Phenotype	Familial aggregation	A	C
7.5 (±0.3)	Huppertz et al., 2016	2535 MZ pairs / 4796 DZ pairs	Multiple survey items	EB		14%/12%[d]	80%/80%[d]
9.8 (±0.4)	Huppertz et al., 2016	2784 MZ pairs / 5223 DZ pairs	Multiple survey items	EB		26%/26%[d]	68%/65%[d]
11.0 (±3.9)	Cai et al., 2006	319 families	Accelerometer	VPA[ab]	18%		
11.2 (±0.5)	Fisher et al., 2010	57 MZ pairs / 60 DZ pairs	Accelerometer	MVPA[ab]		0%	61%
12.3 (±0.4)	Huppertz et al., 2016	5281 MZ pairs / 9348 DZ pairs	Multiple survey items	EB		31%/27%[d]	62%/65%[d]
12.4 (±1.4)	White et al., 2014	72 MZ pairs / 76 DZ pairs	3-day activity record	MVPA		0%/0%[d]	66%/33%[d]
13/14	Stubbe et al., 2005	276 MZ pairs / 370 DZ pairs	Multiple survey items	EB		0%	84%
14.5 (±0.3)	van der Aa et al., 2010	554 MZ pairs / 948 DZ pairs	Multiple survey items	EB		85%/38%[d]	0%/46%[d]
14.6 (±3.3)	Perusse et al., 1989	375 families; 55 MZ pairs / 56 DZ pairs	3-day activity record	MVPA[abc]		0%	12%
14.6 (±0.6)	Huppertz et al., 2016	3325 MZ pairs / 5705 DZ pairs	Multiple survey items	EB		43%/40%[d]	36%/43%[d]
15.0	Beunen & Thomis 1999	43 MZ pairs / 61 DZ pairs	Single survey item	EB		83%/44%[d]	0%/54%[d]
15/16	Stubbe et al., 2005	321 MZ pairs / 442 DZ pairs	Multiple survey items	EB		0%	78%
16.1 (±4.0)	Seabra et al., 2014	2375 families	Baecke Questionnaire	EB[abc]	50-60%		
16.2	Aaltonen et al., 2013	769 MZ pairs / 1743 DZ pairs	Single survey item	EB		52%/52%[d]	19%/24%[d]
16.2 (±0.6)	van der Aa et al., 2010	662 MZ pairs / 969 DZ pairs	Multiple survey items	EB		80%	0%
16.4 (±1.1)	de Moor et al., 2011	1736 families; 656 MZ pairs / 1628 DZ pairs	Multiple survey items	EB		42%/36%[d]	44%/52%[d]
16.7 (±2)	de Geus et al., 2003	69 MZ pairs / 88 DZ pairs	Multiple survey items	MVPA[a]		79%	0%
16.9 (±0.6)	Huppertz et al., 2016	2320 MZ pairs / 3698 DZ pairs	Multiple survey items	EB		56%/49%[d]	27%/31%[d]
16.9 (±5.6)	Maia et al., 2002	203 MZ pairs / 208 DZ pairs	Baecke Questionnaire	EB		68%/40%[d]	20%/28%[d]
17.0 (±2.1)	Boomsma et al., 1989	44 MZ pairs / 46 DZ pairs	Single survey item	EB		64%	0%
17.1	Aaltonen et al., 2013	724 MZ pairs / 1614 DZ pairs	Single survey item	EB		44%/50%[d]	24%/26%[d]
17/18	Stubbe et al., 2005	248 MZ pairs / 395 DZ pairs	Multiple survey items	EB		36%	47%
18.8 (±0.5)	Huppertz et al., 2016	1118 MZ pairs / 1641 DZ pairs	Multiple survey items	EB		79%/49%[d]	4%/19%[d]
18.0 (±2.3)	Koopmans et al., 1994	1593 families: 578 MZ pairs / 1000 DZ pairs	Single survey item	EB		45%	44%
18.1 (±0.7)	van der Aa et al., 2010	488 MZ pairs / 747 DZ pairs	Multiple survey items	EB		72%	0%
18.6	Aaltonen et al., 2013	715 MZ pairs / 1603 DZ pairs	Single survey item	EB		46%/51%[d]	23%/21%[d]

Age group	Refence		Age mean	(SD)	Sample size	Heritability (95% CI)	
7 - 12	Huppertz et al., 2016	boys	7.5	(±0.3)	3694	14	(6, 22)
	Huppertz et al., 2016	girls	7.5	(±0.3)	3637	12	(3, 21)
	Huppertz et al., 2016		9.8	(±0.4)	8006	26	(20, 32)
	Fisher et al., 2010		11.2	(±0.5)	234	0	(-34, 34)
	Huppertz et al., 2016	boys	12.3	(±0.4)	7185	31	(25, 37)
	Huppertz et al., 2016	girls	12.3	(±0.4)	7443	27	(21, 33)
	White et al., 2014		12.4	(±1.4)	518	0	(-23, 23)
	Meta-analysis				**30717**	**20**	**(13, 27)**
13 - 15	Stubbe et al., 2005		13/14		1332	0	(-14, 14)
	van der Aa et al., 2010	boys	14.5	(±0.3)	1162	85	(78, 90)
	van der Aa et al., 2010	girls	14.5	(±0.3)	1476	38	(22, 57)
	Perusse et al., 1989		14.6	(±3.3)	244	0	(-33, 33)
	Huppertz et al., 2016	boys	14.6	(±0.6)	3998	43	(35, 51)
	Huppertz et al., 2016	girls	14.6	(±0.6)	5031	40	(33, 47)
	Beunen & Thomis 1999	boys	15		92	83	(66, 89)
	Beunen & Thomis 1999	girls	15		91	44	(21, 91)
	Stubbe et al., 2005		15/16		1526	0	(-13, 13)
	Meta-analysis				**14952**	**35**	**(17, 52)**
16 - 18	Aaltonen et al., 2013		16.2		5024	52	(42,55)
	van der Aa et al., 2010		16.2	(±0.6)	2726	80	(76, 84)
	de Moor et al., 2011	boys	16.4	(±1.1)	1636	42	(29, 55)
	de Moor et al., 2011	girls	16.4	(±1.1)	1889	36	(24, 48)
	de Geus et al., 2003		16.7	(±2.0)	314	79	(69, 85)
	Huppertz et al., 2016	boys	16.9	(±0.6)	2624	56	(46, 66)
	Huppertz et al., 2016	girls	16.9	(±0.6)	3393	49	(40, 58)
	Maia et al., 2002	boys	16.9	(±5.6)	361	68	(42, 89)
	Maia et al., 2002	girls	16.9	(±5.6)	461	40	(-10, 73)
	Boomsma et al., 1989		17	(±2.1)	180	64	(26, 102)
	Aaltonen et al., 2013	boys	17.1		2137	44	(37, 53)
	Aaltonen et al., 2013	girls	17.1		2539	50	(44, 53)
	Stubbe et al., 2005		17/18		1286	36	(11, 64)
	Huppertz et al., 2016	boys	18.8	(±0.5)	1040	79	(63, 95)
	Huppertz et al., 2016	girls	18.8	(±0.5)	1719	49	(37, 61)
	Koopmans et al., 1994		18	(±2.3)	3156	45	(36, 54)
	van der Aa et al., 2010		18.1	(±0.7)	1864	72	(65, 77)
	Aaltonen et al., 2013	boys	18.6		2105	46	(40, 53)
	Aaltonen et al., 2013	girls	18.6		2551	51	(42, 63)
	Meta-analysis				**37005**	**53**	**(47, 59)**

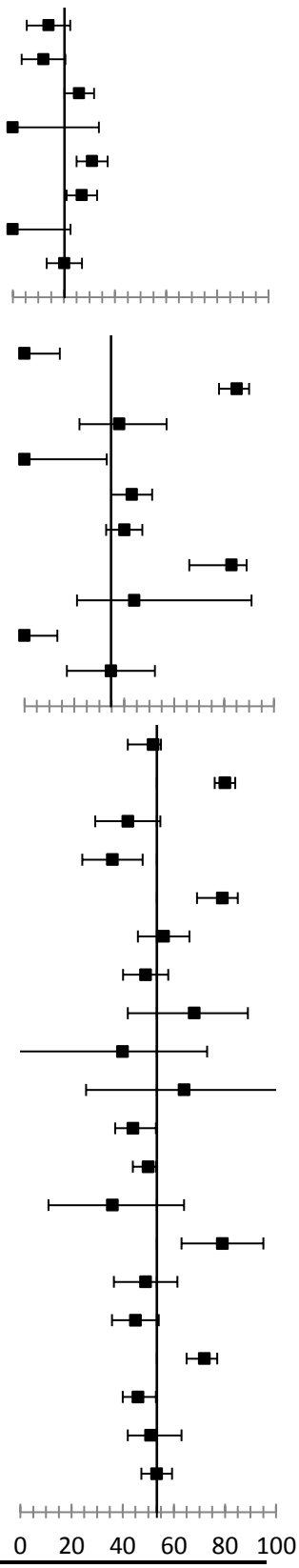


Table 2.3 Results meta-analyses of exercise behavior in three age groups: 7 – 12 year, 13 – 15 year and 16 – 18 year

MOLECULAR GENETIC FINDINGS FOR PHYSICAL ACTIVITY & EXERCISE BEHAVIOR

Studies with spontaneous wheel-running inbred mice-strains and selective breeding in mice for high voluntary wheel-running activity resulted in numerous genomic regions that were associated with physical activity in mice. For instance, Lightfoot et al. (2008) identified four genomic regions that were associated with the distance, duration, and speed of voluntary wheel-running on chromosomes 9 and 13, with the genomic locus for running speed (on chromosome 9) accounting for the largest percentage of phenotypic variance. The research group by Garland found even more loci to be associated with these phenotypes, (Kelly et al., 2010; Nehrenberg et al., 2010) but the only overlap they reported were loci close to the *TYR* gene on chromosome 7 coding for tyrosinase, a precursor for the neurotransmitter dopamine, found be involved in voluntary movement and reward (Rhodes et al., 2005).

In spite of the evidence for a contribution of heritable factors to physical activity from twin and family studies, surprisingly little work has been done to identify the actual genes contributing to this heritability of physical activity and exercise behavior in humans. Even less candidate gene studies have specifically addressed physical activity in children. Lorentz et al. (Lorentzon et al., 2001) found that in a sample of 97 healthy Caucasian girls (mean age 16.9) the A986S polymorphism in the calcium sensor receptor gene (*CASR*) was significantly associated with self-reported physical activity level. This *CASR* gene is involved in the regulation of calcium homeostasis and bone resorption. *CASR* mRNA is also expressed in the hypothalamus of the rat brain, a region that had been associated with regulating motivation. In 7 year old boys, self-reported physical activity level was associated with the Gln223Arg polymorphism in the leptin receptor (*LEPR*) gene, (Richert et al., 2007) known to regulate food intake and energy balance (Elmquist et al., 1998). Physical activity measured for 3 days with an Actiwatch accelerometer in 10 year-olds was associated with variants within the Melanocortin 4 Receptor Gene (*MC4R*), (Cole et al., 2010) a gene associated with weight-related phenotypes. Two studies in 15 and 16 year old children did not find a significant

association of a common variant in the *FTO* gene (rs9939609) and self-reported physical activity, as well as physical activity objectively measured with Actigraphs (Hakanen et al., 2009; Liu et al., 2010).

Candidate genes suffer from the shortcoming that they are based on our current biological knowledge. A more agnostic and open study design to find genetic variants associated with a trait of interest is a genome wide association (GWA) study. Using millions of measured or imputed SNP markers the entire genome is searched for SNP variants that occur more frequently in people with higher levels of the trait of interest compared to people with lower trait level. However, no GWA study in children has been conducted to date. The only GWA study conducted on exercise behavior published by de Moor et al. (De Moor et al., 2009) was entirely based on an adult sample. In 1644 unrelated Dutch and 978 unrelated American adults of European ancestry several novel variants were associated with exercise behavior, mainly in the *PAPSS2* gene. The effect sizes were small, such that these variants did not contribute much to the heritability of exercise behavior and would not have reached significance according to the current GWA standards (Clarke et al., 2011). In hindsight, lessons learned from GWA studies on other complex traits make it likely that this study on exercise behavior was underpowered to detect the many small genetic effects causing heritability (Klein, 2007). Meta-analyses across a total sample size of tens of thousands of individuals will be needed for successful detection of the association of specific genetic variants with a physically active lifestyle.

IMPLICATIONS FOR PEDIATRICS

The evidence that the variance in physical activity and especially exercise behavior are under substantial genetic control does not mean that it is impossible to increase the amount of physical activity and exercise activities to improve sports performance and health in children and adolescents. These findings should, however, contribute to the acknowledgment that the substantial range in physical activity and exercise behavior in population-based samples of children and adolescents will *not* be erased by exercise intervention. We argue that this should never be a goal to begin with: intervention is about shifting the mean of the distribution towards a more favorable value, not about reducing its variance.

To encourage adolescents to stay active, the innate individual differences can be used as a starting point. Children may experience rather different 'gains' when exercising or adopting a physically active lifestyle: by being good at sports some adolescents may gain self-esteem, whereas others who are less good at sports but greatly enjoy the activity or its social aspects reap a different benefit. Acknowledgement of these differences in gains may aid in abandoning population-based strategies and moving towards personalized or family-based intervention strategies. Information on the source of individual differences at different time points during childhood and adolescence can inform type and timing of the optimal intervention approaches. To achieve the same aim of optimizing the appetitive aspects that are specific for that individual and generating realistic person-specific goals, different genotypes may require entirely different exercise programs.

Within the last decades, we have already discovered much on the genetic characteristics of physical activity and exercise behavior. Taken the fast increase in knowledge of genomics and the enhanced technological aids for prolonged physical activity recordings in large scale samples we can expect even more progress in the coming decade. This will lead to better understanding of the genetic and environmental determinants of physical activity and exercise behavior in the young, which in turn will expand our capability to improve pediatric health.

CHAPTER 3

DATA COLLECTION

The Netherlands Twin Register (NTR) was set up more than 25 years ago at the VU University in Amsterdam (Boomsma et al., 2006; van Beijsterveldt et al., 2013; Willemsen et al., 2013). The aim of the NTR is to examine the underlying causes of individual differences in personality, growth, development, disease and risk factors for disease. For the majority of the multiples and their families (parents, siblings, and children) longitudinal data are available. The NTR holds a unique dataset for epidemiological-scaled research on the genetic architecture of behavior and diseases. Subject recruitment and data collection is still ongoing and the longitudinal database is growing. At this moment, more than 200.000 individuals are enrolled in the NTR.

The twin-families that were invited for the laboratory study are part of the Young Netherlands Twin Register (YNTR; van Beijsterveldt et al., 2013). The YNTR recruits twins and their family members upon birth. Shortly after giving birth, mothers receive a first survey with items on pregnancy and birth. At age 2, a survey on growth and achievement of milestones is sent. At ages 3, 7, 9/10, and 12 parents and teachers receive a series of surveys that are targeted at the development of emotional and behavior problems. From age 14 years onward, adolescent twins and their siblings self-report on their behavior problems, health, and lifestyle. In sub-groups of different ages, in-depth phenotyping is conducted for instance for IQ, MRI, growth, hormones, neuropsychological assessments, and cardiovascular measures. This thesis is based on a new extensive laboratory study of an adolescent sub-group of the NTR. In this chapter the data collection for this new study will be described in more detail including response rates and the procedure that was followed.

RECRUITMENT AND SAMPLE

Pilot study

Data collection was started with a pilot phase in which the experimental protocol was tested and fine-tuned. Subjects, mainly from an undergraduate student population, were recruited by means of advertisement throughout the university. They received study credits as part of their curriculum or a €10 gift voucher for their participation. To be eligible for the study, subjects had to have no history of cardiovascular or respiratory disease, and being physically

capable of engaging in exercise activities. This was verified by a short questionnaire by email consisting of questions about health and disease, physical disabilities, history of fainting and palpitations, (family) history on cardiovascular and use of medication. If eligible, a date was set for laboratory testing.

The total sample consisted of 74 subjects: 6 sibling pairs, 2 (monozygotic) twin pairs and 58 singletons having a mean (±SD) age of 21.9 (±4.4). 58 subjects completed the entire protocol; 1 subject did not perform the maximal exercise test (excluded because of a positive family history of sudden cardiac death) and 15 subjects were administered the maximal exercise test only.

After completing the pilot study, the initial protocol was largely retained, except for some minor changes in the order of all the tests included in the protocol (for the final protocol, see Table 3.2).

NTR sample

555 healthy adolescent twin pairs aged between 16 and 18, enrolled in longitudinal survey studies of the Netherlands Twin Register (van Beijsterveldt et al., 2013), were invited to participate in the study on the determinants of adolescent exercise behavior. Siblings of the twins within an age range of 12 – 25 years were also invited. Selection for invitation was based on the availability of longitudinal survey data on zygosity and regular leisure time exercise behavior. The aim was to have sufficient twins present from the entire spectrum of sedentary to vigorous leisure time exerciser and for each zygosity group. We started with a random selection, but if a zygosity group was underrepresented or if there were too little sedentary or vigorous exercisers, invitations were biased towards the underrepresented groups. This was mainly the case for dizygotic twins, siblings, and sedentary subjects. Regarding the latter, we selected twin probands who reported no engagement in exercise behavior on a previously filled out survey. The co-twin was then selected as well, regardless of exercise status. In order to be eligible for the study, subjects had to have no history of cardiovascular or respiratory disease, and being physically capable of engaging in exercise activities. In addition, all invitees had to be able and willing to visit the VU University in Amsterdam for lab testing.

Participants were invited by sending a letter and information brochure (Appendix A) advertising the opportunity to test their fitness in addition to earning a gift voucher. If subjects were under 18, both the parents and under aged twin/siblings received a letter. Within a week of receiving the invitation letter, the invitees or (if invitees were under 18) their parents were contacted by telephone to ask whether they were interested in participating in the study. If so, a short interview was administered consisting of questions about health and disease, physical disabilities, history of fainting and palpitations, (family) history on cardiovascular disease and use of any medication. Exclusion criteria were: less than two members of a family were willing to participate, physical disability preventing participation in moderate to vigorous sports, regular fainting (as a result from moderate to vigorous intensity physical activity), family history of sudden death or known ECG abnormalities, any regular use of cardio active medication or antidepressants. When the invitee proved eligible for the study, an appointment was made.

Between May 2012 and December 2014 a total of 499 subjects from 226 families participated in the study. This corresponds to a response rate of 41% for all invited families. Table 3.1 shows the numbers of families invited and participated. To keep an optimal balance between MZ and DZ twins, males and females, exercises and non-exercisers, and twins and siblings, invitations in several waves of data collection were tailored towards specific groups. One twin pair only completed the first 10 minutes of the protocol. The final sample consisted of 497 subjects (mean age 17.1±1.1) of which 227 complete twin pairs: 58 monozygotic male pairs (MZM, of which 13 pairs participated with 1 sibling and 1 pair with 2 of their siblings), 36 dizygotic male pairs (DZM, of which 3 pairs participated with 1 sibling), 58 monozygotic female pairs (MZF, of which 15 pairs participated with 1 sibling and 2 pairs with 2 of their siblings), 42 dizygotic female pairs (DZF, of which 2 pairs participated with 1 sibling), 33 dizygotic opposite sex pairs (DOS). Two additional sibling pairs participated (without a twin). Five subjects only completed the maximal exercise test (2 MZF pairs of which 1 participated with 1 sibling) one subject (DZF twin) did not perform the maximal exercise test (due to a knee injury), and one subject (sibling of MZM pair) did not perform the treadmill test (because of the lack of proper running shoes during the session). Two subjects (sibling of DZF pair and the male-twin of a DOS pair) did not have Cosmed K4b^2 data and one subject (MZF twin) did not have VU-AMS data due to equipment malfunctioning.

Table 3.1 Response rates NTR sample.

Wave #	Invited families	Participated families	%	Selection tailored towards
Wave 1 (April 2012)	99	49	49.5%	-
Wave 2 (May 2012)	98	50	51.0%	-
Wave 3 (July 2012)	99	13	13.1%	DZ; non-exercisers
Wave 4 (October 2012)	50	16	32.0%	DZM
Wave 5 (January 2013)	88	41	46.6%	siblings; non-exercisers
Wave 6 (March 2013)	87	44	50.6%	siblings
Wave 7 (June 2013)	34	13	38.2%	DZ
Total	555	226	40.7%	

Note: All invitees were selected on the availability of longitudinal survey data. Four additional NTR families volunteered to participate without receiving a formal invitation but by means of word-of-mouth advertising. DZ = dizygotic twins, DZM; dizygotic male twins.

Informed Consent

All subjects above 18 provided written consent and if the subjects were under 18 consent was given by both of their parents/guardians and assent by the subjects. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the VU University Medical Center Amsterdam (NL35634.029.10).

EXPERIMENTAL PROTOCOL LABORATORY STUDY & MEASUREMENTS

Briefing

On arrival at the laboratory, subjects and their parents were briefed about the procedure. Hereafter, the informed consent forms were collected. If present, the parents were asked to wait in the waiting room. The twins were tested in separate experimental rooms.

Body composition and physical fitness tasks

The protocol (Table 3.2) started with measurements of height and weight using a calibrated scale (Omron BF511, Omron Healthcare Europe B.V., The Netherlands). Subsequently, 4

fitness characteristics were examined: balance, handgrip strength, flexibility and explosive strength.

Balance – The Balance Error Scoring System (BESS, Bell et al., 2011) was used to assess balance under 3 testing stances: double leg, single leg (non-dominant leg) and tandem (dominant foot in front of the non-dominant foot in heel-to-toe fashion, weight evenly distributed across both feet) on 2 surfaces (ground and foam pad). During the test, the eyes were closed and the hands were held on the hips. Each condition lasted for 20 seconds. We instructed the subjects that if at any time they fell out of position, they were to return to the test position as quickly as possible. As the subjects performed each 20-second trial, we observed and recorded the number of errors each subject made. An error was defined as opening eyes, lifting hands off the hips, stepping, stumbling or falling out of position, lifting forefoot or heel, abducting the hip by more than 30°, or failing to return to the test position in less than 5 seconds. The total score was the total number of errors across the six 20-sec repeated measures (three stances and two surfaces).

Handgrip strength – Subjects were instructed to hold a dynamometer (Baseline Digital Smedley Hand dynamometer, Fabrication Enterprises Inc., USA) in the dominant hand with arm at the side of the body and elbow at a 90° angle. When ready, the subject was encouraged to squeeze the dynamometer once with maximum effort (in kg), which should be maintained for 5 seconds. This procedure was repeated for the non-dominant hand.

Flexibility – Flexibility was measured using a standard sit-and-reach box (Baseline Sit-and-reach Trunk Flexibility Box, Fabrication Enterprises Inc., USA). Subjects were instructed to sit on the floor with the legs fully extended and the soles of the feet flat against the box. One hand was placed on top of the other, palms down. Then the subject reached forward along the measuring scale on the box as far as possible, without bending the knees. Best out of 3 reaches (in centimeters) was used for subsequent analyses.

Table 3.2 Overview of the experimental protocol.

Activity	Minutes	Measurements
Height/weight	1	
Balance, handgrip strength, flexibility and vertical jump	10	
Connecting VU-AMS	7	Continuous ECG[a], ICG[b] and tri-axial accelerometer signals
Lifestyle interview	10	Exercise behavior, educational attainment, work status, subjective health, medication and contraception use, well-being, smoking, alcohol use, sleep duration.
Delay discounting questionnaire	5	Discounting rate *k*
Baseline: quiet sitting	6	FS[c] AD-ACL[d]
Connecting Cosmed	7	Breath-to-breath values on $\dot{V}O_2$[e], $\dot{V}CO_2$[f] and V_E[g]
Warming up on cycle ergometer at 50W	2	
Cycling on cycle ergometer at 40W or 70W[h]	5	FS RPE[i]
Cycling on cycle ergometer at 60W or 90W	5	FS RPE
Cycling on cycle ergometer at 80W or 110W	5	FS RPE
Cycling on cycle ergometer at 100W or 130W	5	FS RPE
Cool down	1	FS
Recovery: quiet sitting	5	FS AD-ACL
Warming up on treadmill at 0 – 5 km/h	1	
Walking/jogging on treadmill at 5.5 km/h or 6.0 km/h[j]	5	FS RPE
Walking/jogging on treadmill at 6.0 km/h or 6.5 km/h	5	FS RPE
Walking/jogging on treadmill at 6.5 km/h or 7.0 km/h	5	FS RPE
Walking/jogging on treadmill at 7.0 km/h or 7.5 km/h	5	FS RPE
Cool down	1	FS
Recovery: quiet sitting	5	FS AD-ACL
Maximal exercise test	6 – 12	
Cool down	5	FS
Recovery: quiet sitting	>2	
Showering	15	AD-ACL (after showering)

Note .[a]Electrocardiogram; [b]Impedance cardiogram; [c]Feeling Scale; [d]Activation-Deactivation Adjective Checklist; [e]oxygen consumption; [f]carbon dioxide production; [g]minute volume; [h]Females started at 40W, males started at 70W; [i]Rating of Perceived Exertion (Borg Scale); [j]Females started at 5.5 km/h, males started at 6.0 km/h.

Explosive strength – Explosive strength was measured with a vertical jump test that requires the subjects to jump as high as possible, starting from a position of knee bending at a fixed knee angle immediately prior to the jump. Subjects were instructed to jump straight up as much as possible and not go sideways. It was allowed to use the arms to help drive the body upwards. A successful jump was defined as one where at take-off the subjects had the appropriate knee angle and landed their feet within a 10 cm radius of the start position. Jumping height was defined as the vertical displacement between the trunk at the beginning and at the end of the jump measured by the displacement of a measuring tape attached to the subjects' hip and clipped to the floor. Best out of 3 jumps was documented (jumping height in centimeters).

Table 3.3 shows the means and standard deviations (SDs) of age, anthropometrics, and measures of physical fitness for males and females in the pilot and NTR study.

Attachment of the VU-AMS5fs

Next, subjects were equipped with the VU-AMS5*fs* (VU University, Amsterdam, The Netherlands). This device was developed to study autonomic nervous system activity in naturalistic settings (de Geus et al., 1995). The version used here measured the electrocardiogram (ECG) together with the impedance cardiogram (ICG) from five disposable, pregelled Ag/AgCl electrodes (Figure 3.1). Electrode resistance was kept low by cleaning the skin with alcohol and rubbing. A combined ECG/ICG electrode was placed on the sternum over the first rib between the two collarbones. A second ECG electrode was placed at the apex of the heart over the ninth rib on the left lateral margin of the chest approximately 3 cm under the left nipple. A second ICG electrode was placed over the tip of the xiphoid complex of the sternum. Two ICG current electrodes were placed on the back of the cervical vertebra C4 and between thorax vertebras T8 and T9. Due to the portable nature of these devices, the subjects were not restricted in their movement by wearing this during the exercise tests (Figure 3.2A).

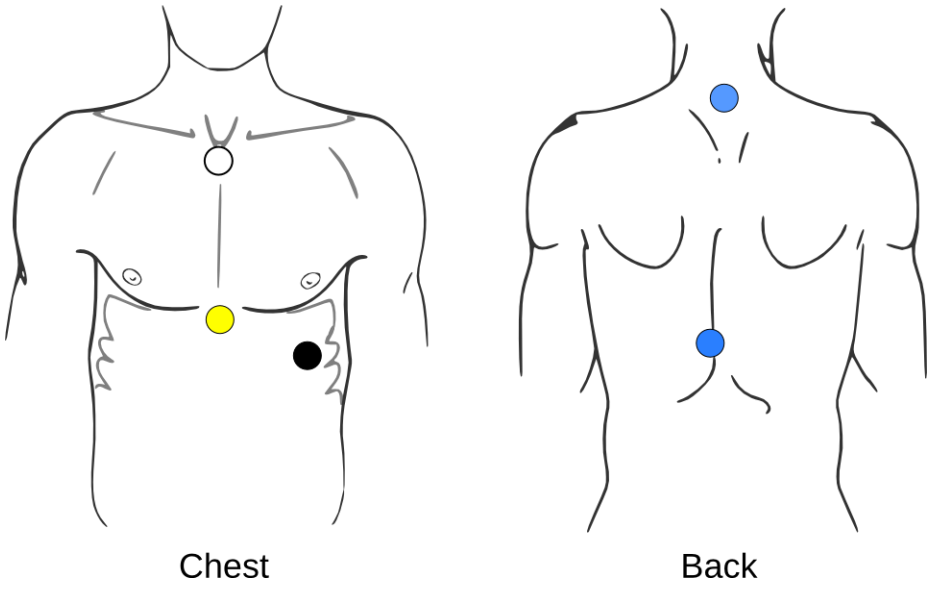


Figure 3.1 Placement of the VU-AMS*5fs* electrodes.

Life style questionnaire

Regular voluntary exercise behavior was measured by a short lifestyle interview (appendix B), in which the subjects indicated what types of regular exercise they were currently involved in. The questions in this interview were structured identical as in our longitudinal surveys in the Netherlands Twin Register (van der Aa et al., 2010). Subjects were asked 1) whether or not they currently participate in exercise activities in leisure time and if so, 2) for how many years, 3) how many months per year, 4) how many times a week, and 5) how many minutes each time. As we were interested in regular exercise activities, we only included activities that were conducted for at least 3 months a year and since at least half a year, thereby excluding holiday specific exercise activities such as sailing camps and skiing. Each activity was recoded into a metabolic equivalent of task (MET) score, based on the compendium of energy expenditure published by Ainsworth. (Ainsworth et al., 2000). A MET is defined as the ratio of work metabolic rate to a standard resting metabolic rate i.e. the energy required to perform an activity relative to the energy that is expended during quiet rest. By multiplying the MET score, the frequency (how many times a week), and the duration of each exercise activity, weekly MET-hours spent on exercise activities were calculated. In addition, subjects were asked to indicate (in minutes) the amount of time spent on walking and cycling (for

transportation) and compulsory physical education classes. Table 3.3 shows the means and SDs of amount of physical activity for males and females in the pilot and NTR study.

Apart from exercise behavior, subjects were queried on their current work status, their general health and well-being and on their smoking habits and alcohol use. Women reported on the regularity of their menstrual cycle and (oral) contraceptive use (for details see Appendix B). A summary of the results is presented in Table 3.4.

Delay discounting questionnaire

Next, subjects were administered a delay discounting questionnaire. Delay-discounting refers to the reduction in the present value of a future reward as the delay to that reward increases. The more remote a future reward is, the lower its present value, and, therefore, the less likely the reward is to be chosen among current alternatives. The monetary-choice questionnaire used here (Appendix C) was based on one developed by Kirby & Marakovic (1996). Subjects were presented a fixed set of 27 choices between smaller, immediate rewards and larger, delayed rewards. For example, on the first trial subjects were asked "Would you prefer €54 today, or €55 in 117 days?" The subject indicated which alternative he or she would prefer to receive by circling the alternative on the questionnaire. The subjects were presented with this questionnaire with the following instruction: “Your job is to imagine that they are real choices, and to decide which choice you would prefer if the choice were real. It is important to keep in mind that this is not a test with right answers. It is your choice – pick whichever you would prefer if the choice were real”. We made sure that every subject understood the instructions. An estimate of a subject's discounting-rate parameter (*k*) can be made from the subject's pattern of choices across the 27 questions. *K* can be thought of as an impulsiveness parameter, with higher values corresponding to higher levels of impulsiveness. For details on the calculations, please see Kirby et al., 1999. Table 3.5 shows the mean and SD of *k* in our sample.

Table 3.3 Means and standard deviations (SDs) of age, anthropometrics, amount of physical activity, and measures of physical fitness for males and females in the pilot and NTR study.

		Males				Females			
		Pilot		NTR		Pilot		NTR	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age (years)		21.6	*5.5*	17.0	*1.1*	22.0	*3.6*	17.2	*1.1*
Anthropo-	Waist circumference (cm)	77.0	*7.0*	74.9	*6.4*	72.5	*5.8*	71.9	*7.7*
metrics	Hip circumference (cm)	90.5	*9.7*	88.7	*7.2*	92.5	*5.8*	87.9	*8.4*
	Height (cm)	180.7	*6.6*	180.3	*8.0*	168.9	*7.0*	168.1	*6.8*
	Weight (kg)	72.4	*10.6*	67.1	*10.6*	63.6	*8.1*	61.6	*9.9*
	BMI ($kg \cdot m^{-1}$)	22.1	*2.4*	20.6	*2.5*	22.3	*2.5*	21.8	*3.2*
	Body fat percentage	18.3	*6.9*	14.0	*5.9*	31.2	*6.7*	29.5	*7.0*
Physical	Walking (min/week)	137.6	*120.0*	40.1	*75.7*	129.2	*89.7*	43.0	*76.8*
Activity	Cycling (min/week)	147.4	*123.9*	233.2	*153.5*	173.1	*136.2*	227.3	*159.2*
	Physical Education (min/week)	157.5	*135.0*	148.3	*137.3*	- [a]	- [a]	132.2	*108.0*
	Exercise Behavior (METs)	28.7	*30.0*	25.9	*22.6*	17.9	*19.0*	19.5	*21.7*
Physical	Resting heart rate (bpm)	76.5	*13.9*	72.4	*11.2*	74.0	*9.3*	75.6	*11.1*
Fitness	Resting systolic BP (mmHg)	125.5	*11.3*	116.9	*10.7*	109.2	*9.2*	111.0	*8.3*
	Resting diastolic BP (mmHg)	70.8	*9.9*	65.7	*8.0*	70.2	*8.6*	67.6	*7.0*
	$\dot{V}O_{2max}$ (mL/min)	3154	*620*	3134	*533*	2304	*404*	2228	*316*
	$\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	43.2	*8.4*	47.0	*6.7*	36.7	*5.7*	36.6	*5.6*
	HR_{max} (bpm)	192.6	*8.6*	195.5	*10.1*	192.2	*10.4*	194.9	*9.3*
	Handgrip strength DH [b] (kg)	44.8	*8.7*	40.0	*8.0*	30.9	*5.6*	29.5	*4.6*
	Handgrip strength NDH [c] (kg)	41.8	*6.7*	36.7	*8.3*	29.1	*5.3*	26.9	*4.5*
	Vertical jump (cm)	45.1	*8.5*	45.9	*6.4*	34.7	*5.0*	35.4	*5.5*
	Flexibility (cm)	23.1	*10.8*	19.8	*9.9*	28.9	*10.4*	29.0	*9.6*
	Balance (errors)	17.0	*7.3*	17.2	*7.6*	13.6	*6.0*	15.1	*6.9*

Note. [a] No cases; [b] DH = dominant hand; [c] NDH = non dominant hand.

Table 3.4 Demographics, lifestyle information, health and well-being of the males and females in the pilot and NTR study.

			Males				Females			
			Pilot		NTR		Pilot		NTR	
			N	*%*	*N*	*%*	*N*	*%*	*N*	*%*
Demo-	Work status	Student	23	92%	238	98%	36	90%	255	99%
graphics		Part time job (12-32h/w)	1	4%	1	0%	2	5%	1	0%
		Full time job (> 32h/w)	1	4%	1	0%	2	5%	1	0%
		Unemployed	0	0%	2	1%	0	0%	0	0%
Lifestyle	Smoking	Yes	2	8%	13	5%	11	28%	14	5%
		No	20	80%	223	92%	28	72%	237	92%
		Quitted	3	12%	6	2%	0	0%	6	2%
	Alcohol use	< 1 units per week	7	28%	86	35%	12	31%	116	45%
		1 - 5 units per week	10	40%	104	43%	13	33%	119	46%
		6 - 10 units per week	5	20%	35	14%	7	18%	19	7%
		11 - 15 units per week	1	4%	8	3%	3	8%	3	1%
		> 16 units per week	2	8%	9	4%	4	10%	0	0%
Health	Subjective	Bad	0	0%	1	0%	0	0%	0	0%
	health	Moderate	0	0%	2	1%	1	3%	6	2%
		Reasonable	3	12%	12	5%	1	3%	16	6%
		Good	19	76%	164	67%	37	95%	197	77%
		Excellent	3	12%	63	26%	0	0%	38	15%
	Medication	No	26	93%	213	88%	37	90%	239	93%
		Psychopharmaca	0	0%	6	247%	1	2%	1	0%
		Antihistamines	0	0%	5	2%	0	0%	5	2%
		Inhalers for asthma	0	0%	6	2%	0	0%	9	4%
		Hormonal contraception					35	90%	136	53%
		Other	0	0%	7	3%	3	7%	3	1%
Wellbeing	Scale 1 - 10	(mean±SD)			8.0±0.7				7.8±0.7	

Table 3.5 Descriptives of *k* (ln transformed).

	Males		Females	
	Mean	*SD*	*Mean*	*SD*
*k*small	-4.50	1.51	-4.24	1.31
*k*med	-4.87	1.54	-4.46	1.35
*k*large	-5.43	1.42	-5.14	1.28
*k*total	-4.93	1.38	-4.61	1.15

Affective response measurements

Subjects were seated comfortably and their baseline affective responses were assessed by the Dutch versions of the Feeling Scale (Hardy & Rejeski, 1989) and the Activation-Deactivation Adjective Checklist (Thayer, 1986). The Feeling Scale (FS) is an 11 point bipolar measure of pleasure-displeasure. The scale ranges from -5 "very bad" to +5 "very good" and has been used as a measure of affective valence in many previous studies on the acute response to exercise (Ekkekakis et al., 2008; Ekkekakis et al., 2011; Hall et al., 2002; Parfitt et al., 2006; Schneider & Graham, 2009) (Appendix D). The Activation-Deactivation Adjective Checklist (AD ACL) is a multidimensional test of various transitory arousal states using a four-point self-rating system: "definitely feel" (4), "slightly feel" (3), "cannot decide" (2) or "definitely do not feel" (1) (Appendix E). This questionnaire is scored by averaging five scores for each subscale: Energy, Tiredness, Tension, and Calmness. Table 3.6 and 3.7 show the means and SDs of the responses on the Feeling Scale and AD ACL collected during this baseline measurement. To measure subjective perception of exercise intensity the Borg's Rating of Perceived Exertion (RPE) (Borg, 1970) was used (Appendix F). The RPE comprises a 15-point scale ranging from 6 to 20, with marks at 7 ("very, very light"), 9 ("very light"), 11 ("fairly light"), 13 ("somewhat hard"), 15 ("hard"), 17 ("very hard") and 19 ("very, very hard").

Table 3.6 Means and standard deviations (SDs) of the responses on the Feeling Scale and Borg Scale (Rate of Perceived Exertion, RPE) and percentage of $\dot{V}O_{2max}$.

	Feeling Scale		RPE		% of $\dot{V}O_{2max}$	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Baseline	3.5	1.2				
Cycle ergometer step 1	3.5	1.0	9.1	1.4	42.3	6.8
Cycle ergometer step 2	3.2	1.1	10.3	1.5	49.2	7.9
Cycle ergometer step 3	2.8	1.3	11.3	1.6	55.6	8.7
Cycle ergometer step 4	2.5	1.4	12.1	1.8	61.7	9.8
Cool down	2.9	1.2			52.8	9.1
Recovery	3.6	1.1			19.4	3.8
Treadmill step 1	3.4	1.0	8.9	1.6	45.6	7.9
Treadmill step 2	3.1	1.2	10.1	1.7	49.9	8.6
Treadmill step 3	2.7	1.4	11.3	1.9	56.8	12.0
Treadmill step 4	2.5	1.5	12.2	2.0	64.0	13.0
Cool down	3.0	1.3			53.6	11.0
Recovery	3.5	1.2			20.0	4.4

Table 3.7 Means and standard deviations (SDs) of the responses on the subscales of the Activation-Deactivation Adjective Check List.

	Energy		Calmness	
	Mean	SD	Mean	SD
Baseline	3.02	0.59	3.41	0.49
Recovery cycle ergometer	3.40	0.53	2.81	0.76
Recovery treadmill	3.40	0.55	2.80	0.81
Recovery maximal exercise test	3.12	0.70	3.27	0.61
	Tiredness		Tension	
	Mean	SD	Mean	SD
Baseline	1.86	0.81	1.22	0.33
Recovery cycle ergometer	1.53	0.55	1.26	0.40
Recovery treadmill	1.60	0.60	1.24	0.40
Recovery maximal exercise test	1.74	0.67	1.15	0.31

Next, subjects remained seated comfortably and were presented with a 6 minute wild life documentary (BBC wild life) to obtain resting heart rate and cardiac vagal control. In addition, resting blood pressure was measured twice (in the 4th and 5th minute, Omron HEM-907 digital blood pressure monitor). The mean of these two measurements was used as baseline blood pressure measure. Table 3.3 shows the means and SDs of blood pressure and HR.

Attachment of the Cosmed K4b^2

A telemetric gas exchange system (Cosmed K4b^2, Cosmed Benelux, Nieuwegein, The Netherlands) was attached to the subject to record breath-by-breath oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). During the course of the experiment, the main sample unit and the battery pack were attached to the back of the subject (Figure 3.2B and 3.2C). Before each test, the O_2/CO_2 analysis system was calibrated using ambient air and a gas mixture that had an O_2 concentration of 16% and a CO_2 concentration of 5%. The calibration of the turbine flowmeter was performed by using a 3-liter syringe (all according to the manufacturer's instructions).

Submaximal exercise tests

Two exercise test were conducted (in fixed order) on an electromechanically braked Lode cycle ergometer (type Corival) and a Lode treadmill (type Valiant) at fixed loads that are below the intensity of the ventilatory threshold for most adolescents (Table 3.2). The first session on the cycle ergometer started with a 2-minute warming up period, followed by 4 incremental stages of 5 minutes each (males: 70W, 90W, 110W, 130W; females: 40W, 60W, 80W, 100W). Subjects were instructed to pedal at fixed rounds per minute (RPM): between 60 and 70 RPM. The test ended with a 1-minute cooling-down phase, followed by a 5-minute recovery period. The second session on the treadmill consisted of a 1-minute warm-up period, followed by 4 incremental stages of 5 minutes each (males: 6, 6.5, 7 and 8 km/h; females: 5.5, 6, 6.5 and 7 km/h). Again, the test ended with a 1-minute cooling-down phase, followed by a 5-minute recovery period.

To ensure that the intensity of every stage was below the intensity of the ventilatory threshold for most adolescents, the ratio of the oxygen consumption and carbon dioxide production ($\dot{V}CO_2/\dot{V}O_2$) was monitored. This respiratory exchange ratio (RER) can be used to

estimate the ventilatory threshold (Solberg et al., 2005). This threshold is passed when exhalation of CO_2 exceeds inhalation of O_2, which is visualized by a RER > 1.00. For each test the load of each stage was adjusted when necessary to keep the intensity below an RER of 1.00.

In order for the subjects to reach a steady-state during the 4 steps of the submaximal exercise protocol, FS and RPE responses were collected in the last minute (after 4 minutes) of every step (Figure 3.3). During the cooling down, FS response was collected in the last 15 seconds. During the recovery phases, the AD ACL and FS response was collected after respectively ~2 and 5 minutes of quiet sitting. The RPE responses were collected in every last minute of each incremental step of the submaximal cycle ergometer and treadmill protocol (after 4 minutes). Figure 3.4 illustrates the changes in $\dot{V}CO_2$ and $\dot{V}O_2$ across the entire experimental protocol for one of the subjects. The arrows indicate when subjects were asked to fill out the AD ACL, report on their current affective state (FS) and rate of perceived exertion (RPE).

Maximal exercise test

Finally, an incremental maximal exercise test was conducted on a cycle ergometer to establish $\dot{V}O_{2max.}$ The work rate was increased every minute until exhaustion while subjects pedaled at 60-100 RPM. In this protocol males started at 75 Watt with increments of 25 Watt per minute. For females stage one started at 70 Watt and work load was increased by 20 Watt per minute. Adjustments to this protocol (higher increasing workloads every step) were done by experienced researchers based on the exercise status, age, height and weight of the subject. The test was terminated when the subject was not able to keep RPM above 50 despite serious attempts. After cessation of the test, the gas exchange measurement system was removed and every subject completed a mandatory cool-down phase on the cycle ergometer of 5 minutes on a low, individually chosen work rate. One minute and five minutes after the end of the maximal exercise test subjects were asked to indicate how they felt on the FS. After 5 minutes the subjects were asked to sit down for at least two minutes but otherwise long enough for the heart rate to reach values below 120. Hereafter, the VU-AMS was removed and subjects were allowed to take a shower. After showering, the subjects were asked to fill out the AD ACL for the last time (Table 3.2; Figure 3.4).

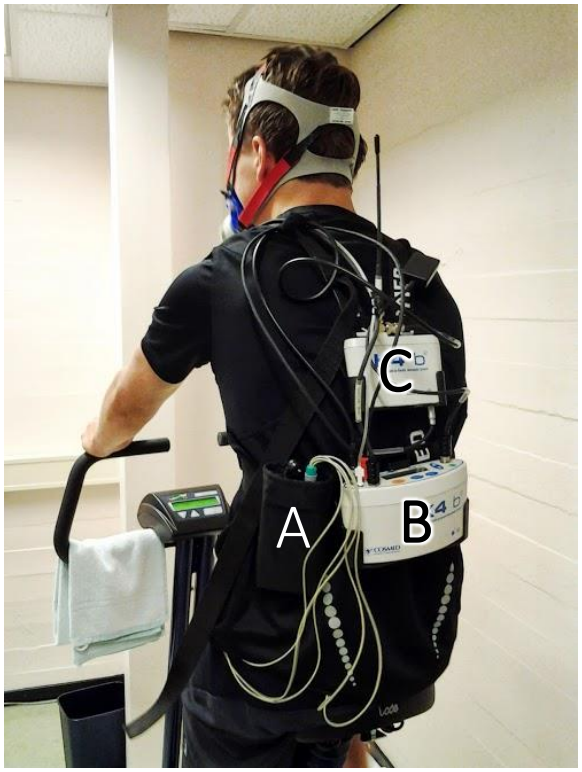


Figure 3.2 Attachment of the VU-AMS and Cosmed K4b[2]. A) VU-AMS; B) Cosmed main unit; C) Battery pack.

Figure 3.3 Feeling Scale responses collected during the exercise tests.

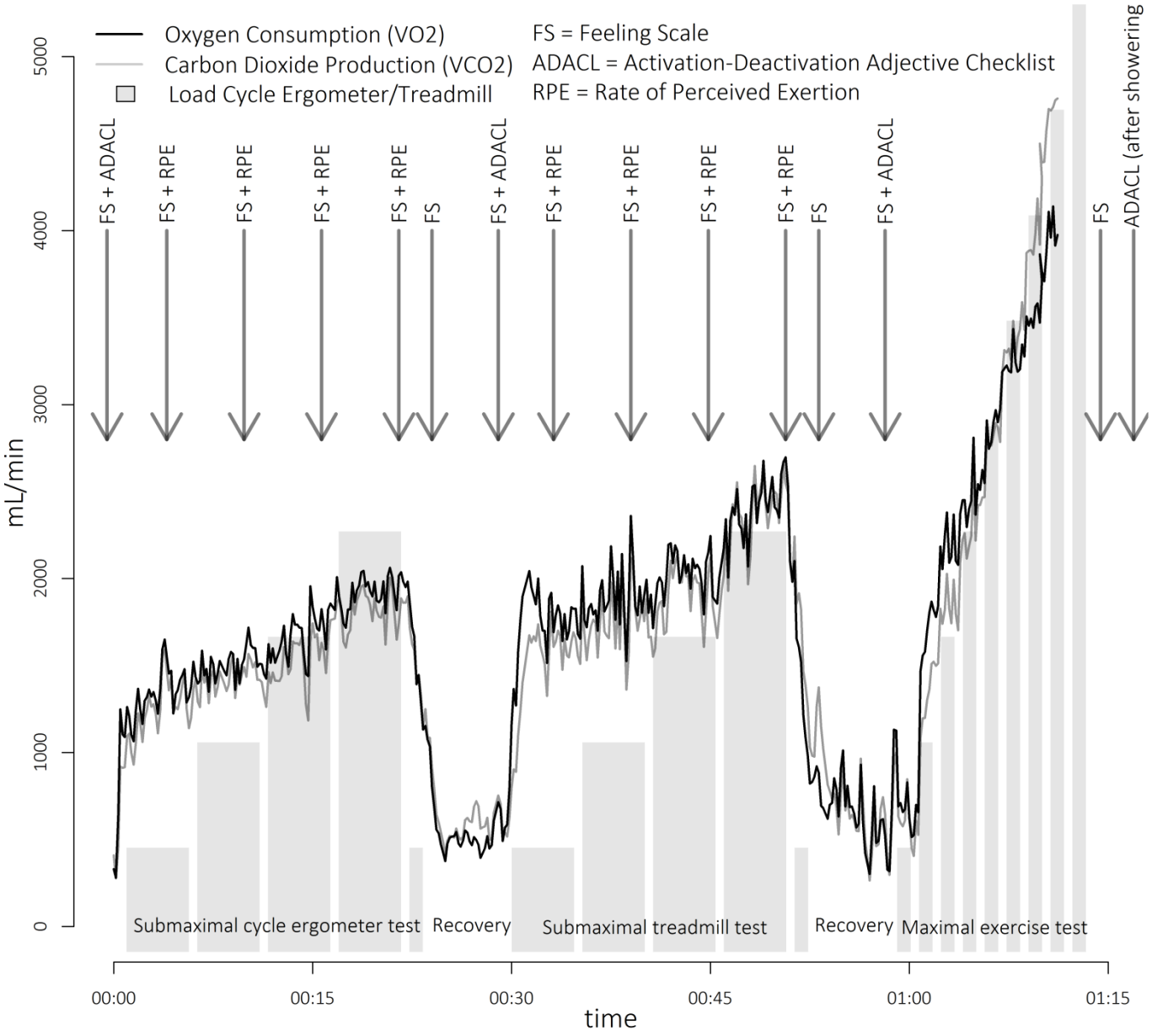


Figure 3.4 Changes in $\dot{V}O_2$ and $\dot{V}CO_2$ across the entire experimental protocol of a randomly selected subject. The two submaximal exercise tests on the cycle ergometer and treadmill and the final maximal exercise test are clearly visible as $\dot{V}O_2$ and $\dot{V}CO_2$ increase when subjects start exercising. The arrows indicate when this subject was asked to fill out the AD ACL, report on their current mood (FS) and rate of perceived exertion (RPE).

Table 3.3 shows the mean and SD of $\dot{V}O_{2max}$ for both males and females. In addition, Table 3.5 shows the means and SDs of the responses on the Feeling Scale and Borg Scale (Rate of Perceived Exertion, RPE) collected during the exercise tests and percentage of $\dot{V}O_{2max}$, whereas Table 3.7 summarizes the means and SDs of the responses on the subscales of the AD ACL.

Report and reward

The subjects were provided with a report with their personal scores on the fitness tests, their resting heart rate and blood pressure values, height, weight and BMI and maximal oxygen uptake (for an example see Appendix G). In addition, they received their gift voucher and/or study credit.

DNA Collection & Zygosity determination

Buccal DNA samples were collected for 15 pilot subjects and for 482 NTR subjects (14 subjects refused). Buccal sampling was done by the subjects themselves at home. A week before the lab testing, all subjects received 16 cotton swabs and four test tubes filled with 2 ml of solution and an instruction folder (Appendix H). The subject was asked to collect buccal swabs on 4 occasions during two days. On the day of testing the samples were collected by the experimenter. The samples were stored in a dark and cool place before sending to the laboratory. After genome-wide genotyping at the molecular genetics lab, the twins (or the parents, when subjects were under 18) were informed on their zygosity status by means of a letter.

FOLLOW-UP: SURVEY ON EXERCISE STATUS

In July and August 2015, all 499 subjects were sent an online questionnaire on their current exercise behavior, (if relevant) the highest level attained in competitive sports, sports injuries, subjective exercise ability, daily physical activity and sedentary behavior (Appendix I). When subjects failed to take the survey online, the survey was done by telephone. Five subjects had unsubscribed from the Netherlands Twin Register and were therefore not available for the follow-up survey. Twenty-two subjects were lost to follow-up due to missing contact

information. Forty-two did not fill out the questionnaire after several reminders or refused to participate. Complete follow-up data was available for 430 subjects (which corresponds to a response rate of 88%); 49 MZM pairs (of which 10 twin pairs participated with 1 sibling and 1 pair with 2 siblings); 26 DZM pairs (of which 1 participated with 1 sibling); 45 MZF pairs (of which 10 twin pairs participated with 1 sibling and 1 pair with 2 siblings); 37 DZF pairs (of which 2 participated with 1 sibling); 28 DOS pairs, 2 (non-twin) sibling pairs and 29 singletons.

CHAPTER 4

HERITABILITY OF THE AFFECTIVE RESPONSE TO EXERCISE AND ITS CORRELATION TO EXERCISE BEHAVIOR

PUBLISHED AS

Schutte NM, Nederend I, Hudziak JJ, Bartels M & de Geus EJC (2017)

Heritability of the affective response to exercise and its correlation to exercise behavior

Psychology in Sports & Exercise 31, 139-148

ABSTRACT

Individual differences in adolescent exercise behavior are strongly influenced by genetic factors. The affective response to exercise is a potential source of these genetic influences. To test its role in the motivation to exercise, we estimated the heritability of the affective responses during and after exercise and the overlap with the genetic factors influencing regular voluntary exercise behavior. 226 twin pairs and 38 siblings completed two submaximal exercise tests on a cycle ergometer and a treadmill and a maximal exercise test on a cycle ergometer. Affective responses were assessed by the Feeling Scale (FS), Borg's Rating of Perceived Exertion (RPE) and the Activation-Deactivation Adjective Checklist (AD ACL). Multivariate structural equation modeling was used to estimate heritability of the affective responses during and after submaximal and maximal exercise and the (genetic) correlation with self-reported regular voluntary exercise behavior over the past year. Genetic factors explained 15% of the individual differences in FS responses during the cycle ergometer test, as well as 29% and 35% of the individual differences in RPE during the cycle ergometer and treadmill tests, respectively. For the AD ACL scales, heritability estimates ranged from 17% to 37% after submaximal exercise and from 12% to 37% after maximal exercise. Without exception, more positive affective responses were associated with higher amounts of regular exercise activity ($.15 < r < .21$) and this association was accounted for by an overlap in genetic factors influencing affective responding and exercise behavior. We demonstrate low to moderate heritability estimates for the affective response during and after exercise and significant (genetic) associations with regular voluntary exercise behavior. These innate individual differences in the affective responses to exercise should be taken into account in interventions aiming to motivate adolescents to adopt and maintain regular exercise.

INTRODUCTION

Regular physical activity is a key contributor to adolescents' health (Janssen & Leblanc, 2010). However, the majority of youngsters does not engage in regular exercise at the recommended level, despite efforts of governments and health care organizations promoting exercise (Martinez-Gonzalez et al., 2001; Troiano et al., 2008). To create a successful intervention, one must have knowledge about the underlying predictors of a physically active lifestyle. One of the potential motivational mechanisms underlying exercise behavior is the affective response immediately during exercise and shortly after cessation of an exercise bout (Ekkekakis et al., 2013; Ekkekakis et al., 2011).

Affect refers to an individual's core of all valenced states: good versus bad, pleasure and displeasure, positive and negative (Ekkekakis et al., 2013; Ekkekakis et al., 2011). In contrast to the persistent general belief that exercise is enjoyable for everyone, strong individual differences are found in the affective responses during and after exercise. Whereas some individuals indeed report an increase in pleasure or no change, others report reduced pleasure or negative changes in affect (Ekkekakis et al., 2005; Ekkekakis et al., 2011; Van Landluyt et al., 2000; Welch et al., 2007). Based on the principles of instrumental conditioning, the repeated affective responses to exercise activities could be a powerful determinant of the formation of stable behavioral habits. If the affective response is on balance positive, people are likely to maintain the behavior and become regular exercisers. However, if the net affective response is not favorable, people are at risk of dropping out and becoming non-exercisers. In keeping with this theoretical expectation, a more favorable affective response during exercise was found to be associated with the intention to engage in voluntary exercise (Kwan & Bryan, 2010; Ruby et al., 2011) and greater actual participation in (voluntary) moderate to vigorous exercise (Dunton & Vaughan, 2008; Rhodes & Kates, 2015; Schneider et al., 2009; Williams et al., 2008; Williams et al., 2012). A better understanding of the determinants of the affective response to exercise may therefore be paramount to creating successful exercise interventions.

The net affective response during and shortly after exercise may reflect a mixture of multiple aversive and appetitive effects. Examples of immediate aversive effects are exercise-related fatigue related to muscle pain, respiratory exertion and monoamine depletion (Davis & Bailey,

1997). After exercise, cardiovascular activation levels may be uncomfortably high for a prolonged period, paired to lingering muscle fatigue and central fatigue (Ament & Verkerke, 2009). More complex aversive effects may involve the fear for embarrassment and injuries (Huppertz et al., 2014b; Rhodes et al., 1999; Skelton & Beyer, 2003; Vartanian & Shaprow, 2008). These aversive effects may be balanced by the rewarding effects which are governed by the mesolimbic reward system that involves dopaminergic (Beaulieu & Gainetdinov, 2011) and endocannabinoid (Solinas et al., 2007) pathways. More complex appetitive effects can involve a sense of accomplishment or distraction from worry or feelings of anxiety (Anderson & Shivakumar, 2013) during but also after exercise cessation. Shortly after exercise activities, sympathetic withdrawal may temporarily reduce the physiological sensitivity to stress (Chen & Bonham, 2010; Hsu et al., 2015).

De Geus and de Moor (2008) have hypothesized that these individual differences in part reflect differences in genetic sensitivity to the psychological effects of exercise (de Geus & de Moor, 2008). A significant genetic contribution of the affective responses to exercise could explain the now well-documented heritability of voluntary exercise behavior which peaks at 82% in late adolescence (Huppertz et al., 2012) and remains in play throughout adulthood with heritability estimates of around 42% (de Moor et al., 2011). Genetic variants influencing the affective exercise response could do so in part by an effect on the so-called 'activity drive' (Lerman et al., 2002; Lightfoot et al., 2004; Rowland, 1998). This activity drive can be conceptualized as an innate motivation to be physical active in the classical Hullian sense, not different from sex drive, hunger or thirst. Just as the glucostat cells and the baroreflex that keep sugar and blood pressure level constant at an optimal level, the activity-stat could keep a person's energy expenditure at an optimal level, but that level may differ significantly across individuals dependent on genotype (Lightfoot et al., 2004; Swallow et al., 1998). The activity-stat could influence the net balance of positive and negative affective responses during and after a bout of exercise as the fulfillment of drives is intrinsically rewarding.

Other factors known to influence exercise behavior could further modulate the affective response to exercise. Positive attitudes and expected health benefits may lead the individual to endure an unfavorable balance between the aversive and appetitive effects, as may a strong ability to self-regulate. Both self-regulation traits and attitude are associated with exercise behavior and/or physical activity (Dishman et al., 2014; Dishman et al., 2015; Hagger

et al., 2002; Rhodes & Smith, 2006). Moreover, attitudes have been shown to be heritable (Huppertz et al., 2014b) and the psychological concept of self-regulation might also be a shaped by genetic factors (Posner & Rothbart, 2009). Increased sensitivity to punishment as seen in neuroticism, aversion to arousal as seen in introversion, or reward-seeking behavior as seen in extraversion and sensation seeking, all heritable personality traits, may further modulate the affective response to exercise accounting for the association of personality with exercise behavior (de Moor et al., 2006).

A final important contributor to the net affective response to exercise is exercise ability and/or trainability. Activities that one is good at are likely to be pursued in leisure time. Performing better at exercise than others, or gaining more rapidly when exposed to comparable training regimes, will lead to feelings of competence, whereas lower levels of performance and trainability might lead to disappointment or shame (particularly when the exercise is performed in a competitive context). A large body of literature has confirmed self-efficacy, the belief and conviction that one can perform a given activity at an adequate level of performance, is a powerful determinant of whether someone engages in and adheres to an exercise program (Dishman et al., 2005; McAuley & Blissmer, 2000; Nigg, 2001). Self-efficacy may be an especially strong factor in adolescence, when the sensitivity to one's own relative ranking among peers may be largest.

The present study aims to test the hypothesis that the affective responses during and after exercise show significant heritability in adolescence. Secondly, it aims to test the hypothesis by De Geus & de Moor (2008) that the genetic factors underlying this heritability partly overlap with the genetic factors underlying regular voluntary exercise behavior. To test these two hypotheses, the affective state was repeatedly measured in a large adolescent sample of twins and siblings during and after graded (sub)maximal exercise tests. Regular voluntary exercise behavior over the past year was characterized in these subjects by a lifestyle interview. In a twin study, the intrapair resemblance for a trait is compared between genetically identical (monozygotic, MZ) and non-identical (dizygotic, DZ) twins. We expect that MZ twins resemble each other more than DZ twins in affective responses to exercise, providing evidence for genetic influences on this response. In a bivariate extension of the twin design, cross-trait/cross-twin correlations can be further used to compute the correlation between genetic factors influencing these two traits. We expect a significant genetic

correlation between adolescent exercise behavior and the exercise-induced affective response showing that they are influenced by shared genetic factors.

METHODS

Subjects

Healthy adolescent twin pairs aged between 16 and 18 and their siblings (age range 12 – 25) from the Netherlands Twin Register (van Beijsterveldt et al., 2013) were invited to participate in a study on the determinants of adolescent exercise behavior. A complete dataset was available for 499 subjects: 115 monozygotic pairs (MZ) and 111 dizygotic pairs (DZ), and 35 of their singleton siblings. Six additional non-twin sibling pairs participated. All subjects provided written consent and if the subjects were under 18 consent was given by both of their parents/guardians. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the VU Medical Center Amsterdam (NL35634.029.10).

Measures

Regular voluntary exercise behavior was measured by a short lifestyle interview, in which the subjects indicated what types of regular exercise they were currently involved in. The questions in this interview were structured identical as in our longitudinal surveys used by the Netherlands Twin Register (van der Aa et al., 2010). Subjects were asked 1) whether or not they currently participate in exercise activities in leisure time and if so, 2) for how many years, 3) how many months per year, 4) how many times a week, and 5) how many minutes each time. Activities that were related to transportation (walking and cycling) and compulsory education classes were excluded. As we were interested in regular voluntary exercise activities, we only included activities that were conducted for at least 3 months a year and since at least half a year, thereby excluded holiday specific exercise activities such as sailing camps and skiing. Each activity was recoded into a metabolic equivalent of task (MET) score, based on the compendium of energy expenditure published by Ainsworth. (Ainsworth et al., 2000). A MET is defined as the ratio of work metabolic rate to a standard resting metabolic rate i.e. the energy required to perform an activity relative to the energy that is expended during quiet rest. By multiplying the MET score, the frequency (how many times a week), and

the duration of each exercise activity, weekly MET-hours spent on exercise activities were calculated.

Affective responses to exercise were assessed by the Dutch versions of the Feeling Scale (Hardy & Rejeski, 1989) and the Activation-Deactivation Adjective Checklist (Thayer, 1986). The Feeling Scale (FS) is an 11-point bipolar measure of pleasure-displeasure. The scale ranges from -5 "very bad" to +5 "very good" and has been used in many studies on the affective response to exercise (Ekkekakis et al., 2008; Ekkekakis et al., 2011; Hall et al., 2002; Parfitt et al., 2006; Schneider & Graham, 2009). Figure 4.1a shows the scores on the Feeling Scale of 6 randomly selected subjects for every step of a submaximal exercise test. The area above the curve was calculated for every subject during exercise (for details see Figure 4.1b) (using the *polyarea* function in Matlab (Matlab 2014a, The MathWorks Inc., Natick, Massachusetts, USA). These scores were recoded so that negative scores were associated with a larger decrease on the FS during the exercise tests. The Activation-Deactivation Adjective Checklist (AD ACL) is a multidimensional test of transitory arousal states using a four-point self-rating system: "definitely feel" (4), "slightly feel" (3), "cannot decide" (2) or "definitely do not feel" (1). As the subjects experienced some trouble with understanding three of the items "placid" and "wakeful" and "intense", these items were left out of the analyses. This questionnaire is scored by averaging five scores for each subscale: Energy, Tiredness, Tension, and Calmness. Finally, to measure subjective exercise intensity The Borg's Rating of Perceived Exertion (RPE) (Borg, 1970) was used: A 15-point scale ranging from 6 to 20, with marks at 7 ("very, very light"), 9 ("very light"), 11 ("fairly light"), 13 ("somewhat hard"), 15 ("hard"), 17 ("very hard") and 19 ("very, very hard"). The sum of the scores for every submaximal exercise test was used for analyses. These scores were recoded so that higher scores were associated with less exertion (feeling better, less exhausted).

To account for potential effects of differences in the relative intensity of exercise compared to a person's maximal exercise capacity, oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were recorded continuously by means of a telemetric gas exchange system (Cosmed K4b^2, Cosmed Benelux, Nieuwegein, The Netherlands) during the submaximal and maximal exercise tests. Breath-by-breath $\dot{V}O_2$ data were exported and deviant breaths were removed by excluding the breaths that were more than 3 standard deviations from the mean.

Procedure

On arrival at the laboratory, height and weight were measured, the life-style interview was completed, and baseline FS and AD ACL responses were obtained. Next, two submaximal exercise tests were conducted (in fixed order) on an electromechanically braked Lode cycle ergometer (type Corival) and a Lode treadmill (type Valiant) at fixed loads that are typically below the intensity of the ventilatory threshold (VT) for most adolescents. The first session on the cycle ergometer started with a 2-minute warming up period, followed by 4 incremental stages of 5 minutes each (males: 70 Watt (W), 90W, 110W, 130W; females: 40W, 60W, 80W, 100W). Subjects were instructed to pedal at fixed revolutions per minute (RPM): between 60 and 70 RPM. The test ended with a 1-minute cooling down phase, followed by a 5-minute recovery period. The second session on the treadmill consisted of a 1-minute warm-up period, followed by 4 incremental stages of 5 minutes each (males: 6, 6.5, 7 and 8 km/h; females: 5.5, 6, 6.5 and 7 km/h). Again, the test ended with a 1-minute cooling-down phase, followed by a 5-minute recovery period. In order for the subjects to reach a steady-state during the 4 steps of the submaximal exercise protocol, FS and RPE responses were collected in the last minute (after 4 minutes) of every step. During the cooling down phase (1 minute), FS response was collected in the last 15 seconds. During the recovery phases, the AD ACL and FS responses were collected after respectively ~2 and 5 minutes of sitting quietly.

To ensure that the subjects did not exercise at vigorous intensities, the ratio of the oxygen consumption and carbon dioxide production ($\dot{V}CO_2/\dot{V}O_2$) was monitored. This respiratory exchange ratio (RER) can be used to estimate the blood lactate-based anaerobic threshold (Solberg et al., 2005). This threshold is passed when exhalation of CO_2 exceeds inhalation of O_2, which is visualized by a RER > 1.00. The load of each stage was adjusted when necessary to keep the intensity of the final stage of each submaximal test below an RER of 1. For subjects who showed an RER above 1.0 during the submaximal tests, FS and AD ACL responses for that submaximal were set to missing (including the cool down and recovery period).

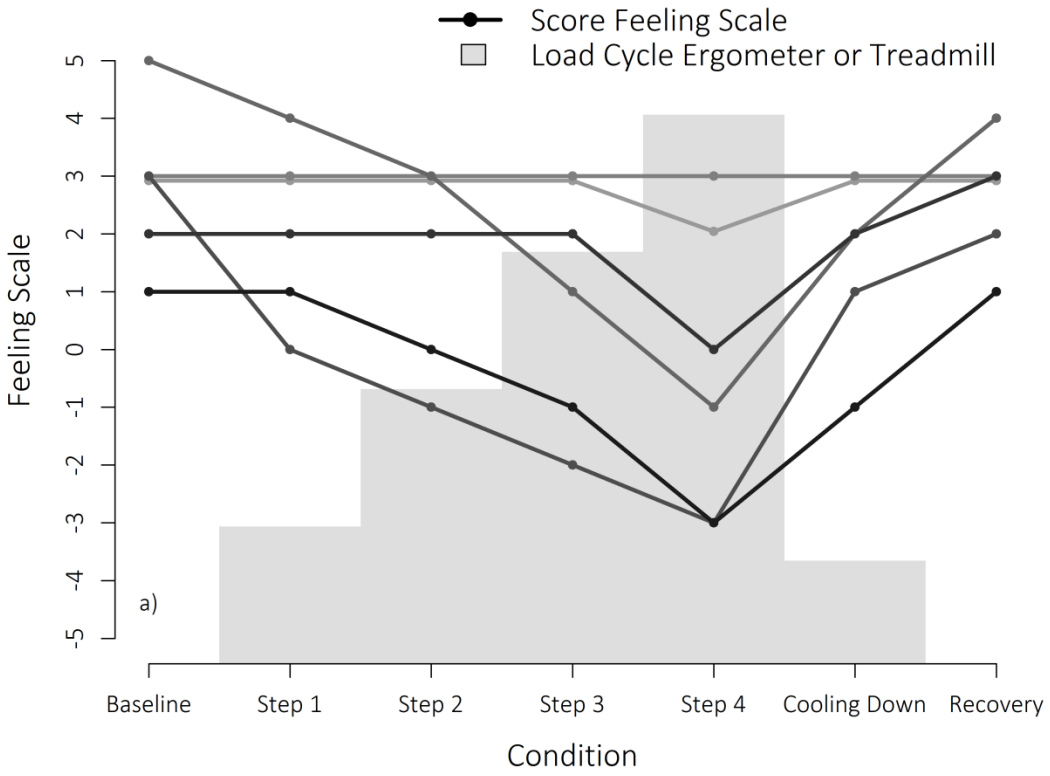
Finally, an incremental maximal exercise test was conducted on a cycle ergometer to establish $\dot{V}O_{2max}$. The work rate was increased every minute until exhaustion (see Chapter 6 for

measurement details on $\dot{V}O_{2max}$). On average 25.2 ± 7.6 minutes after the end of the maximal exercise test and a shower the AD ACL was filled out a final time.

Statistical analyses

The classical twin design compares the intrapair resemblance between two types of sibling relationships; genetically identical twins or monozygotic (MZ), the result of division of a single fertilized egg during an early stage in embryonic development, and non-identical twins or dizygotic (DZ), resulting from two separate fertilized eggs. Consequently, MZ twins are genetically identical, whereas DZ twins share on average 50% of their genetic make-up. Twin studies decompose all phenotypic variance of a trait into sources of genetic influences ('A'), shared environmental influences (influences shared with other family members e.g. upbringing; referred to as 'C'), dominant genetic influences ('D') and person-specific influences (influences that are unique to the individual; referred to as 'E'). An important assumption is that the shared environmental effects are independent of zygosity (and thus equal for both MZ and DZ twins).

Modeling of the twin and sibling data was performed using structural equation modeling (SEM) in OpenMx (Boker et al., 2011) under R (R Development Core Team, 2011) with the raw-data ML procedure for estimation of the parameters. For all analyses, a threshold of $p < 0.05$ was considered for statistical significance. Given the relative small sample size, with no power to test for sex-differences, and since (non-twin) siblings share, like DZ twins, on average 50% of their genes, parameter estimates were constrained to be equal for males and females and for DZ twins and siblings. Main effects of baseline measurements, sex and age and highest percentage of $\dot{V}O_{2max}$ reached during the submaximal tests on mean levels of the affective responses were considered in the model. In addition, for the AD ACL responses collected after the maximal test, we included the main effect of the time between the maximal exercise test and the final measurement of the AD ACL (in minutes) when modeling the mean AD ACL response.


Score Feeling Scale
Load Cycle Ergometer or Treadmill
Feeling Scale
5
4
3
2
1
0
-1
-2
-3
-4
-5
a)
Baseline
Step 1
Step 2
Step 3
Step 4
Cooling Down
Recovery
Condition

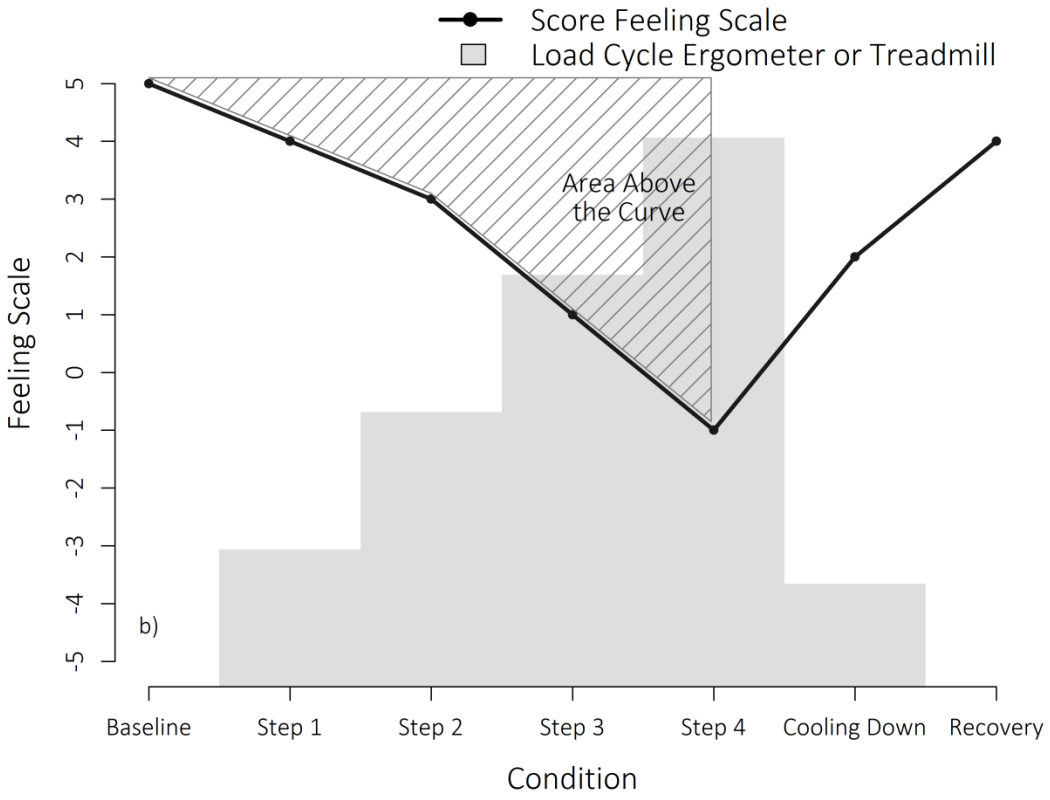

Score Feeling Scale
Load Cycle Ergometer or Treadmill
Area Above
the Curve
Feeling Scale
5
4
3
2
1
0
-1
-2
-3
-4
-5
b)
Baseline
Step 1
Step 2
Step 3
Step 4
Cooling Down
Recovery
Condition

Figure 4.1 An example of the decline and increase in Feeling Scales responses during a submaximal exercise test. a) Feeling Scale response of 6 randomly selected subjects during a submaximal exercise test; b) Example of the quantification of the Feeling Scale response during a submaximal exercise test in a randomly selected subject. The hatched area is used as the Feeling Scale response for this subject.

First, twin-sibling correlations were estimated with univariate saturated models in OpenMx. In a saturated model, all parameters (means, variances) are estimated freely. Next, total phenotypic variance of FS, RPE and AD ACL responses was decomposed into sources of additive genetic variance (A), dominant genetic variance (D) or shared environmental variance (C) and person-specific environmental variance (E) to test which sources of variance significantly contribute to the phenotype and estimate their best value. Since C and D effects cannot be estimated simultaneously in the classical twin model, the ratio of the MZ correlations to the DZ correlations was used to determine which model (ACE or ADE) is most appropriate.

Significance of the variance components was tested by constraining them to zero (for instance, comparing model ACE versus a submodel AE, in which the C component was fixed to zero). These submodels were compared by hierarchic χ^2 tests. The χ^2statistic is computed by subtracting log-likelihood (-2LL) of a submodel from the -2LL of the original model (χ^2= -2LL$_{\text{original model}}$ – -2LL$_{\text{submodel}}$). This χ^2statistic is distributed with degrees of freedom (*df*) equal to the difference in the number of parameters estimated in the two models ($\Delta df = df_{\text{original model}} - df_{\text{submodel}}$). If the difference test is significant, the constraints on the submodel cause a significant deterioration of the fit of the model (Rijsdijk & Sham, 2002).

The phenotypic and cross-trait/cross twin correlations for FS, RPE and AD ACL responses with regular exercise were estimated in bivariate models: an analysis of two variables to determine the relationship between them. When phenotypic correlations proved significant, genetic (r_A) and environmental (r_E) correlations were calculated to determine how much of the genetic influence on two variables is common to both. Finally, a multiple regression analysis was run in STATA to determine the amount of variance in exercise behavior explained by the FS, RPE and AD ACL responses while taking into account familial relatedness.

RESULTS

Descriptives

For 16 subjects affective responses collected during the submaximal cycle ergometer test (10) or submaximal treadmill (6) test were set to missing, and for 7 subjects affective responses collected during both tests were set to missing, because they showed an RER above 1.0 during these exercise tests. Table 4.1 shows the means and standard deviations (SDs) of age, body composition, regular exercise and $\dot{V}O_{2max}$. Tables 4.2 shows the means and SDs of the FS and RPE responses, $\dot{V}O_2$ expressed as a percentage of $\dot{V}O_{2max}$ for every step of the experiment. As the intensity of the submaximal tests increased, the percentage of $\dot{V}O_{2max}$ at which the subjects were exercising increased accordingly. The mean FS responses showed a decline when load was increasing, whereas the subjects reported a higher mean RPE. During the cool down and recovery phase, mean FS responses increased reflecting a return to a more positive affective state. The means and SDs and the Cronbach's alpha for the four subscales of the AD ACL are shown in Table 4.3. The mean score for Energy increases during the recoveries of the submaximal tests, but decreases after the maximal exercise test. Calmness and Tiredness show a reverse pattern, whereas the scores for Tension seem to be stable over the course of the experiment. In the current study, Cronbach's alpha was sufficient for Energy, Calmness and Tiredness (.60 – .87), but low for Tension (.34 – .48).

Table 4.1 Means and standard deviations of age, body composition, regular exercise and $\dot{V}O_{2max}$.

	Male (*N* = 242)		Female (*N* = 257)	
	Mean	*SD*	*Mean*	*SD*
Age	17.1	1.4	17.3	1.5
Height (cm)	180.5	8.1	168.1	6.9
Weight (cm)	67.6	10.8	61.7	10.0
BMI ($kg \cdot m^{-1}$)	20.7	2.5	21.8	3.2
Voluntary Exercise Behavior (METs/week)	25.8	22.6	19.5	21.9
$\dot{V}O_{2max}$ in mL/min	3134.5	542.8	2232.1	329.2
$\dot{V}O_{2max}$ in mL/min/kg	46.9	6.9	36.7	5.6

Table 4.2 Means and standard deviations (SD) of the Feeling Scale, RPE and percentage of $\dot{V}O_{2max}$ during and after submaximal exercise.

	Feeling Scale		RPE		% of V̇O2max		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Interquartile range*
Baseline	3.53	1.21					
Cycle ergometer step 1	3.55	1.01	9.10	1.42	41.76	6.42	8.56
Cycle ergometer step 2	3.25	1.07	10.27	1.51	48.52	7.33	9.31
Cycle ergometer step 3	2.87	1.23	11.17	1.51	54.78	7.99	10.44
Cycle ergometer step 4	2.56	1.42	11.97	1.78	60.64	8.89	11.60
Cool down	2.98	1.21			51.90	8.32	11.02
Recovery	3.36	1.06			19.01	3.63	4.70
Treadmill step 1	3.43	1.14	8.87	1.53	45.27	7.80	10.17
Treadmill step 2	3.11	1.21	10.10	1.77	49.42	8.37	10.49
Treadmill step 3	2.78	1.41	11.14	1.88	54.64	9.77	12.32
Treadmill step 4	2.54	1.50	12.06	1.96	61.98	11.36	13.95
Cool down	3.00	1.30			52.25	10.03	12.67
Recovery	3.50	1.18			19.62	4.11	5.43

Table 4.3 Means and standard deviations of the AD ACL subscales Energy, Calmness, Tiredness and Tension after (sub)maximal exercise.

	Energy			Calmness		
	Mean	*SD*	*Cronbach's alpha*	*Mean*	*SD*	*Cronbach's alpha*
Baseline	3.02	0.59	0.78	3.42	0.49	0.66
Recovery cycle ergometer	3.40	0.52	0.81	2.85	0.73	0.78
Recovery treadmill	3.40	0.55	0.83	2.83	0.80	0.65
Recovery maximal exercise test	3.11	0.71	0.87	3.29	0.60	0.68
	Tiredness			Tension		
	Mean	*SD*	*Cronbach's alpha*	*Mean*	*SD*	*Cronbach's alpha*
Baseline	1.85	0.80	0.82	1.21	0.33	0.34
Recovery cycle ergometer	1.51	0.54	0.60	1.25	0.38	0.42
Recovery treadmill	1.59	0.59	0.84	1.23	0.39	0.48
Recovery maximal exercise test	1.74	0.67	0.78	1.15	0.31	0.41

Heritability of the affective responses to exercise

Table 4.4 shows the MZ and DZ/sibling correlations and genetic modelling results. For FS response during cycling, the dominant genetic factor (D) was not significant ($p > .05$) and heritability estimate (A) was 15% (95% CI: 0% – 31%). For FS responses during the submaximal treadmill test, the DZ/sibling correlation was higher than half the MZ correlation and the ACE model could be simplified by dropping either A or C, with a CE model giving the best fit. C explained 19% (95% CI: 8% – 30%) of the total variance in FS responses during the submaximal treadmill test. For RPE responses during both submaximal exercise tests, C could be dropped from the model and heritability estimates of 29% (95% CI: 13% – 43%) and 35% (95% CI: 20% – 48%) were found. Person-specific environmental influences explained a substantial portion of the variance in all FS and RPE responses.

For energy and Calmness the MZ correlations were higher than DZ/sibling correlations and the AE model provided the best fit, except for Energy measured during the recovery of the treadmill test for which a significant C component was found (22%). For Energy measured after the cycle test and the maximal exercise test, heritability estimates were 37%. For Calmness, heritability estimates increased over the course of the experiment, ranging from 19% after the submaximal cycle ergometer test to 36% after the maximal exercise test. Tiredness showed no evidence of genetic influences after the submaximal tests, but 32% of the differences in Tiredness after the maximal exercise test could be explained by genetic factors. Finally, heritability estimate of Tension after the submaximal cycle ergometer tests was 24%, but no evidence of genetic influences were found for Tension after the other two exercise tests.

Correlations to regular exercise behavior

Table 4.5 shows the phenotypic correlations between the responses on the FS and the RPE and regular exercise. Significant correlations were found for the FS responses and the (reverse-coded) RPE responses with voluntary exercise behavior ($.15 < r < .21$). A larger decrease in scores on the FS during the exercise test was associated with lower values of regular exercise. Significant genetic correlations for FS and RPE with voluntary exercise behavior were $r_G = .34$ (FS during cycle ergometer test), $r_G = .22$ (RPE, cycle ergometer), and r_G

= .36 (RPE, treadmill). Environmental correlations were small (.04 < r_E < .14) and not significant.

Significant correlations were found for voluntary exercise behavior with the four subscales of the AD ACL measured during the recovery of the cycle ergometer test. Subjects with higher exercise status reported higher values of Energy (r = .15) and Calmness (r = .12) and lower values of Tiredness (r = -.11) and Tension (r = -.11). After the submaximal treadmill test, only Energy and Calmness correlated significantly with voluntary exercise behavior (r = .11 and .17 respectively). After the maximal exercise test, only Calmness showed a significant correlation with exercise behavior (r = .12). That the same genetic variants may influence both exercise behavior and affective responding to exercise was confirmed by examination of the genetic correlation. Significant genetic correlations were found for voluntary exercise behavior with Energy measured during the recovery of the submaximal cycle ergometer test (r_G =.28), Calmness measured during the recovery of all three exercise tests (r_G = .27, r_G = .41, and r_G = .22 for the cycle ergometer, treadmill and maximal exercise test respectively) and Tension measured during the recovery of the submaximal cycle ergometer (r_G = -24). Again, environmental correlations were small (-.05 < r_E < .03) and not significant.

A multiple regression analysis (corrected for familial relatedness) showed that all FS, RPE and AD ACL responses, explained 11.1% of the variance in exercise behavior. When including only the FS, RPE and AD ACL responses collected during and after the submaximal cycle ergometer test, 6.3% of the variance in exercise behavior could be explained. For the treadmill test and maximal exercise test this was 9.5%.

Table 4.4 Twin correlations (95% CI) and standardized estimates (95% CI) of additive (A) genetic influences, dominant (D) genetic influences or shared environmental (C), and person-specific environmental (E) influences on the Feeling Scale and RPE responses and AD ACL subscales Energy, Calmness, Tiredness and Tension.

			r_{MZ}	$r_{DZ/sibling}$	ACE/ADE model				Best fitting	
					A	C	D	E		
Cycle ergometer	*Ex.*	FS	.20 (.02, .36)	.01 (-.15, .16)	.00 (.00, .29)		.19 (.00, .35)	.81 (.65, .98)	**A = .15 (.00, .31)**	**E = .85 (.69, 1)**
		RPE	.25 (.06, .43)	.20 (.04, .34)	.08 (.00, .41)	.17 (.00, .34)		.75 (.59, .89)	**A = .29 (.13, .43)**	**E = .71 (.57, .87)**
	Rec.	Energy	.35 (.17, .50)	.24 (.09, .38)	.25 (.00, .50)	.10 (.00, .36)		.65 (.50, .82)	**A = .37 (.23, .51)**	**E = .63 (.49, .78)**
		Calmness	.24 (.05, .40)	.03 (-.11, .18)	.00 (.00, .32)		.22 (.00, .38)	.78 (.62, .95)	**A = .19 (.02, .34)**	**E = .81 (.66, .98)**
		Tiredness	.10 (-.10, .29)	-.02 (-.17, .13)	.00 (.00, .23)		.09 (.00, .27)	.91 (.73, 1)	**E = 1**	
		Tension	.26 (.08, .41)	.10 (-.05, .25)	.31 (.00, .38)		.09 (.00, .41)	.75 (.59, .91)	**A = .24 (.08, .38)**	**E = .76 (.62, .92)**
Treadmill	*Ex.*	FS	.23 (.07, .38)	.14 (-.01, .29)	.12 (.00, .38)	.11 (.00, .30)		.77 (.62, .92)	**C = .19 (.08, .30)**	**E = .81 (.70, .92)**
		RPE	.33 (.20, .48)	.20 (.05, .34)	.23 (.00, .47)	.10 (.00, .36)		.67 (.53, .83)	**A = .35 (.20, .48)**	**E = .65 (.52, .80)**
	Rec.	Energy	.23 (.04, .40)	.23 (.09, .36)	.01 (.00, .39)	.21 (.00, .33)		.78 (.61, .90)	**C = .22 (.10, .33)**	**E = .78 (.67, .90)**
		Calmness	.21 (.04, .34)	.09 (-.06, .24)	.16 (.00, .35)		.05 (.00, .37)	.79 (.63, .94)	**A= .21 (.05, .35)**	**E = .79 (.65, .95)**
		Tiredness	.11 (-.07, .27)	.13 (-.02, .27)	.00 (.00, .28)	.13 (.00, .24)		.87 (.72, .99)	**E = 1**	
		Tension	.17 (-.02 .34)	.03 (-.17, .12)	.00 (.00, .25)		.13 (.00, .30)	.87 (.70, 1)	**E = 1**	
Maximal exercise	*Rec.*	Energy	.39 (.24 .52)	.07 (-.09, .22)	.06 (.00, .48)		.33 (.00, .52)	.61 (.48, .76)	**A = .37 (.22, .49)**	**E = .63 (.51, .78)**
		Calmness	.38 (.23, .51)	.14 (-.03, .29)	.11 (.00, .48)		.27 (.00, .51)	.62 (.49, .78)	**A = .36 (.21, .49)**	**E = .64 (.51, .79)**
		Tiredness	.33 (.15, .48)	.16 (.01, .30)	.32 (.00, .46)		.00 (.00, .44)	.68 (.54, .83)	**A = .32 (.17, .46)**	**E = .68 (.54, .83)**
		Tension	.09 (-.10 .27)	.11 (-05, .27)	.00 (.00, .27)	.11 (.00, .23)		.89 (.73, 1)	**E = 1**	

Note. Ex. = Exercise test; Rec. = Recovery

Table 4.5 Correlations of voluntary regular exercise with the Feeling Scale and RPE responses and AD ACL subscales Energy, Calmness, Tiredness and Tension

			Correlation to voluntary exercise behavior
Cycle ergometer	*Exercise*	FS	**.18 (.08, .27)**
		RPE	**.15 (.06, .25)**
	Recovery	Energy	**.15 (.05, .25)**
		Calmness	**.12 (.03, .21)**
		Tiredness	**-.11 (-.20, -.01)**
		Tension	**-.11 (-.20, -.00)**
Treadmill	*Exercise*	FS	**.21 (.11, .30)**
		RPE	**.21 (.11, .30)**
	Recovery	Energy	**.11 (.01, .21)**
		Calmness	**.17 (.08, .26)**
		Tiredness	-.06 (-.15, .03)
		Tension	-.08 (-.17, .01)
Maximal exercise	*Recovery*	Energy	.01 (-.09, .10)
		Calmness	**.12 (.03, .22)**
		Tiredness	-.05 (-.15, .04)
		Tension	-.07 (-.17, .02)

Note. Significant correlations in bold.

DISCUSSION

The main aims of this study were to test the significance of a genetic contribution to the affective response to exercise and a genetic contribution to its relationship with regular exercise behavior. Results confirmed that the individual differences in affective responses in our adolescent sample during and after two submaximal exercise tests and a maximal exercise test could partly be explained by genetic factors. Heritability of the affective exercise response varied between 12% and 37%. This suggests that the well-documented individual differences in exercise-induced affective responses at moderate to vigorous (but not severe) intensities (Ekkekakis et al., 2005; Ekkekakis et al., 2011; Van Landluyt et al., 2000; Welch et al., 2007) should not solely be sought in environmental factors. In addition, more positive affective responses were associated with higher amounts of regular exercise activity ($.15 < r < .21$) and significant genetic correlations were found between higher amounts of regular voluntary exercise behavior and affective responses measured with the Feeling Scale during

exercise and the AD ACL subscales Energy and Calmness after cessation of exercise. This supported our hypothesis that there is an overlap in the genetic variants causing favorable affective exercise responding and the genetic variants influencing voluntary exercise behavior.

The results of this study are in keeping with De Geus & de Moor (2008) who predicted a role for the genetic variants influencing the affective response to exercise in the heritability of exercise behavior. However, as longitudinal follow-up of long-term exercise behavior of these adolescents was not available, reverse causality cannot be ruled out. In reverse causality, the genetic correlation arises because the genetic variants influencing exercise behavior could become part of the heritability of the affective response if regular exercise itself sensitizes regular exercisers to the appetitive effects of exercise or desensitizes them to the aversive effects. Furthermore, a third scenario is that the same genetic variants independently influence the affective response and the tendency to become a regular exerciser. An example of such genetic pleiotropy would be genetic variants influencing vagally mediated heart rate recovery from exercise. Such recovery may be an important factor determining both the affective response to exercise as well as exercise ability which in turn will reinforce regular exercise behavior.

Mixtures of these three causal scenarios may be at play as well, e.g. there may be bidirectional causality in the presence of pleiotropy. Training studies could help resolve causality, but might suffer from selection bias, as they are typically conducted in sedentary individuals (regular exercisers would not show meaningful changes). Twin studies can resolve causality in unselected population-based samples if the sample size is sufficiently large to detect environmental correlations (de Moor et al., 2008), but might be a challenging undertaking for the relatively involved experimental protocol used here. Below 5000 twin pairs, the power to detect a significant environmental correlation between affective responses and exercise behavior is poor (Stubbe & de Geus, 2009). Mendelian Randomization would be a very good alternative strategy to resolve causality as this technique detects causal effects in an unbiased manner (Davey Smith & Hemani, 2014; Lawlor et al., 2008). However, this approach would need (a set of) genetic variants influencing only the affective response to

exercise and ideally also a set of genetic variants influencing only exercise behavior. As many large cohorts have genetic data paired to data on voluntary exercise behavior, the latter will become available in time through a meta-analysis of cohort-specific genome-wide association analyses. For genetic variants influencing the affective response to exercise, a candidate gene approach seems the only feasible approach.

A future challenge is to identify the specific genes underlying the heritability of the affective response to exercise, to test their predictive value for the adoption of regular exercise behavior and their usefulness in personalizing exercise intervention. To do so studies need to have measured affective exercise responses in designs as used in the present study as well as having collected DNA materials. A study by Bryan et al. (2007) reported that the brain-derived neurotrophic factor (*BDNF*) gene, a peptide with a broad influence on the vascular, muscular and central nervous system, moderated the effect of exercise on mood, heart rate, and perceived exertion in a sample of healthy exercisers. Moreover, the *BDNF* gene might also be associated with intrinsic motivation during exercise (Caldwell Hooper et al., 2014). In inactive but healthy adults, Karoly et al. (2012) found two single nucleotide polymorphisms (SNPs) in the fat mass and obesity-associated protein gene (*FTO*) gene related to positive affect change during exercise (Karoly et al., 2012). Other candidate gene studies aimed at exercise behavior have already focused on the feelings of reward that are governed by the dopaminergic and cannabinoid reward systems in mesolimbic circuits. Genetic variation in these circuits might indeed explain the heritability of affective responses to exercise. Previous studies reported an effect of genetic variants in dopaminergic genes on voluntary physical activity in animals (Knab & Lightfoot, 2010), but for humans the dopaminergic connection is less well established (Huppertz et al., 2014a; Jozkow et al., 2013; Simonen et al., 2003)

Exercise also generates aversive responses. Genetic variation in brain circuits governing punishment or pain and fatigue may be as relevant as reward (Ekkekakis, 2003; Ekkekakis, 2003) but they have been much less studied to date. Our study was no exception: here we deliberately chose to measure affective states below or close to the VT, i.e. the range where displeasure is not yet very strong. As many subjects engaged in regular leisure time exercise activities will stay below this intensity threshold, individual differences in (dis)pleasure

experienced at such intensities could be important determinants of voluntary maintenance of regular exercise behavior. This neglects the potential importance of the increase in interindividual variation in affective responding at intensities just above the VT, when the supply of energy through oxygen must be supplemented by anaerobic metabolism and the physiological steady-state is challenged (Ekkekakis, 2003). We confirm the emergence of stronger individual differences with increased intensity as is reflected by the increase in standard deviations of the FS (Table 4.2). Exercising above the VT (but below the maximum steady-state lactate concentration) may therefore increase the genetic variance in affective response beyond that seen below the VT. Indeed, when calculating MZ and DZ/sibling correlations for FS for every step of the submaximal test separately, the difference between these correlations was increasing with intensity (with MZ correlations increasing; data not shown). Future studies should confirm the expectation that affective responses to exercise above the VT are driven by genetic factors to a substantial extent.

Some further limitations of the study must be addressed. Two different submaximal tests were performed on a cycle ergometer and a treadmill. Although the use of more than one exercise mode adds to the robustness of the findings and increases external validity, these laboratory conditions still do not reflect daily settings in which an individual is exercising. The type of exercise e.g. aerobic or anaerobic exercise, individual or in teams, time of the day and whether it is done outdoors rather than indoor as in the current study, might all have an influence on how one feels during and after exercise. Furthermore placing the treadmill test always in fixed order after the cycling test could have influenced the affective responses during the treadmill test even if Dutch adolescents are very used to cycling and the recovery time was enough to reach resting $\dot{V}O_2$ values. Most importantly, affective responses may have been influenced by the prospect of a maximal exercise test that would have to be completed at the end of the session. Many other studies on this topic use a separate day for maximal exercise testing which has the added advantage that workloads can be standardized exactly as a percentage of $\dot{V}O_{2max}$. However, our subjects were not students recruited in the typical way from a single high school or college, but came from the entire country as they were selected from a nation-wide twin register. This meant substantial travel for most of them in a period when many of them were engaged in their final school year (with closing examination

determining their further careers). To reduce burden on the subjects and also for logistic reasons only a single measurement day was therefore deemed possible. Apart from creating a potential foreshadowing influence on the affective response to the submaximal exercise tasks, this may have led subjects to have been too exhausted to perform optimally at the maximal exercise test. However, comparison of the $\dot{V}O_{2max}$ predicted from the submaximal tests to the actual peak $\dot{V}O_2$ attained during the maximal exercise test suggest that such underestimation will have been mild and only mildly affect rank order of aerobic fitness levels (Schutte et al., 2016a).

The acknowledgement of the existence of individual differences in affective response to exercise is key to the innovation of exercise programs. Moderate heritability estimates of these parameters do show that it may be harder to engage some people in exercise than others, but does not suggest that we should stop trying. It simply suggests that we should not close our eyes to human genetic variation. In the population at large, regular leisure time exercise seems associated with better mental health largely through pleiotropic genetic effects (Schutte et al., 2014). The longer term beneficial psychological effects of exercise appear to be more easily unlocked by some genetic profiles than by others. This may well be linked to the heritability of the psychological responses for a single bout of exercise, as tested in the present study in adolescents. Favourable genetic profiles may for instance cause a larger sensitivity to the rewarding or a smaller sensitivity to the punishing effects of broad classes of activities, including exercise. For some individuals, exercising may be associated with a strong 'feel good' experience and constitute an excellent short-term coping strategy that helps to unwind more rapidly from daily pressures experienced in the school, job or home environment. For others, the aversive effects of exercise, at least in the forms that they tried so far, may greatly overwhelm the rewarding effects, causing them to drop-out. These individuals might benefit more from an individualized exercise intervention, in which the appetitive aspects for an individual should be emphasized and the aversive aspects reduced as much as possible.

As the motivation to adopt and maintain regular exercise is key to a better public health, genetic pathways underlying individual differences in the affective responses to exercise

should remain an important target for research. A main future challenge is to identify the specific genes underlying the heritability of the affective response to exercise, to test their predictive value for the adoption of regular exercise behavior in adolescence and in other age ranges as well as their usefulness in personalizing exercise intervention.

CHAPTER 5

DIFFERENCES IN ADOLESCENT PHYSICAL FITNESS: A MULTIVARIATE APPROACH & META-ANALYSIS

PUBLISHED AS

Schutte NM, Nederend I, Hudziak JJ, de Geus EJC & Bartels M (2016)

Differences in adolescent physical fitness: a multivariate approach & meta-analysis

Behavior Genetics 46,217-227

ABSTRACT

Physical fitness can be defined as a set of components that determine exercise ability and influence performance in sports. This study investigates the genetic and environmental influences on individual differences in explosive leg strength (vertical jump), handgrip strength, balance, and flexibility (sit-and-reach) in 227 healthy monozygotic and dizygotic twin pairs and 38 of their singleton siblings (mean age 17.2 ± 1.2). Heritability estimates were 49% (95% CI: 35% – 60%) for vertical jump, 59% (95% CI: 46% – 69%) for handgrip strength, 38% (95% CI: 22% – 52%) for balance, and 77% (95% CI: 69% – 83%) for flexibility. In addition, a meta-analysis was performed on all twin studies in children, adolescent and young adults reporting heritability estimates for these phenotypes. Fifteen studies, including results from our own study, were meta-analyzed by computing the weighted average heritability. This showed that genetic factors explained most of the variance in vertical jump (62%; 95% CI: 47% – 77%, *N* = 874), handgrip strength (63%; 95% CI: 47% – 73%, *N* = 4516) and flexibility (50%; 95% CI: 38% – 61%, *N* = 1130) in children and young adults. For balance this was 35% (95% CI: 19% – 51%, *N* = 978). Finally, multivariate modeling showed that the phenotypic correlations between the phenotypes in current study (.07 < *r* < .27) were mostly driven by genetic factors. It is concluded that genetic factors contribute significantly to the variance in muscle strength, flexibility and balance; factors that may play a key role in the individual differences in adolescent exercise ability and sports performance.

INTRODUCTION

Physical fitness can be defined as a set of components that influences exercise ability and performance in sports (Caspersen et al., 1985). Because exercise ability may be a major driver of voluntary exercise behavior (Bryan et al., 2007; de Geus & de Moor, 2008) it is important to understand the sources of variation in physical fitness. Studies on physical fitness have focused on maximal oxygen consumption, as aerobic fitness is a major determinant of exercise ability. However, exercise ability also entails muscular strength, flexibility, and motor control, all of which play an important role in health (Baranowski et al., 1992; Ortega et al., 2008). Several easy to perform tests exist that have been shown to provide reliable and valid indicators of these traits.

Muscle strength is defined as the maximal force that can be generated by a specific muscle or muscle group during a single movement. Measurements of muscle strength typically focus on the force generated by the elbow flexors or the knee extensors, typically at different angles of elbow flexion or knee extension. Strength can be measured with the muscle remaining at a fixed length (isometric) or while contracting (dynamic). The handgrip test, an easy and reliable measure, is by far the most commonly used measure for assessing isometric strength in epidemiological studies (Bohannon et al., 2011). For dynamic explosive strength, the vertical jump has been the most widely used test. *Balance* is a performance-related fitness component that relates to the maintenance of a stable body position (Caspersen et al., 1985) which is maintained by both sensory and motor systems (Tresch, 2007). It can be measured using the Balance Error Scoring System (BESS) that is commonly used by researchers and clinicians and has a moderate to good reliability (Bell et al., 2011). *Flexibility*, defined as the ability of a specific muscle or muscle group to move freely through a full range of motion, can be assessed by the sit-and-reach test (reaching forward as far as possible from a seated position).

The two main factors that can influence individual differences in physical fitness are innate biological differences and environmental factors. The latter can be subdivided in influences shared with other members of the family (shared environmental influences) and person-specific or unique environmental influences, which includes error measurement but also comprises person specific exercise participation, training, and coaching. A design that is often

used for partitioning total variance in genetic, shared environmental, and person-specific environmental components is the classical twin design. In a twin study, intrapair resemblance between two types of twin relationships is compared; genetically identical (monozygotic, MZ) and non-identical (dizygotic, DZ) twins. If the MZ resemblance for physical fitness is comparable to the DZ resemblance and non-zero, this constitutes evidence for shared environmental influences on the phenotype under study. If the MZ resemblance for physical fitness is higher than the DZ resemblance this constitutes evidence for genetic influences on the phenotype. Previous twin studies showed that genetic factors account for a substantial part of the variation in the aforementioned components of physical fitness.

Table 5.1 provides an overview of twin studies conducted in children, adolescents or young adults (published in English). It reports twin correlations and/or heritability estimates of similar or comparable components of physical fitness. Although the sample sizes are small for most studies, results consistently show moderate to high heritability estimates for vertical jump (ranging from 47% to 83%) (Chatterjee & Das, 1995; Kovar, 1976; Maes et al., 1996). For the handgrip test, heritability estimates range from 32% to 77% in children and young adults (Kovar, 1976; Okuda et al., 2005; Silventoinen et al., 2008; Venerando & Milani-Comparetti, 1970). Four studies report moderate heritability estimates for balance (24% to 46%) (Maes et al., 1996; Vandenberg, 1962; Williams & Gross, 1980). Although the balance tests used were slightly different, all were indicators of static body balance ability. Finally, 18% to 55% of the variation in flexibility (as measured by the sit-and-reach test) in children and young adults could be explained by genetic influences (Chatterjee & Das, 1995; Maes et al., 1996; Okuda et al., 2005). In addition, the study by Maes et al. detected significant shared environmental influences on flexibility. Taken together, the existing studies confirm a role for genetic influences on the individual differences in physical fitness but estimates vary widely. This may reflect the modest sample sizes used in most studies, with one clear exception for handgrip (Silventoinen et al., 2008). Meta-analysis could be helpful to provide a more robust estimate of the heritability of components of physical fitness.

A further theme that has not been extensively addressed is the extent of overlap between the genetic factors influencing these varied fitness phenotypes. Only a few studies provided some information on the (genetic) co-variation between components of physical fitness. Two studies reported moderate phenotypic correlations for muscle strength measures such as

isometric strength measured by handgrip and knee extension. Multivariate modeling of these traits showed that part of the genes affecting muscle strength may be common to isometric strength measured by handgrip and knee extension (Silventoinen et al., 2008; Tiainen et al., 2004).

The four tests used in our study are derived from earlier work in fitness test batteries with the aim of constructing 'unrelated' components of health- and performance related fitness (Simons et al., 1969). Although Simons showed that these four fitness tests loaded on different factors, moderate phenotypic correlations (ranging from 0 to .54) between these tests were found in the 16 to 19 year olds. In a multivariate design it is possible to explore the source of covariance between these phenotypes. Information on the genetic association between various measures of physical fitness might be useful for meta-analyses over genetic association studies to examine the association of genetic variants with physical fitness.

To summarize, there is evidence for genetic influences on muscle strength (handgrip and vertical jump), balance, and flexibility but heritability estimates vary across samples. Multivariate genetic analyses on all four parameters have not been reported. To replicate and expand the literature on the genetic architecture of physical fitness components, we estimated the heritability of muscle strength measures (vertical jump and handgrip strength), balance and flexibility in a large sample of adolescent twins and their siblings and these estimates were incorporated in a meta-analysis on the heritability of muscle strength, flexibility and balance. Finally, in a multivariate design, the source of covariance among these fitness components was examined.

Table 5.1 Overview of heritability studies of vertical jump, hand grip, balance and flexibility.

Phenotype	Study	Sample[a]	Age range	r_{MZ}	r_{DZ}	A	C	E
Vertical jump	Kovar, 1976	• 17 MZ • 13 DZ	11 – 25			83%		
	Chatterjee & Das, 1995	• 30 MZ • 24 DZ	10 – 27	.85	.21	71%		
	Maes et al., 1996	• 43 MZ • 61 DZ	10	.65	.28	47%[b] 78%[c]	-	53%[b] 22%[c]
Handgrip	Venerando & Milani–Comparetti, 1970	• 24 MZ • 24 DZ	9 – 17			32%		
	Kovar, 1976	• 17 MZ • 13 DZ	11 – 25			63%		
	Okuda et al., 2005	• 90 MZ • 68 DZ	10 – 14	.78	.49	77%	-	23%
	Silventoinen et al., 2008	• 1682 MZ • 1864 DZ	16 – 25	.66	.35	66%	3%	31%
Balance[d]	Vandenberg, 1962	• 40 MZ • 30 DZ	14 – 18			24%		
	Williams & Gross, 1980[e]	• 22 MZ • 41 DZ	11 – 18	.51	.33	27%		73%
	Maes et al., 1996	• 43 MZ • 61 DZ	10	.46	.32	46%	-	54%
Flexibility[f]	Chatterjee & Das, 1995	• 30 MZ • 24 DZ	10 – 27	.73	.60	18%		
	Maes et al., 1996	• 43 MZ • 61 DZ	10	.84	.54	38%[b] 50%[c]	32%[b] 42%[c]	30%[b] 8%[c]
	Okuda et al., 2005	• 90 MZ • 68 DZ	10 – 14	.58	.29	55%		44%

Note. Only unadjusted estimates are reported, except for Silventoinen et al. (2008) and Tiainen et al. (2004) (age-adjusted results). A dash indicates that this component could be dropped from the model. If empty, only A was reported. [a]number of twin pairs [b]Males; [c]Females; [d]different balance tests, but all indicators of static body balance; [e]longitudinal study; only baseline results are shown here; [f]flexibility measured with the sit-and-reach test.

METHODS

Subjects

548 healthy adolescent twin pairs aged between 16 and 18, enrolled in longitudinal survey studies of the Netherlands Twin Register (van Beijsterveldt et al., 2013), were invited to participate in the study on the determinants of adolescent exercise behavior. Siblings of the twins within an age range of 12 – 25 years were also invited. Selection for invitation was based on the availability of longitudinal survey data on zygosity and regular leisure time exercise behavior. The aim was to have sufficient twins present from the entire spectrum of sedentary to vigorous leisure time exerciser and for each zygosity group. We started with a random selection, but if a zygosity group was underrepresented or if there were too little sedentary or vigorous exercisers, invitations were biased towards the underrepresented groups. In order to be eligible for the study, subjects had to have no history of cardiovascular or respiratory disease, and being physically capable of engaging in exercise activities.

Subjects were invited by sending a letter advertising the opportunity to test their fitness in addition to earning a gift voucher. All invitees had to be able and willing to visit the VU University in Amsterdam for lab testing. The final sample consisted of 227 complete twin pairs: 59 monozygotic male pairs (MZM), 36 dizygotic male pairs (DZM), 57 monozygotic female pairs (MZF), 42 dizygotic female pairs (DZF), 33 dizygotic opposite sex pairs (DOS) and 38 of their singleton siblings. Two additional sibling pairs participated (without a twin), resulting in a sample size of 498 subjects. Mean age at time of the laboratory assessment was 17.2 ± 1.2.

All subjects above 18 provided written consent and if the subjects were under 18 consent was given by both of their parents/guardians and assent by the subject. All study procedures were reviewed and approved by the Medical Ethics Review Committee of the VU University Medical Center Amsterdam (NL35634.029.10).

Components of physical fitness

On arrival at the laboratory, height and weight (Omron BF511, Omron Healthcare Europe B.V., The Netherlands) were measured. Subsequently, 4 fitness characteristics were examined: vertical jump, handgrip strength, balance, and flexibility.

Vertical jump Explosive strength was measured with a vertical jump test that requires the subjects to jump as high as possible, starting from a position of knee bending at a fixed knee angle immediately prior to the jump. Subjects were instructed to jump straight up as much as possible and not go sideways. It was allowed to use the arms to help drive the body upwards. A successful jump was defined as one where at take-off the subjects had the appropriate knee angle and landed their feet within a 10 cm radius of the start position. Jumping height was defined as the vertical displacement between of the trunk at the beginning and at the end of the jump measured by the displacement of a tapeline attached to the subjects' hip and a clipped to the floor. Best out of 3 jumps was documented (jumping height in centimeters).

Handgrip strength Subjects were instructed to hold a dynamometer (Baseline Digital Smedley Hand dynamometer, Fabrication Enterprises Inc., USA) in the dominant hand with arm at the side of the body and elbow at a 90° angle. When ready, the subject was encouraged to squeeze the dynamometer once with maximum effort (in kg), which should be maintained for about 5 seconds.

Balance The Balance Error Scoring System (BESS) (Bell et al., 2011) was used to assess balance under 3 testing stances: double leg, single leg (non-dominant leg) and tandem (dominant foot in front of the non-dominant foot in heel-to-toe fashion, weight evenly distributed across both feet) on 2 surfaces (ground and foam pad). During the test, the eyes were closed and the hands were held on the hips. Each condition lasted for 20 seconds. We instructed the subjects that if at any time they fell out of position, they were to return to the test position as quickly as possible. As the subjects performed each 20-second trial, we observed and recorded the number of errors each subject made. An error was defined as opening eyes, lifting hands off hips, stepping, stumbling or falling out of position, lifting forefoot or heel, abducting the hip by more than 30°, or failing to return to the test position in less than 5 seconds. The total score was the total number of errors. For every subject, this number was recalculated (finals score was subtracted from 60) as such that a better balance was associated with a higher score.

Flexibility Flexibility was measured using a standard sit-and-reach box (Baseline Sit-and-reach Trunk Flexibility Box, Fabrication Enterprises Inc., USA). Subjects were instructed to sit on the floor with the legs fully extended and the soles of the feet flat against the box. One hand was

placed on top of the other palms down. Then the subject reached forward along the measuring scale on the box as far as possible, without bending the knees. Best out of 3 reaches (in centimeters) was used for subsequent analyses.

Genetic analyses

Genetic structural equation modeling in OpenMx (Boker et al., 2011) under R (R Development Core Team, 2011) was used with the raw-data ML procedure for estimation of parameters. For all analyses, a threshold of $p < 0.05$ was considered for statistical significance. First, a so-called saturated model that estimated all parameters freely (a) was fitted to the data. Given the relative small sample size, with no power to test for sex-differences, and since (non-twin) siblings share, like DZ twins, on average 50% of their genes, parameter estimates were constrained to be equal for males and females and for DZ twins and siblings. Main effects of sex and age and Body Mass Index (BMI) on mean levels of components of physical fitness were considered in the model since these factors are associated with strength (Chatterjee & Chowdhuri, 1991).

Cross-trait/cross-twin correlations and their 95% confidence intervals were estimated for the MZ and DZ twins/siblings. Subsequently, 4 univariate models and a 4-variate Cholesky decomposition were fitted to the data to decompose the phenotypic statistics into sources of additive genetic variance/covariance (A), dominant genetic variance/covariance (D) or shared environmental variance/covariance (C) and person-specific environmental variance/covariance (E). Since C and D effects cannot be estimated simultaneously in the classical twin model, the ratio of the MZ correlations to the DZ correlations was used to determine which model (ACE or ADE) is most appropriate. Significance of variance-covariance components was tested by comparing the model including the specific component (e.g. ADE) to a model in which the component is constraint to be equal to zero (e.g. AE). The pattern of the factor loadings on the latent genetic and environmental factors in a Cholesky decomposition reveals a first insight into the etiology of covariances between the physical fitness components.

Meta-Analyses

In order to collect all studies on the heritability of the four components of physical fitness under study, a search of the electronic databases ISI Web of Knowledge and PubMed was conducted using *handgrip / muscle strength / vertical jump / explosive strength / flexibility / sit-and-reach / balance* and *genes / heritability / twin(s)* as key words. In addition, the reference lists of these articles were inspected. Articles (all-year) published in English and reporting twin correlations and/or heritability estimates of the vertical jump test, handgrip strength, balance and flexibility (sit-and-reach test) in a sample of children, adolescents and/or young adults up to the age of 30 were included, provided that these phenotypes were roughly comparable (i.e. protocol) to the phenotypes measured in the current study. These papers are shown in Table 5.1. For all studies, the univariate and unadjusted correlations and/or estimates were extracted, except for the study by Silventoinen et al. (2008) and Tiainen et al. (2004), who reported age-adjusted estimates only. While not all studies reported twin correlations, they did include an estimate of the heritability; therefore the meta-analyses were based on the heritability estimates. By weighing these heritability estimates from all studies by the number of subjects, the weighted average heritability can be computed using Microsoft Excel (2010) (Li et al., 2003; Neyeloff et al., 2012). When the standard errors (SEs) or confidence intervals (CIs) of the heritability estimates were not reported, these were calculated using the SEs or CIs from studies who did report these statistics (Li et al., 2003). All studies reported one (equated) heritability estimate for males and females, except for Maes et al. (1996). These heritability estimates for males and females were treated if these were independent samples. Results from the current study were also included in the meta-analyses. For consistency, univariate models were fitted to our four phenotypes and the resulting heritability estimates were used in the meta-analyses. The I^2 statistic was used to assess heterogeneity and was calculated as $(Q - df)/Q$, where Q is Cochran's heterogeneity statistic and df the degrees of freedom (Higgins & Thompson, 2002).

RESULTS

Descriptives

Means and standard deviations for the fitness components of males and females are shown in Table 5.2. BMI ($kg \cdot m^{-1}$) of this sample was (mean ± SD) 20.6 ± 2.5 for males and 21.8 ± 3.3 for females, comparable to the average 17 year olds in The Netherlands (Schonbeck et al., 2011). Males outperformed females for the vertical jump ($p < .001$) and handgrip ($p < .001$), whereas females performed better for balance ($p < .001$) and flexibility ($p < .001$). As expected, significant age effects were found on vertical jumping ($p = .011$) and handgrip ($p < .001$). Additionally, significant effects of BMI on mean levels of vertical jump ($p = .026$), handgrip ($p = .041$) and balance ($p = .011$) were detected. Because of significant sex, age, and BMI effects on the mean these factors were taken into account in further model fitting.

Table 5.2 Means and standard deviations of vertical jump, handgrip strength, balance and flexibility in males in females.

	Male (*N* = 243)		Female (*N* = 255)	
	Mean	*SD*	*Mean*	*SD*
Vertical jump (cm)	45.8	6.4	35.5	5.4
Handgrip strength (kg)	40.2	8.0	29.5	4.7
Balance[a]	45.1	6.6	47.1	6.6
Flexibility (cm)	19.8	10.1	29.0	9.7

Note. [a]see main text for details on balance-measurements.

Table 5.3 shows the phenotypic correlations (95% confidence intervals) in the upper panel. Vertical jump was significantly associated with handgrip (.27) and flexibility (.10) but not with balance. Better balance was associated with higher scores on the handgrip (.15) and flexibility test (.10). No association between handgrip and flexibility was found.

Table 5.3 Phenotypic and cross-twin/cross-trait correlations (95% CI) for vertical jump, handgrip strength, balance and flexibility estimated from the saturated model

	Phenotypic correlations			
	Vertical jump	Handgrip strength	Balance	Flexibility
Vertical jump	1.00			
Handgrip strength	.27 (.18, .37)	1.00		
Balance	.07 (-.03, .16)	.15 (.05, .25)	1.00	
Flexibility	.10 (.01, .20)	.08 (-.02, .18)	.10 (.01, .19)	1.00
	MZ correlations			
	Vertical jump	Handgrip strength	Balance	Flexibility
Vertical jump	.53 (.39, .64)			
Handgrip strength	.19 (.02, .36)	.58 (.44, .69)		
Balance	.08 (-.09, .25)	.23 (.06, .38)	.34 (.16, .49)	
Flexibility	.10 (-.07, .25)	.10 (-.06, .25)	.03 (-.13, .18)	.79 (.71, .85)
	DZ/sibling correlations			
	Vertical jump	Handgrip strength	Balance	Flexibility
Vertical jump	.20 (.05, .34)			
Handgrip strength	.07 (-.07, .21)	.27 (.13, .40)		
Balance	.04 (-.10, .17)	-.03 (-.18, .11)	.22 (.07, .35)	
Flexibility	.10 (-.04, .24)	-.12 (-.27, .04)	.09 (-.06, .24)	.31 (.16, .44)

For all fitness components, the MZ correlation was higher than the DZ/sibling correlation (diagonal components of the lower two panels of Table 5.3), suggesting a genetic effect. For vertical jump, handgrip, and flexibility, the DZ/sibling correlations were less than half the MZ correlations, so shared environment factors were not further considered as a source of variance for these fitness parameters. Cross-twin/cross-trait correlations (off-diagonal correlations in Table 5.3) showed that in MZ twins handgrip was significantly associated with vertical jump (.19) and balance (.23), but not in DZ twins/siblings, suggesting a common set of genes influencing these components of physical fitness. Furthermore, flexibility was not significantly associated with vertical jump, handgrip and balance in MZ twins and DZ twins/siblings. The negative DZ twin/sibling correlations between handgrip strength and balance and handgrip strength and flexibility were not significant and most likely the result of a relatively small sample size.

Univariate results

Model fitting for vertical jump, handgrip and flexibility started with an ADE model. Dominant genetic influences were not significant (p > .05) and were dropped from the model. Heritability estimates were 49% (95% CI: 35% – 60%) for vertical jump, 59% (95% CI: 46% – 69%) for handgrip and 77% (95% CI: 69% – 83%) for flexibility. For balance, modeling started with an ACE model, as twin correlations for balance suggested the presence of shared environmental influences. However, C could be dropped from the model (p < .05). Genetic factors explained 38% (95% CI: 22% – 52%) of the variance in balance in our sample. The remaining variance in the four phenotypes was accounted for by person-specific environmental factors.

Meta-analyses

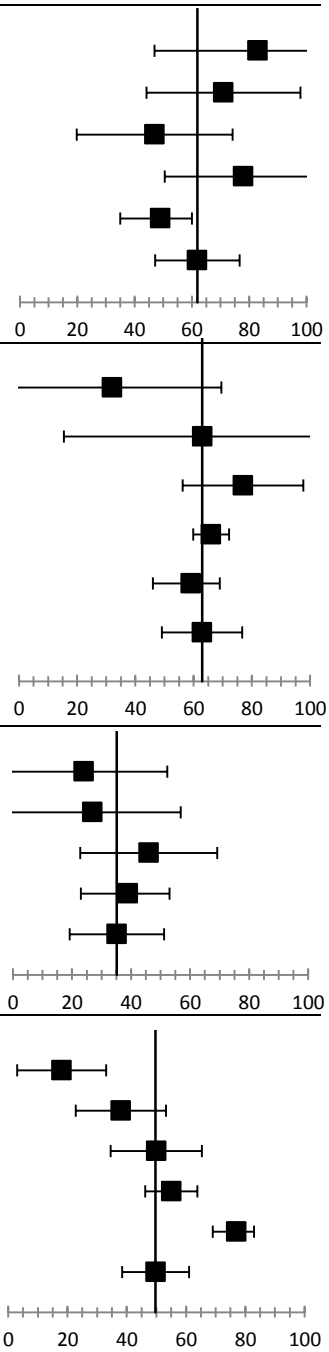
The results of the meta-analyses are presented in Table 5.4. For vertical jump, results from four studies (the study by Maes et al. resulted in two sex-specific estimates) were used, including the current study. The heritability estimates of these studies are represented in the graph on the right as squares (with 95% CIs) and the bottom square shows the weighted average heritability estimate of 62% (95% CI: 47% – 77%). This estimate falls within the CIs of all studies, except for the current study. The majority of variance in vertical jump in this combined child and young adult sample (N = 874) can be explained by genetic factors. Handgrip measured in a combined sample of 9 – 25 year olds (N = 4516) showed a weighted average heritability estimate of 63% (95% CI: 47% – 73%). This estimate falls within the CIs of all studies included in this meta-analysis. For balance, a weighted average heritability estimate of 35% (95% CI: 20% – 41%, N = 1704) was found, which falls perfectly in the CIs of the included studies. Four studies reported a heritability estimate for flexibility in children and young adults, ranging from 18% to 77% (current study). The meta-analytic weighted average heritability of 50% (%95 CI: 38% – 61%, N = 1130) falls outside the CIs of these two heritability estimates. The meta-analyses for handgrip and balance showed low heterogeneity (I^2 = 18% and 0%). However, high I^2 values were detected for vertical jump and flexibility (46% and 94%), suggesting that differences in studies are not caused by sampling error only.

Multivariate analyses

Based on the overall correlational structure, multivariate model fitting was started with an ADE model. Dominant genetic influences were not significant (p = .659). Standardized components from this final model for additive genetic and person-specific environmental influences on the four components of physical fitness and their covariances are presented in Table 5.5. The diagonals in the upper panel show the heritability estimates for the four phenotypes from the multivariate model. The off-diagonal values show that the majority of the phenotypic correlations between the phenotypes under study could be explained by genetic factors (74% to 99%) except for vertical jump and flexibility, of which environmental factors explained more than half of the phenotypic correlation (53%). Significant genetic correlations were found for handgrip and vertical jump (r_G = .46, 95% CI: .27 − .65), handgrip and balance (r_G = .32, 95% CI: .11 − .52) and balance and flexibility (r_G = .18, 95% CI: .01 − .37). In addition, a significant environmental correlation was found between vertical jump and flexibility (r_E = .22, 95% CI: .04 − .38).

Table 5.4 Heritability estimates (95% CI) of the studies used in the meta-analyses. In bold the weighted average heritability estimate (95% CI).

Phenotype	Study	Sample Size	Heritability
Vertical Jump	Kovar, 1976	60	83 (47, 119)
	Chatterjee & Das, 1995	108	71 (44, 98)
	Maes et al., 1996[a]	105	47 (20, 74)
	Maes et al., 1996[b]	103	78 (51, 106)
	Current study	498	49 (35, 60)
	Meta-analysis	874	62 (47, 77)
Handgrip	Venerando & Milani–Comparetti, 1970	96	32 (-6, 70)
	Kovar, 1976	60	63 (15, 111)
	Okuda et al., 2005	316	77 (56, 98)
	Silventoinen et al., 2008	3546	66 (60, 72)
	Current study	498	59 (46, 69)
	Meta-analysis	4516	63 (47, 73)
Balance	Vandenberg, 1962	146	24 (-4, 52)
	Williams & Gross, 1980	126	27 (-3, 57)
	Maes et al., 1996	208	46 (23, 69)
	Current study	498	38 (22, 52)
	Meta-analysis	978	35 (19, 51)
Flexibility	Chatterjee & Das, 1995	108	18 (3, 33)
	Maes et al., 1996[a]	105	38 (23, 53)
	Maes et al., 1996[b]	103	50 (35, 65)
	Okuda et al., 2005	316	55 (46, 64)
	Current study	498	77 (69, 83)
	Meta-analysis	1130	50 (38, 61)



Note. [a]Males; [b]Females. All confidence intervals are calculated based on sample size.

Table 5.5 Standardized estimates (95% CI) for additive genetic (A) and person-specific environmental influences (E) on the four components of physical fitness and their covariance based on the full AE Cholesky model.

	A			
	Vertical jump	Handgrip strength	Balance	Flexibility
Vertical jump	.49 (.36, .61)			
Handgrip strength	.85 (.55, 1)	.60 (.48, .70)		
Balance	.83 (0, 1)	.99 (.49, 1)	.39 (.23, .52)	
Flexibility	.47 (0, .89)	.94 (.64, 1)	.74 (.09, 1)	.78 (.70, .83)
	E			
	Vertical jump	Handgrip strength	Balance	Flexibility
Vertical jump	.51 (.39, .64)			
Handgrip strength	.15 (0, .45)	.40 (.30, .52)		
Balance	.17 (0, 1)	.01 (0, .51)	.61 (.48, .77)	
Flexibility	.53 (.11, 1)	.06 (0, 1)	.26 (0, .91)	.22 (.17, .30)

DISCUSSION

To examine the heritability of and genetic co-variation between various components of physical fitness, genetic models were fit to data from 498 late-adolescent twins and their siblings. Univariate modeling showed that a moderate to large part of the individual differences in components of physical fitness is accounted for by genetic differences between individuals. The remaining variance was accounted for by person-specific environmental effects. Muscle strength, flexibility, and balance all contribute to exercise ability and performance in sports (Caspersen et al., 1985; Gleim & McHugh, 1997; Hrysomallis, 2011; Ruiz et al., 2006). Strength and flexibility are not only performance-related but also health-related (Baranowski et al., 1992; Caspersen et al., 1985). Lower levels of these components measured in childhood and adolescence are associated with cardiovascular risk factors, such as hypercholesterolemia or hypertension in adulthood (Ortega et al., 2008; Wedderkopp et al., 2003). For instance, data from the AVENA study showed an association of lower scores of maximal handgrip and explosive strength in adolescent females and a cardiovascular risk score (Garcia-Artero et al., 2007). Balance, on the other hand, is considered mainly a performance-related fitness component (Caspersen et al., 1985). It showed a moderate

heritability estimate of 38% compared to strength and flexibility, demonstrating that most of the variance can be explained by person-specific environmental factors. These findings confirm findings by Maes et al. (1996) that components of physical fitness that are only performance-related are less under genetic control than components that are both performance and health-related.

The heritability estimates found in the current study were confirmed in meta-analyses of all studies reporting on the heritability of these phenotypes in twin samples under age 30 with some notable differences. For balance, all studies, including the current study, report more or less similar heritability estimates (24% to 46%) and showed a rather homogenous picture, whereas the meta-analysis of flexibility showed heterogeneity as two out of five studies report an estimate significantly lower (Chatterjee & Das, 1995) or higher (current study) than the meta-analytic heritability estimate of 50%. A source of this variation might be the age of the subjects as Okuda et al. and Maes et al. measured flexibility in children and reported a lower heritability compared to the current study in late-adolescents. Chatterjee & Das measured flexibility in subjects with a much wider age range (10 – 27) and found a heritabilty estimate of only 18%. However, after adjustment for age, this estimate increased to 50% (Chatterjee & Das, 1995). For muscle strength meta-analyses resulted in weighted average heritability estimates of 62% for vertical jump and 63% for handgrip strength. This estimate generally fell within the confidence intervals of all the studies, despite the wide range of heritability estimates of the included studies (47% − 83% for vertical jump and 32% − 77% for handgrip). Our study, however, reports a 13% lower heritability for vertical jump. Taken together, from the analyses presented we conclude that at least half of the variance in vertical jump, handgrip strength and flexibility and a substantial part of the variance in balance in children and young adults (< 30yr) can be explained by genetic factors.

Environmental factors that are shared by the twins (such as the family environment) do not seem to play a major role in explaining individual differences in physical fitness components in our late-adolescent sample. As the correlations for DZ twins/siblings were low and non-significant, shared environment factors were not further considered as a source of variance for these fitness parameters. This does not rule out a small contribution of shared environmental influences, as this is hard to detect in samples of this size, even if the power to detect shared environmental influences was increased by adding siblings to the design. Of

interest, twin studies on voluntary exercise behavior show that the influence of these shared environmental factors is significant at young ages, but decreases or has completely disappeared when reaching adolescence (Huppertz et al., 2012; Stubbe et al., 2005; van der Aa et al., 2010). Twin correlations in Table 5.1 do suggest the presence of shared environmental influences as the DZ/sibling correlations for handgrip, balance and flexibility were higher than half the MZ correlations. In the current study, the DZ/sibling correlation for balance was higher than half the MZ correlation. Only two studies (Maes et al., 1996; Silventoinen et al., 2008) reported a significant contribution of shared environmental factors. Posthuma & Boomsma (2000) showed that to detect shared environmental factors with a power of 80%, a sample size of more than 2000 individuals is needed (extended twin design) to detect shared environmental influences (when A = 20% – 50% and C = 10% – 20%). A very large study on handgrip estimated C at 3% (Silventoinen et al., 2008) in adolescents and young adults. Our sample size, and even most of those accrued in the meta-analyses were too small to detect such small C effects (Posthuma & Boomsma, 2000). Of note, when C is dropped from the model, resemblance between the twin and co-twin/sibling will be modeled as A. As a result, the variance that is attributed to genetic factors might be slightly overestimated (with smaller 95% CIs) in small samples, which, in turn, might have biased our meta-analytic heritability estimate.

In the current study, some of the physical fitness components were moderately, but significantly, associated to each other although they reflect different dimensions of physical fitness (Simons et al., 1969). These cross-trait associations are mostly driven by genetic factors (the association between vertical jump and handgrip strength and between handgrip strength and balance) or person-specific environmental factors (association between vertical jump and flexibility). Silventoinen (2008) reported genetic correlations of .43 up to .54 for handgrip, knee extension and elbow flexion. In addition, Tiainen (2004) showed that that handgrip and knee extension strength are measures under the control of the same genetic component. Furthermore, high genetic correlations (.62 to .91) were reported for maximal isometric, concentric and eccentric muscle strength and muscle cross-sectional area of the elbow flexors (De Mars et al., 2007). Genetic correlations in our sample ranged from .18 to .46. The genetic overlap between vertical jump strength and handgrip strength in our sample can be explained from a muscle biology viewpoint, as both explosive and isometric strength

are dependent on the cross-sectional area of the contributing muscles. The specific genetic factors contributing to vertical jump might entail muscle coordination strategy, the percentage of type II fibers and elastic components.

A major future challenge is to identify the specific genes underlying the heritability of these four components of physical fitness. Candidate genes studied have focused on insulin-like growth factor- and myostatin-related genes and genes involved in inflammatory factors. Linkage analyses revealed several additional regions of interest in the genome, although individual genes could not be identified as yet (see Thomis and Aerssens, 2012 for a review). One of the most studied polymorphisms is the R577X variation in the *ACTN3* gene. This gene seems to influence the performance of fast skeletal muscle fibers and *ACTN3* XX homozygotes may have modestly lower skeletal muscle strength in comparison with R-allele carriers (Yang et al., 2003). No large-scale genome-wide association (GWA) studies have been conducted on these phenotypes, which have proven to be a successful approach to understanding the heritability of many health-related risk factors and disease (Flint, 2013; Visscher et al., 2012). This is unfortunate, because the components of physical fitness used in this study are relatively easy to measure (compared to for example maximal oxygen consumption) in large samples and show substantial heritability, suggesting that a GWA meta-analysis effort could be successful. Moreover, the moderate but significant genetic association between handgrip and vertical jump suggests that meta-analysis over genetic association studies that use comparable traits is valid, and that the traits do not need to be exactly similar to capture the latent genetic factors.

Some limitations must be considered while interpreting our results. An important assumption underlying twin studies is that twins are fully representative compared to the general population. Silventoinen et al. reported that singletons showed extra variation in weight and strength measured compared to twins, which could lead to inflated heritability estimates (Silventoinen et al., 2008). Furthermore, the siblings in our study had a very wide age range (12 – 25) which may be a problem as the younger siblings may still be pubertal, compared to the rest of the subjects. Inter-individual variation in maturation is an established factor that affects strength and power. However, when we tested for possible effects of these maturational differences between the twins and younger siblings by repeating the analysis with a restriction on age (no siblings) comparable results emerged (data not shown). The

meta-analyses for vertical jump and flexibility showed moderate to high heterogeneity, indicating that differences between studies are not caused by sampling error only, but may also reflect population-specific differences. Although we aimed for including only studies in which fitness tests conceptually measured the same phenotype, differences in testing procedures might also add to the heterogeneity. In addition, it may be argued that in our meta-analyses it might not be justified to compare samples with different age ranges, due to differences in biological maturity in children and young adults. However, there are limited studies on the heritability of these components of physical fitness and combining the samples will increase power. Moreover, most studies presented in Table 5.1 had sample sizes too small to detect or account for gender differences, therefore gender differences were not taken into account when performing the meta-analyses. Finally, whereas we aimed to standardize the protocol as much as possible, differences in leg-muscle warm up and back stretching might partly explain the significant environmental association between vertical jump and flexibility.

To summarize, the analyses performed in this study confirm a significant contribution of genetic factors to the four physical fitness components and to their association. Understanding the genetic basis of fitness parameters may help us to understand the individual differences in regular voluntary exercise behavior, which show substantial heritability, particularly at the end of adolescence (Huppertz et al., 2012). Individual differences in muscle strength and flexibility co-determine late-adolescent exercise ability (Caspersen et al., 1985; Gleim & McHugh, 1997; Hrysomallis, 2011; Ruiz et al., 2006). Above-average exercise ability will allow an individual to gain more in exercise performance than others and this may lead to enhanced feelings of competence in exercise and sports. These rewarding effects will support longer term maintenance of exercise behavior. Vice versa, aversive mood effects which could be induced by below-average performance have been found to predict drop out from an exercise program (Williams et al., 2008). Physical fitness can be therefore a major driver of voluntary exercise behavior (Bryan et al., 2007; de Geus & de Moor, 2008). Increased efforts to unravel the molecular genetic pathways underlying the heritability of fitness parameters are direly needed.

CHAPTER 6

A TWIN-SIBLING STUDY AND META-ANALYSIS ON THE HERITABILITY OF MAXIMAL OXYGEN CONSUMPTION

PUBLISHED AS

Schutte NM, Nederend I, Hudziak JJ, Bartels M, de Geus EJC (2016)

A twin-sibling study and meta-analysis on the heritability of maximal oxygen consumption

Physiological Genomics 48(3), 210-219

ABSTRACT

Large individual differences exist in aerobic fitness in childhood and adolescence, but the relative contribution of genetic factors to this variation remains to be established. In a sample of adolescent twins and siblings (*N* = 479), heart rate (HR) and maximal oxygen uptake ($\dot{V}O_{2max}$) were recorded during the climax of a graded maximal exercise test. In addition, $\dot{V}O_{2max}$ was predicted in two graded submaximal exercise tests on the cycle ergometer and the treadmill, using extrapolation of the HR/$\dot{V}O_2$ curve to the predicted HR_{max}. Heritability estimates for measured $\dot{V}O_{2max}$ were 60% in mL/min and 55% for $\dot{V}O_{2max}$ in mL/min/kg. Phenotypic correlations between measured $\dot{V}O_{2max}$ and predicted $\dot{V}O_{2max}$ from either submaximal treadmill or cycle ergometer tests were modest ($.57 < r < .70$), in part because of the poor agreement between predicted and actual HR_{max}. The majority of this correlation was explained by genetic factors, therefore the submaximal exercise tests still led to very comparable estimates of heritability of $\dot{V}O_{2max}$. To arrive at a robust estimate for the heritability of $\dot{V}O_{2max}$ in children to young adults, a sample size weighted meta-analysis was performed on all extant twin and sibling studies in this age range. Eight studies, including the current study, were meta-analyzed and resulted in a weighted heritability estimate of 59% (mL/min) and 72% (mL/min/kg) for $\dot{V}O_{2max}$. Taken together, the twin-sibling study and meta-analyses showed that from childhood to early adulthood genetic factors determine more than half of the individual differences in $\dot{V}O_{2max}$.

INTRODUCTION

Maximal oxygen uptake ($\dot{V}O_{2max}$) is defined as the highest rate of oxygen consumption during maximal intensity exercise performed until exhaustion (Kenney et al., 2012) and is considered a good index of aerobic fitness and endurance capacity. Direct measurement of oxygen consumption and carbon dioxide production during the climax of a graded maximal exercise test is the golden standard to measure $\dot{V}O_{2max}$. Large individual differences exist in maximal exercise test derived $\dot{V}O_{2max}$, and, although these are significantly correlated to the regular exercise status of a subject, this correlation is not as strong as generally assumed. Various measures of total physical activity or regular leisure time sports and exercise behavior generally show only modest association with $\dot{V}O_{2max}$ (Aadahl et al., 2007; Bonen & Shaw, 1995; Siconolfi et al., 1985; Talbot et al., 2000). The variation in baseline $\dot{V}O_{2max}$ in sedentary subjects is often already much larger than the training-induced increase over this baseline, which is on average only about 25% (Church et al., 2007; Grant et al., 1995; Payne & Morrow, Jr., 1993; Wilmore et al., 2001). Training furthermore increases rather than decreases the individual differences seen at baseline, as the $\dot{V}O_{2max}$ response to training itself shows large variation (Bouchard & Rankinen, 2001; Skinner et al., 2001).

The above pattern suggests an important role for innate factors in the population variation in $\dot{V}O_{2max}$ and twin and family studies seem to confirm this (Bouchard et al., 1986; Bouchard et al., 1998; Fagard et al., 1991; Klissouras, 1971; Klissouras et al., 1973; Lesage et al., 1985; Lortie et al., 1982; Maes et al., 1996; Montoye & Gayle, 1978; Mustelin et al., 2011; Sundet et al., 1994). Table 6.1 provides an overview of correlations among relatives i.e. monozygotic (identical, MZ) and dizygotic (fraternal, DZ) twins, siblings and parents with their offspring. The monozygotic twin correlations in Table 6.1 range from .62 to .95. The dizygotic (DZ) twin correlations, sibling correlations and parent-offspring correlations also vary substantially across studies, but are systematically lower than the MZ twin correlations. In line with the variability in twin correlations, heritability estimates have varied widely. Possible sources of this variation are differences in the age of the subjects, differential approaches to adjustment for body mass and/or body composition, training status of the subjects, or differences in protocol or fitness equipment (i.e. cycle ergometer or treadmill) that was used to measure or predict $\dot{V}O_{2max}$ between the various studies. A major source, however, seems to be the rather modest sample sizes. As is clear from Table 6.1 there are only two studies with large samples

(Lortie et al., 1982; Sundet et al., 1994) but both these larger studies used a submaximal instead of a maximal exercise test. These tests do not measure $\dot{V}O_{2max}$ directly, but predict it from an exercise test that is halted at a predetermined point (certain percent of the predicted maximal heart rate) below the maximal exercise capability of the individual. Since it does not demand $\dot{V}O_{2max}$ measurement during exhaustive exercise, the submaximal exercise test is better suited in larger (genetic) epidemiological studies. However, it is currently unknown whether a submaximal exercise test correctly captures the genetic factors influencing $\dot{V}O_{2max}$.

One of the most used submaximal exercise test is the nomogram of Åstrand, which requires cycling on a constant individually chosen work rate. $\dot{V}O_{2max}$ is predicted using the steady-state heart rate (HR) achieved after 6 minutes (Åstrand & Rhyming, 1960). This method has clear limitations as results may be influenced by individual differences in submaximal HR at a given work rate due to training status, resting HR and body composition. Estimated $\dot{V}O_{2max}$ with this method showed correlations in the range of .47 and .82 with measured $\dot{V}O_{2max}$ in adult populations (Cink & Thomas, 1981; Ekblom-Bak et al., 2014; Jette, 1979; Kasch, 1984; Siconolfi et al., 1982). More promising is the $\dot{V}O_{2max}$ prediction using a graded submaximal exercise protocol in which the intensity increases at regular intervals up to but never exceeding a certain percent of the maximal heart rate (HR_{max}). $\dot{V}O_{2max}$ can be obtained by extrapolating the HR/$\dot{V}O_2$ curve to the predicted HR_{max}, allowing for individual differences in $\dot{V}O_2$/HR slope. This estimation method showed correlations in the range of .76 and .98 with measured $\dot{V}O_{2max}$ in adult populations (Ekblom-Bak et al., 2014; Grant et al., 1995; Legge & Banister, 1986), although it is sensitive to the protocol used. Submaximal tests on a cycle ergometer yield lower predicted $\dot{V}O_{2max}$ values than tests on a treadmill (Grant et al., 1995; Mays et al., 2010).

Adolescent $\dot{V}O_{2max}$ has been measured in parent-offspring studies using submaximal exercise tests (Lesage et al., 1985; Lortie et al., 1982) but a striking omission in Table 6.1 is adolescent twin studies using a maximal exercise test to examine $\dot{V}O_{2max}$ in an adolescent population. The aim of the current study is to address this gap in the extant literature. In a large sample of adolescent twins and siblings, HR and $\dot{V}O_2$ were recorded during the climax of a graded maximal exercise test. $\dot{V}O_{2max}$ was further predicted from two graded submaximal exercise tests on the cycle ergometer and the treadmill, using extrapolation of the HR/ $\dot{V}O_2$ curve to the predicted HR_{max}. This allowed us to address our second aim: to test the extent to which

Table 6.1 Overview of genetic studies on V̇O2max conducted in a twin and/or family design.

Study	Subjects	$\dot{V}O_{2max}$ measurements	r_{MZ}	r_{DZ}	$r_{sibling}$	$r_{parent-offspring}$	Heritability
Klissouras et al., 1971	• 15 MZ • 10 DZ • Age: 10 ± 2	• Maximal exercise test • Treadmill • mL/min	.91	.44			93%
Klissouras et al., 1973	• 23 MZ • 16 DZ • Age: 27 ± 14	• Maximal exercise test • Treadmill • mL/min/kg	.95	.36			
Montoye & Gayle 1978	• 93 father-son pairs • 70 brother pairs • Age: 10 – 69	• <39y Maximal exercise test • >39y Submaximal exercise test (until HR=160) • Treadmill • L/min • Corrected for age, weight, skinfolds			.18	.34	
Lortie et al., 1982	• 594 parent-offspring pairs • 223 sibling pairs • Age: 42 ± 4 (parents) • Age: 15 ± 3 (children)	• Submaximal exercise test (until HR=150/170) • Treadmill • mL/min • mL/min/kg • VO_{2max} predicted • Corrected for sex, age, skinfolds, physical activity, cigarette smoking and SES			.36[a] .33[b]	.21[a] .17[b]	
Lesage et al., 1985	• 96 parent-offspring pairs • 39 sibling pairs • Age: 43 ± 5 (parents) • Age: 16 ± 4 (children)	• Maximal exercise test • Treadmill • mL/min • mL/min/kg • Corrected for sex and age			.14[a] .19[b]	.06[a] .03[b]	
Bouchard et al., 1986	• 106 MZ • 66 DZ • 27 sibling pairs • Age: 22 ± 3	• Maximal exercise test • Cycle ergometer • mL/min/kg • Corrected for sex and age	.70	.51	.41		38%

Study	Subjects	$\dot{V}O_{2max}$ measurements	r_{MZ}	r_{DZ}	$r_{sibling}$	$r_{parent\text{-}offspring}$	Heritability
Fagard et al., 1991	• 29 MZ • 19 DZ • Age: 22 ± 4	• Maximal exercise test • Cycle ergometer • mL/min • mL/min/kg • Only males, restricted age range	.77[a] .77[b]	.05[a] .04[b]			77% 68%
Sundet et al., 1994	• 436 MZ • 622 DZ • Age: late teens/ early twenties	• Submaximal exercise test (until HR=140) • Cycle ergometer • mL/min/kg • VO_{2max} predicted[c] • Only males, restricted age range	.62	.29			62%
Maes et al., 1996	• 43 MZ • 61 DZ • 84 fathers • 97 mothers • Age: 39 ± 4 (parents) • Age: 10 (children)	• Maximal exercise test • Treadmill • L/min • Restricted age range	.75	.32		.25 / .31[d]	69% / 87%[e]
Bouchard et al., 1998	• 125 sons • 134 daughters • 85 fathers • 85 mothers • Age: 52 ± 5 (parents) • Age: 25 ± 6 (children)	• Maximal exercise test • Cycle ergometer • mL/min • Corrected for sex and age			.36	.14 / .36[d]	59%
Mustelin et al., 2011	• 59 MZ • 92 DZ • Age: 27 ± 2	• Maximal exercise test • Cycle ergometer • mL/min • Corrected for sex, restricted age range	.64	.21			65%

Note. r_{MZ} = Monozygotic twin correlation; r_{DZ} = Dizygotic twin correlation; $r_{sibling}$ = Sibling correlation; $r_{parent\text{-}offspring}$ = Parent-offspring correlation; [a] mL/min; [b] mL/min/kg; [c] Predicted VO_{2max} was transformed to a categorical score from 1 to 9. The correlations are based upon those categorical scores; [d] father-child correlation/mother-child correlation; [e] heritability estimate for males/heritability estimate for females.

the genetic factors influencing measured $\dot{V}O_{2max}$ during a maximal exercise test overlap with those influencing predicted $\dot{V}O_{2max}$ from submaximal exercise tests. Information on the genetic overlap between measured and predicted $\dot{V}O_{2max}$ can reveal whether they can be used interchangeably in genetic association studies to examine the association of aiming to identify the genetic variants underlying $\dot{V}O_{2max}$. A high degree of overlap would mean that submaximal exercise tests, which are easier to implement in large scale genetic studies, might suffice for such studies. Twin correlations, heritability of the measured and predicted $\dot{V}O_{2max}$ as well as the genetic covariance among these parameters were estimated in a multivariate design. We hypothesize that a substantial part of the variation in $\dot{V}O_{2max}$ in our adolescent sample is explained by genetic factors. As previous studies in adults showed high correlations between $\dot{V}O_{2max}$ predicted using a graded submaximal exercise protocol and measured $\dot{V}O_{2max}$, we expect moderate to high phenotypic correlations, and a significant contribution of genes to this correlation. Finally, a sample size weighted meta-analysis was performed on the univariate analysis obtained from all twin studies in the age range of 10 to 30 years (including the current study) that measured $\dot{V}O_{2max}$, aiming to arrive at a more robust estimate for the heritability of this crucial trait in exercise physiology.

METHODS

Sample

Healthy adolescent twin pairs aged between 16 and 18 and their siblings (age range 12 – 25) from the Netherlands Twin Register (van Beijsterveldt et al., 2013) were invited to participate in a study on the determinants of adolescent exercise behavior. Selection for invitation was based on the availability of longitudinal survey data on zygosity and regular leisure time exercise behavior. The aim was to have sufficient individuals present from the entire spectrum of sedentary to vigorous leisure time exerciser and for each zygosity group. We started with a random selection, but if a zygosity group was underrepresented or if there were too few sedentary or vigorous exercisers, invitations were biased towards the underrepresented groups. This was mainly the case for sedentary subjects; twins who reported no engagement in exercise behavior on a previously filled out survey were selected for invitation. The co-twin was then selected as well, regardless of her or his exercise status.

In order to be eligible for the study, subjects had to have no history of cardiovascular or respiratory disease, and being physically capable of engaging in exercise activities.

Participants were invited by sending a letter advertising the opportunity to test their fitness in addition to earning a gift voucher. All invitees had to be able and willing to visit the VU University in Amsterdam for lab testing. For the current study, a complete dataset was available for 479 subjects: 221 complete twin pairs: 112 monozygotic pairs (MZ) and 109 dizygotic pairs (DZ) and 33 of their singleton siblings. In addition, two non-twin sibling pairs participated. This sample size should be sufficient to detect univariate genetic influences with a power of 80% (assuming substantial heritability estimates of 60%, based on previous studies) (Posthuma & Boomsma, 2000).

All subjects provided written informed consent and if the subjects were under 18 consent was given by both of their parents/guardians. All study procedures submitted to and approved by the Medical Ethics Review Committee of the VU University Medical Center Amsterdam (NL35634.029.10).

Procedure

On arrival at the laboratory, height and weight were measured and a short lifestyle interview was completed, including detailed questions on current levels of regular exercise. Next, two exercise test were conducted (in fixed order) on an electromechanically braked Lode cycle ergometer (type Corival) and a Lode treadmill (type Valiant) at fixed loads that are below the intensity of the ventilatory threshold for most adolescents.

The first session on the cycle ergometer started with a 2-minute warming up period, followed by 4 incremental stages of 5 minutes each (males: 70W, 90W, 110W, 130W; females: 40W, 60W, 80W, 100W). Subjects were instructed to pedal at fixed rounds per minute (RPM): between 60 and 70 RPM. The test ended with a 1-minute cooling-down phase, followed by a 5-minute recovery period. The second session on the treadmill consisted of a 1-minute warm-up period, followed by 4 incremental stages of 5 minutes each (males: 6, 6.5, 7 and 8 km/h; females: 5.5, 6, 6.5 and 7 km/h). Again, the test ended with a 1-minute cooling-down phase, followed by a 5-minute recovery period. To ensure that the intensity of every stage was below the intensity of the ventilatory threshold for most adolescents, the ratio of the oxygen

consumption and carbon dioxide production ($\dot{V}CO_2/\dot{V}O_2$) was monitored. This respiratory exchange ratio (RER) can be used to estimate the ventilatory threshold (Solberg et al., 2005). This threshold is passes when exhalation of CO_2 exceeds inhalation of O_2, which is visualized by a RER > 1.00. For each test the load of each stage was adjusted when necessary to keep the intensity below an RER of 0.95.

Finally, an incremental maximal exercise test was conducted on a cycle ergometer to establish $\dot{V}O_{2max.}$ The work rate was increased every minute until exhaustion while subjects pedaled at 60-100 RPM. In the standard protocol male started at 75 Watt with increments of 25 Watt per minute. For females stage one started at 70 Watt and work load was increased by 20 Watt per minute. Adjustments to this protocol (higher increasing workloads every step) were done by experienced researchers based on the exercise behavior, age, height and weight of the subject. The test was terminated when the subject was not able to keep RPM above 50 despite serious attempts. After cessation of the test, every subject completed a mandatory cool-down phase on the cycle ergometer of 5 minutes on a low, individually chosen work rate.

Measurements

Regular exercise behavior Leisure time exercise behavior was measured by a short lifestyle interview, in which the subjects indicated what types of regular sports or exercise activities they were involved in. Subjects were asked to indicate for each activity for how many years the subject participated in the activity, for how many months a year, how many times a week, and how many minutes each time. Each activity was recoded into a metabolic equivalent (MET) score, based on the compendium of energy expenditure (Ainsworth et al., 1993). By multiplying the MET score, the frequency, and the duration of each exercise activity, weekly MET-hours spent on exercise activities were calculated for each subject. We only included activities that were conducted for at least 3 months a year and since at least half a year (thereby excluding ski holidays, sailing camps, and similar). In addition, subjects were asked to indicate how much time per week was spent on physical activity related to active transportation (walking, cycling) and compulsory physical education classes, but MET-hours spent on these activities were kept separate and not used in our index of voluntary exercise behaviour in leisure time.

Gas exchange Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were recorded breath-by-breath by means of a telemetric gas exchange system (Cosmed K4b^2, Cosmed Benelux, Nieuwegein, The Netherlands). During the course of the experiment, the main sample unit and the battery pack were attached to the back of the subject. Before each test, the O_2/CO_2 analysis system was calibrated using ambient air and a gas mixture that had an O_2 concentration of 16% and a CO_2 concentration of 5%. The calibration of the turbine flowmeter was performed by using a 3 liter syringe (all according to the manufacturer's instructions). Figure 6.1 illustrates the changes in $\dot{V}CO_2$ and $\dot{V}O_2$ across the entire experimental protocol for a pair of MZ and a pair of DZ twins.

Heart rate The electrocardiogram (ECG) was recorded continuously with the VU-AMS5fs device (VU University, Amsterdam, The Netherlands). This device was developed to study autonomic nervous system activity in naturalistic settings (de Geus et al., 1995). The version used here measured the ECG together with the impedance cardiogram (ICG) from five disposable, pre-gelled Ag/AgCl electrodes. Due to the portable nature of this device, the subjects were not discomforted by wearing this on the hip during the exercise tests. Heart rate was obtained from the ECG by an automated R-wave peak detector in the VU-AMS software suite (VU-DAMS version 3.1, VU University, Amsterdam, the Netherlands, www.vu-ams.nl) and shown online during testing. Data analysis was based on automated offline scoring of the R-waves, with suspicious inter beat intervals (too short or too long taken the local mean and variance) corrected by interpolation or excluded by marking these beats as artifacts during visual inspection of the ECG signal.

Data processing

Measuring $\dot{V}O_{2max}$ during maximal exercise To obtain VO_{2max}, only $\dot{V}O_2$ data with a corresponding RER of at least 1.10 was selected to ensure good effort above the intensity of the ventilatory threshold. Breath-by-breath VO_2 data was cut into 20-second blocks. For every 20 second block, the mean $\dot{V}O_2$ was calculated, after discarding deviant breaths. $\dot{V}O_{2max}$ was determined as the highest mean value of $\dot{V}O_2$ of all the 20-second blocks. The maximal HR in that specific block was taken as corresponding HR_{max}.

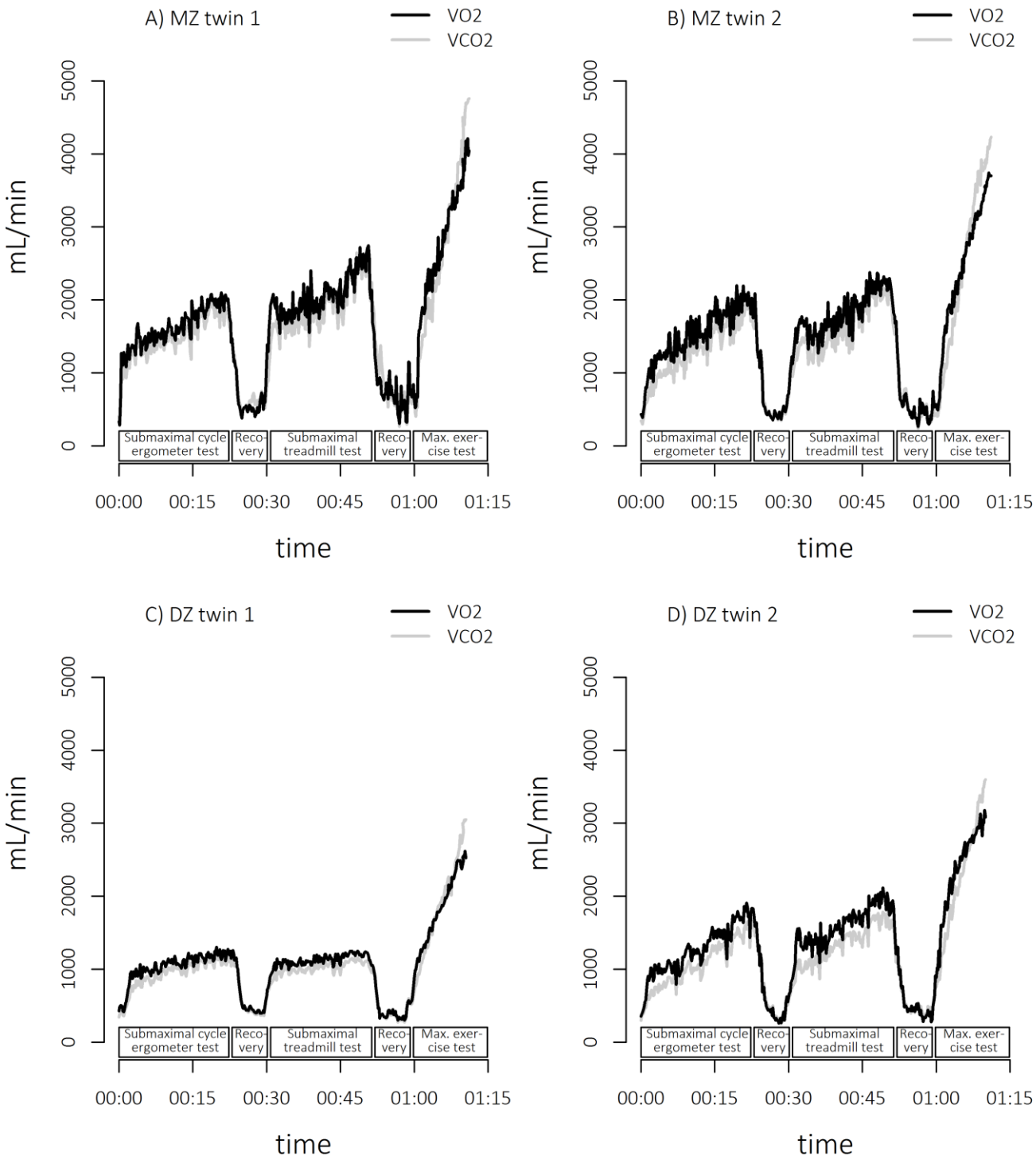


Figure 6.1 Changes in $\dot{V}O_2$ and $\dot{V}CO_2$ across the entire experimental protocol for a pair of MZ (A and B) and a pair of DZ twins (C and D). The two submaximal exercise tests on the cycle ergometer and treadmill and the final maximal exercise test are clearly visible as $\dot{V}O_2$ and $\dot{V}CO_2$ increase when subjects start exercising. The MZ twins resemble each other more than DZ twins in absolute $\dot{V}O_2$ and $\dot{V}CO_2$.

Predicting $\dot{V}O_{2max}$ from submaximal exercise To predict $\dot{V}O_{2max}$, breath-by-breath $\dot{V}O_2$ data and beat-to-beat HR data were synchronized and the mean of every 5-second block was calculated for submaximal cycle and treadmill exercise tests separately. Using the univariate regression function in SPSS (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp), the relationship between $\dot{V}O_2$ (dependent variable) and HR (independent variable) was examined and a slope and intercept were calculated for every subject for the submaximal cycle ergometer test as well as for the submaximal treadmill test. Using these parameter estimates together with HR_{max} the predicted $\dot{V}O_{2max}$ was calculated for every subject. Because we wanted to test the feasibility of using submaximal tests only, HR_{max} was obtained by using the formula 208 – 0.7*age (Tanaka et al., 2001) rather than using the actual measured HR_{max}, although analyses were repeated using the actual measured HR_{max}.

Genetic analyses

Genetic structural equation modeling was done in OpenMx (Boker et al., 2011) under R (R Development Core Team, 2011) with the raw-data ML procedure for estimation of parameters. For all analyses, a threshold of $p < 0.05$ was considered for statistical significance. All $\dot{V}O_{2max}$ values were Z-transformed. Since (non-twin) siblings share, like DZ twins, on average 50% of their genes, parameter estimates were constrained to be equal for DZ twins and siblings. First, a trivariate model that estimated all parameters freely (a saturated model) was fitted, including the measured $\dot{V}O_{2max}$ and the $\dot{V}O_{2max}$ predicted from the submaximal cycle and treadmill test. Main effects of sex and age on mean levels of these phenotypes were considered in the model. The significance of these covariates was tested by comparing the model including the specific component to a model in which the component is constraint to be equal to zero. These nested submodels were compared by hierarchic χ^2 tests. The χ^2statistic is computed by subtracting log-likelihood (–2LL) for a reduced model from the -2LL for the full model (χ^2= $-2LL_{full\ model} - -2LL_{reduced\ model}$). This χ^2statistic is distributed with degrees of freedom (*df*) equal to the difference in the number of parameters estimated in the two models ($\Delta df = df_{full\ model} - df_{reduced\ model}$). If the difference test is significant the constraints on the reduced model cause a significant deterioration of the fit of model. Twin and cross-twin/cross-trait correlations and their 95% confidence intervals were estimated for the MZ and DZ twins/siblings.

Subsequently, a trivariate Cholesky decomposition was fitted to the data. This decomposition model decomposes the total phenotypic variance into sources of additive genetic variance/covariance (A), dominant genetic variance/covariance (D) or shared (familial) environmental variance/covariance (C) and person-specific environmental variance/covariance (E). The C and D effects cannot be estimated simultaneously in a twin/sibling model. Therefore, the ratio of the MZ correlations to the DZ/sibling correlations was used to determine which model (ACE or ADE) is most appropriate. The significance of the variance-covariance components was tested by comparing the model including the specific component to a model in which the component is constraint to be equal to zero.

Meta-analysis

A search of the electronic databases ISI Web of Knowledge and PubMed was conducted using the key words: *maximal oxygen uptake, $\dot{V}O_{2max}$, aerobic capacity, aerobic performance, cardiorespiratory (fitness) and genes, heritability, twin(s), family* (date last searched: January 2015). Furthermore, the reference lists of these articles were inspected. Articles published in English, and reporting twin, sibling and/or parent-offspring correlations and corresponding sample sizes, and with subjects with an age < 30y were selected. Only articles in which $\dot{V}O_2$ was measured in a maximal exercise protocol or predicted using a submaximal exercise protocol were included. All twin and sibling correlations of these articles (including the current study) were included in a sample size weighted meta-analysis for $\dot{V}O_{2max}$ expressed in mL/min and $\dot{V}O_{2max}$ expressed in mL/min/kg. Twin correlations from the current study were calculated in univariate models without the siblings to be comparable to the twin correlations included in the meta-analysis.

In OpenMx, a variance decomposition model was fitted to the twin correlations (weighted for sample size) to estimate the influence of additive genetic (A) and shared environmental influences (C) on $\dot{V}O_{2max}$ in mL/min and $\dot{V}O_{2max}$ in mL/min/kg according to the approach of Bartels et al. (2003). First, the twin and sibling correlations were used to estimate the genetic and environmental influences for each study separately. Subsequently, all studies were taken together to estimate one weighted heritability estimate for $\dot{V}O_{2max}$. These two models were compared using the hierarchic χ^2 test. A significant deterioration of the fit of model indicated significant heterogeneity across the studies (Bartels et al., 2003). We repeated the meta-

analysis by excluding the study by Sundet et al. (1994) that used predicted $\dot{V}O_{2max}$ from submaximal exercise testing to also provide a weighted heritability estimate of actual measured $\dot{V}O_{2max}$.

RESULTS

General descriptives

Means and standard deviations for measured $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ predicted from the submaximal cycle and treadmill test, and measured and predicted HR_{max} of males and females are shown in Table 6.2. Fifteen subjects did not meet the RER > 1.10 criterion. For 9 of these subjects there was no sufficient evidence that they did exercise until exhaustion according to the experimental researcher report and/or the HR_{max} was less than 85% of HR_{max}. Therefore, these 9 subjects and their coinciding twin/sibling were excluded from further analyses involving measured $\dot{V}O_{2max}$. The final sample size consisted of 463 subjects. Means and standard deviations of minutes spent on walking, cycling, physical education class and MET scores for leisure time exercise behavior are presented in Table 6.2. Although the age-range was small, significant age effects ($\dot{V}O_{2max}$ increases with age) were found on $\dot{V}O_{2max}$ in mL/min ($p < .001$), but not for $\dot{V}O_{2max}$ expressed in mL/min/kg. Both predicted and measured $\dot{V}O_{2max}$ were higher in males than in females (all $p < .001$). Furthermore, males were more engaged in weekly exercise behavior ($p = .002$). As expected, weekly leisure time exercise behavior correlated significantly with $\dot{V}O_{2max}$, but the correlation was modest: $r = .28$ with $\dot{V}O_{2max}$ in mL/min and $r = .34$ (both $p < .001$) with $\dot{V}O_{2max}$ mL/min/kg. The correlation between measured $\dot{V}O_{2max}$ and weekly minutes of cycling was significant ($r = .25$ for $\dot{V}O_{2max}$ expressed in mL/min and $r = .22$ for $\dot{V}O_{2max}$ expressed in mL/min/kg, both $p < .001$), whereas the correlations between measured $\dot{V}O_{2max}$ and weekly minutes of walking or weekly hours of physical education class were only small ($-.12 < r < .03$).

Table 6.2 Means and standard deviations (SD) of measured and predicted $\dot{V}O_{2maxl}$ in mL/min and mL/min/kg, measured and predicted HR, and minutes per week spent on walking and cycling (transportation), physical education class and leisure time exercise behavior in METs of males and females.

		Males (N = 233)		Females (N = 230)	
		Mean	*SD*	*Mean*	*SD*
Body composition	Height (cm)	180.4	7.8	168.3	6.6
	Weight (cm)	67.3	10.3	61.8	9.7
	BMI ($kg \cdot m^{-1}$)	20.6	2.5	21.8	3.3
$\dot{V}O_{2max}$ in mL/min	Measured	3132	540	2240	316
	Predicted from cycle ergometer test	2933	648	2021	389
	Predicted from treadmill test	2968	606	2029	400
$\dot{V}O_{2max}$ in mL/min/kg	Measured	46.9	6.9	36.7	5.6
	Predicted from cycle ergometer test	43.8	8.1	33.0	6.0
	Predicted from treadmill test	44.5	8.1	34.1	6.3
Heart rate (bpm)	Resting Heart Rate	72.6	11.4	75.6	11.2
	Maximal Heart Rate Measured	195.4	10.1	195.2	8.9
	Maximal Heart Rate Predicted (Tanaka)	196.1	0.8	195.9	0.9
Regular exercise	Walking (minutes/week)	38.9	71.8	43.5	79.8
	Cycling (minutes/week)	233.1	156.3	209.4	163.3
	Physical education (minutes/week)	151.6	139.9	132.4	112.4
	Leisure-time exercise (METs/week)	25.7	22.5	19.2	22.1

Correlation between measured and predicted $\dot{V}O_{2max}$

Measured $\dot{V}O_{2max}$ in mL/min showed a correlation of .70 (95% CI: .65 – .75) with $\dot{V}O_{2max}$ predicted from the submaximal cycle test and .64 (95% CI: .58 – .70) with $\dot{V}O_{2max}$ predicted from the treadmill test. Likewise, measured $\dot{V}O_{2max}$ in ml/min/kg was significantly correlated with $\dot{V}O_{2max}$ predicted from the submaximal cycle test (r = .61, 95% CI: .55 – .68) and with $\dot{V}O_{2max}$ predicted from the submaximal treadmill test (r = .57, 95% CI: .50 – .64). In spite of the significant relationship between predicted and measured $\dot{V}O_{2max}$, Bland Altman plots in Figure 6.2 show considerably discrepancy between these measures, expressed in mL/min. Regression of the mean of the two measurements (measured and predicted $\dot{V}O_{2max}$) on the difference between the two values (y-axis), showed that the discrepancy increases as

absolute $\dot{V}O_{2max}$ increases. In males the absolute differences in measured and predicted $\dot{V}O_{2max}$ were larger than in females. A potential source of error was the use of an age-predicted HR_{max}. Absolute mean differences between measured (195 ± 10) and predicted HR_{max} (202 ± 1) were greater than zero ($p < .001$). Repeating the analyses with measured HR_{max} significantly improved the correlation of measured to predicted $\dot{V}O_{2max}$ from the submaximal cycle test (in mL/min $r = .76$, 95% CI: .72 – .90; in mL/min/kg $r = .69$, 95% CI: .64 – .74) and to predicted $\dot{V}O_{2max}$ from the treadmill test (in mL/min $r = .71$, 95% CI: .66 – .76; in mL/min/kg $r = .65$, 95% CI: .59 – .70).

Genetic analyses

The twin and cross-twin/cross-trait correlations of measured $\dot{V}O_{2max}$ and predicted $\dot{V}O_{2max}$ are presented in Table 6.3. For $\dot{V}O_{2max}$ in mL/min, MZ correlations ($r = .61$ for measured $\dot{V}O_{2max}$ and $r = .67$ and $r = .65$ for the $\dot{V}O_{2max}$ predicted from the submaximal cycle and treadmill tests) almost twice as high as the DZ/sibling correlation ($r = .26$, $r = .45$ and $r = .37$). When the MZ resemblance is higher than the DZ resemblance this constitutes evidence for genetic influences on $\dot{V}O_{2max}$. For $\dot{V}O_{2max}$ in mL/min/kg, twin correlations were also higher for MZ twins ($r = .53$, $r = .58$ and $r = .59$) than for DZ twins/siblings ($r = .43$, $r = .52$ and $r = .38$) but much less than half, providing evidence for genetic as well as shared environmental factors underlying familial aggregation. The cross-twin/cross-trait correlations (off-diagonal correlations in Table 6.3) were higher for MZ twins than for DZ twins/siblings for all phenotypes suggesting genetic influences on the covariance between measured $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ predicted from the submaximal cycle and treadmill tests.

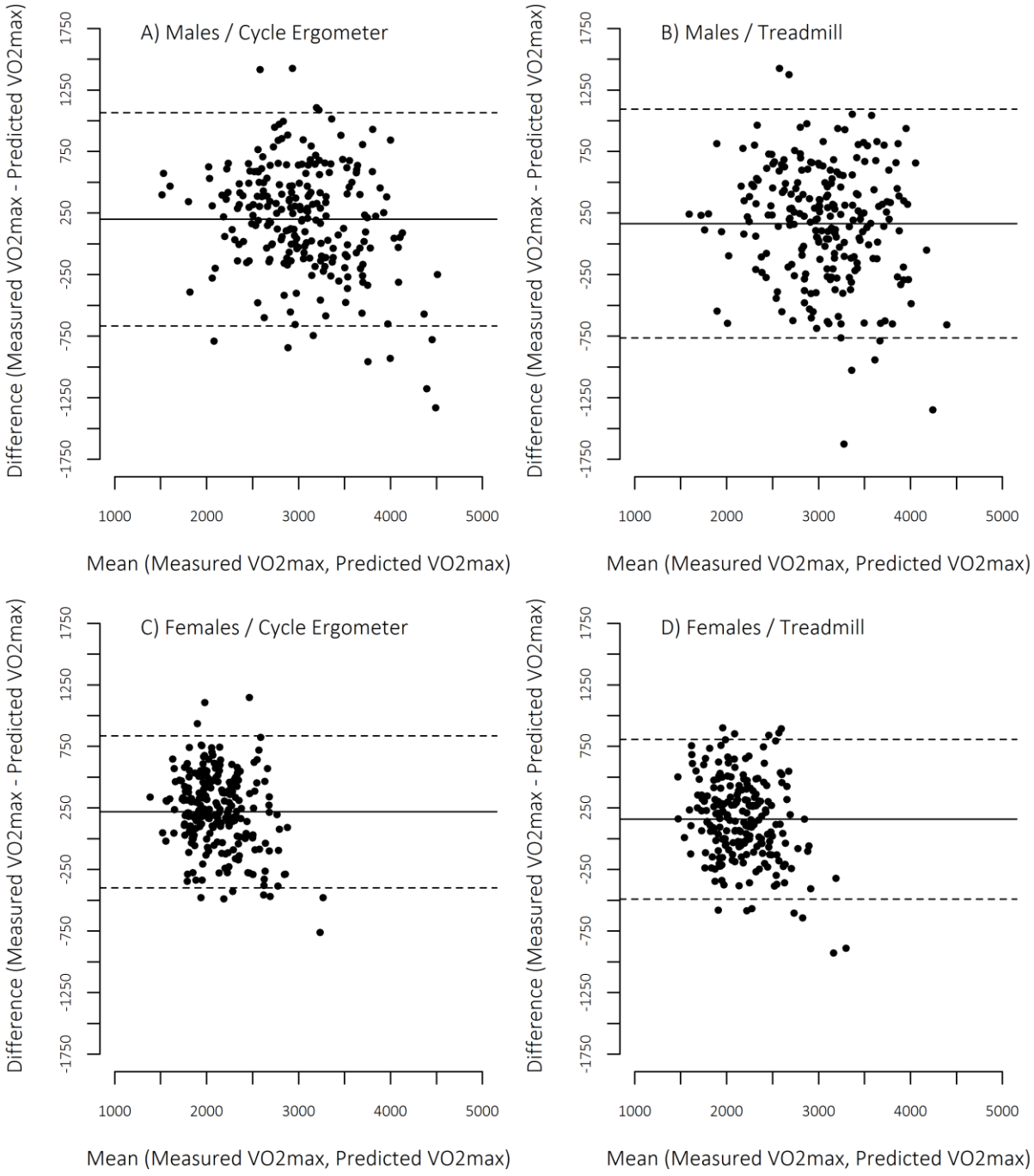


Figure 6.2 Bland-Altman plots for VO_{2max} in mL/min. The x-axis shows the mean of the two measurements (measured and predicted $\dot{V}O_{2max}$) and the y-axis the difference between the two values. The solid line represents the mean difference. The dotted lines represent the average difference ± 1.96 standard deviation of the difference. A) Male $\dot{V}O_{2max}$ submaximal cycle test; B) Female $\dot{V}O_{2max}$ submaximal cycle test; C) Male $\dot{V}O_{2max}$ submaximal treadmill test; D) Female $\dot{V}O_{2max}$ submaximal treadmill test.

Table 6.3 Twin (diagonal) and cross-twin/cross-trait (off diagonal) correlations (95% CI) estimated from the saturated model for measured $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ predicted from the submaximal cycle and treadmill tests.

	V̇O2max (mL/min)		
	Measured	Predicted cycle test	Predicted treadmill test
	MZ correlations		
Measured	.61 (.50, .70)		
Predicted cycle test	.59 (.51, .66)	.67 (.57, .75)	
Predicted treadmill test	.53 (.44, .61)	.69 (.62, .74)	.65 (.54, .73)
	DZ/sibling correlations		
Measured	.26 (.11, .40)		
Predicted cycle test	.45 (.37, .53)	.45 (.34, .55)	
Predicted treadmill test	.43 (.35, .51)	.53 (.45, .60)	.37 (.23, .49)
	V̇O2max (mL/min/kg)		
	Measured	Predicted cycle test	Predicted treadmill test
	MZ correlations		
Measured	.53 (.40, .63)		
Predicted cycle test	.52 (.43, .59)	.58 (.47, .68)	
Predicted treadmill test	.44 (.35, .53)	.63 (.56, .69)	.59 (.46, .68)
	DZ/sibling correlations		
Measured	.43 (.29, .55)		
Predicted cycle test	.45 (.36, .54)	.52 (.41, .61)	
Predicted treadmill test	.42 (.32, .50)	.53 (.45, .61)	.38 (.24, .50)

Genetic modeling started with an ACE model, as in all cases the DZ/sibling correlation was higher than half the MZ correlation, except for measured $\dot{V}O_{2max}$ in mL/min. Shared environmental influences were not significant for measured and predicted $\dot{V}O_{2max}$ in mL/min ($X^2(6) = 10.8$, $p = .096$). For $\dot{V}O_{2max}$ in mL/min/kg, shared environmental factors were not significant for the measured $\dot{V}O_{2max}$, but for predicted $\dot{V}O_{2max}$ a small but significant effect of shared environmental factors was detected. Standardized components from the best fitting model for additive genetic and shared and person-specific environmental influences on measured and predicted $\dot{V}O_{2max}$ and their covariances are presented in Table 6.4. Heritability estimates for measured $\dot{V}O_{2max}$ were 60% (95% CI: 47% – 69%) and 55% (95% CI: 43% – 64%) for $\dot{V}O_{2max}$ in mL/min (Table 6.4a) and mL/min/kg respectively (Table 6.4b). Heritability estimates for predicted $\dot{V}O_{2max}$ ranged from 47% for $\dot{V}O_{2max}$ in mL/min/kg to 67% for $\dot{V}O_{2max}$ in mL/min (both predicted from the cycle test). Shared environmental influences were small and

not significant for $\dot{V}O_{2max}$ in mL/min. For $\dot{V}O_{2max}$ in mL/min/kg, however, 12% (95% CI: 4% – 19%) of the variance in $\dot{V}O_{2max}$ predicted from the cycle protocol and 4% (95% CI: 4% – 19%) of the variance in $\dot{V}O_{2max}$ predicted from the treadmill protocol could be explained by shared environmental influences.

Table 6.4 Standardized estimates (95% CI) for additive genetic (A), shared environmental (C) and person-specific environmental (E) influences on measured $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ predicted from the submaximal cycle and treadmill tests and their covariances in (a) mL/min and (b) mL/min/kg.

a)	V̇O2max (mL/min)		
	Measured	Predicted cycle test	Predicted treadmill test
	Additive genetics (A)		
Measured	**.60 (.47, .69)**		
Predicted cycle test	.76 (.63, .85)	**.67 (.60, .75)**	
Predicted treadmill test	.70 (.56, .81)	.76 (.65, .84)	**.64 (.53, .72)**
	Unique environment (E)		
Measured	.40 (.31, .55)		
Predicted cycle test	.24 (.15, .37)	.33 (.25, .43)	
Predicted treadmill test	.30 (.19, .44)	.24 (.16, .35)	.36 (.28, .47)

b)	V̇O2max (mL/min/kg)		
	Measured	Predicted cycle test	Predicted treadmill test
	Additive genetics (A)		
Measured	**.55 (.43, .64)**		
Predicted cycle test	.70 (.55, .82)	**.47 (.32, .60)**	
Predicted treadmill test	.62 (.46, .75)	.61 (.45, .75)	**.55 (.42, .66)**
	Shared environment (C)		
Measured	-		
Predicted cycle test	-	.12 (.04, .19)	
Predicted treadmill test	-	.09 (.01, .16)	.04 (.00, .10)
	Unique environment (E)		
Measured	.44 (.35, .56)		
Predicted cycle test	.31 (.19, .46)	.41 (.32, .52)	
Predicted treadmill test	.37 (.24, .54)	.30 (.21, .43)	.40 (.31, .52)

Note. Heritability estimates in **bold**.

Significant genetic correlations were found for measured $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ predicted from the submaximal cycle test (r = .84 (95% CI: .76 – .91) for $\dot{V}O_{2max}$ in mL/min and .81 (95% CI: .68 – .95) for $\dot{V}O_{2max}$ in mL/min/kg). Measured $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ predicted from the submaximal treadmill test showed a genetic correlation of .73 (95% CI: .62 – .82) and .63 (95% CI: .48 – .76) for $\dot{V}O_{2max}$ in mL/min and in mL/min/kg respectively. A genetic correlation > 0 indicates that traits are influences by common genes. Therefore, these correlations suggest that the three $\dot{V}O_{2max}$ measures largely reflect the same set of underlying genetic variants. Furthermore, 61% – 76% of the phenotypic correlations between measured $\dot{V}O_{2max}$ and $\dot{V}O_{2max}$ predicted from the submaximal cycle and treadmill tests could be explained by genetic factors.

Meta-analysis

The literature search and screening resulted in 11 articles (see Table 6.1). Four studies were excluded from the meta-analysis. The studies by Montoye & Gayle (1978), Lortie et al. (1982), Lesage et al. (1985), and Bouchard et al. (1998) were parent-offspring studies and were excluded from the analysis because cohort effects and shared environment could be affecting the correlations. Moreover, whereas other studies either corrected for sex and age or used single-sex or age-restricted samples, Montoye & Gayle (1978) and Lortie et al. (1982) additionally corrected for skinfold thickness, physical activity, cigarette smoking and social-economic status (SES).

The seven studies included in the meta-analysis showed MZ twin correlations for $\dot{V}O_{2max}$ ranging from .62 to .95 whereas the DZ and sibling correlations were much lower (.04 to .51). In the study by Sundet et al. (1994), $\dot{V}O_{2max}$ was predicted using extrapolation of the $\dot{V}O_2$/HR slope, whereas the rest of the studies reported measured $\dot{V}O_{2max}$ values. All of these remaining studies corrected the $\dot{V}O_{2max}$ values for sex when the sample comprised both males and females, except for two studies by Klissouras (Klissouras, 1971; Klissouras et al., 1973). The age range in most studies was very restricted and two studies with a broader range corrected for age (Bouchard et al. (1986) and the current study). Univariate twin correlations (without siblings, estimated from a saturated model) from the current study were used in the meta-analysis (r_{MZ} = .58, r_{DZ} = .29 and r_{MZ} = .54, r_{DZ} = .38 for $\dot{V}O_{2max}$ in mL/min and mL/min/kg respectively).

Heterogeneity testing showed that all studies on the heritability of $\dot{V}O_{2max}$ expressed in mL/min (combined sample of 1088 individuals) could be taken together ($\chi^2(4) = 5.8$, $p = .218$) and a sample size weighted heritability estimate of 59% (95% CI: 52% – 66%) was found. For $\dot{V}O_{2max}$ expressed in mL/min/kg a weighted heritability estimate of 64% (95% CI: 60% – 69%) was found in a combined sample size of 3120 individuals, but heterogeneity testing showed that these studies could not be simply taken together ($\chi^2(4) = 12.2$, $p = .016$). Repeating the analysis without Sundet et al. (in which $\dot{V}O_{2max}$ was predicted) removed heterogeneity in the estimates of the four remaining studies ($p = .098$) and increased the weighted heritability estimate to 72% (N = 1004). For both $\dot{V}O_{2max}$ expressed in mL/min as $\dot{V}O_{2max}$ expressed in mL/min/kg, shared environmental influences were not significant ($p > .05$).

DISCUSSION

The main purpose of this paper was to estimate the heritability of aerobic fitness in an adolescent population, as assessed by $\dot{V}O_{2max}$ measured during a maximal exercise test. In concordance with previous literature, $\dot{V}O_{2max}$ was only moderately correlated with regular exercise behavior in leisure time. Genetic analysis revealed that 60% of the total variance in measured $\dot{V}O_{2max}$ in mL/min and 55% of the total variance in measured $\dot{V}O_{2max}$ in mL/min/kg can be explained by genetic factors.

In addition to measuring $\dot{V}O_{2max}$ during the climax of a graded maximal cycle ergometer test, $\dot{V}O_{2max}$ was predicted from submaximal tests on a cycle ergometer and a treadmill using extrapolation of the heart rate/oxygen uptake (HR/$\dot{V}O_2$) curve to the predicted HR_{max}. Only a moderate phenotypic relationship was found between predicted $\dot{V}O_{2max}$ and measured $\dot{V}O_{2max}$ in the current study ($.57 < r < .70$). This was lower than had been reported in previous studies using adult subjects (Ekblom-Bak et al., 2014; Grant et al., 1995; Legge & Banister, 1986). This difference can in part be attributed to the poor agreement between predicted and actual HR_{max}. Although there is substantial evidence that maximal heart rate is age-related in adults, it has been suggested that HR_{max} might be age-independent in children and adolescents (Rowland, 1996). When repeating the analysis using the *measured* HR_{max}, the phenotypic correlations between the measured $\dot{V}O_{2max}$ and the predicted $\dot{V}O_{2max}$ indeed increased. Nonetheless, correlations remained below those found for adults, suggesting that, apart from

the higher individual variation in HR_{max}, the variability in the HR/$\dot{V}O_2$ relationship may also be higher in adolescents.

In spite of the moderate phenotypic correlation to measured $\dot{V}O_{2max}$, heritability estimates from multivariate genetic analyses showed that heritability estimates for predicted $\dot{V}O_{2max}$ (46% to 67%) were very similar to those obtained for measured $\dot{V}O_{2max}$. For $\dot{V}O_{2max}$ in mL/min, the heritability estimates were higher than measured $\dot{V}O_{2max}$, but for $\dot{V}O_{2max}$ in mL/min/kg the heritability estimates were as high (treadmill test) or lower (cycle ergometer test) than measured $\dot{V}O_{2max}$. However, as all heritability estimates are within the confidence interval of measured $\dot{V}O_{2max}$, the differences were not significant. Moreover there was a substantial overlap in the genetic factors influencing predicted and measured $\dot{V}O_{2max}$. That genetic effects on $\dot{V}O_{2max}$ can be reliably estimated from submaximal tests is important as submaximal tests may be more amenable in large-scale studies. The graded maximal exercise test requires strenuous physical activity from the subject, which produces discomfort, and cannot be attained by or poses a health risk for some subgroups of the population (e.g. sedentary individuals, young children, elderly or patients suffering from cardiovascular or respiratory disease). It may also lead to a larger selection bias when recruiting volunteers from population-based samples (like twin registries) as not all subjects may be willing to exercise to exhaustion. This favors the participation of regular exercisers over sedentary subjects to exercise testing studies which will lead to biased estimates of both mean and variance in $\dot{V}O_{2max}$. The use of submaximal tests may lead to samples that are more representative of the general population.

Our sample size weighted meta-analysis on all heritability studies in children, adolescents and young adults to date showed that 59% (when expressed in mL/min) (N = 1088) and 72% (when expressed in mL/min/kg) (N = 1004) of the variance in measured $\dot{V}O_{2max}$ can be explained by genetic influences. All studies converge on the absence of detectable shared environmental factors (C). Shared environmental influences, including the family environment, were also low and not significant in the current study (except for predicted $\dot{V}O_{2max}$ expressed in mL/min/kg) but the power to detect C was low, even after adding siblings of the twins to the design. Power analysis suggests that our sample size had to be at least twice as big for C to be detected with 80% power (Posthuma & Boomsma, 2000). This leads us

to suggest that shared environmental influences on adolescent $\dot{V}O_{2max}$ cannot be excluded but at best play a very modest role.

The overarching conclusion from our (meta-)analyses is that $\dot{V}O_{2max}$ is a highly heritable phenotype from childhood to young adulthood. Heritability is likely to continue into adulthood, but there were no middle-aged or older adult twin samples that could be included in our meta-analysis. We did find four studies that measured $\dot{V}O_{2max}$ in parents and offspring. In these parent-offspring designs, however, heritability estimation can be affected by cohort effects, since different genetic variants affecting aerobic fitness can be expressed at different ages. To get a complete picture of the heritability of $\dot{V}O_{2max}$ across the whole life-span, twin studies focusing on middle-aged and older samples are direly needed.

A limitation of our study is that we cannot currently determine the exact contribution of the two different components that make up the heritability of $\dot{V}O_{2max}$: genetic factors that contribute to baseline (untrained) performance levels and those related to 'gain' in $\dot{V}O_{2max}$ (i.e. genetic factors contributing to aerobic trainability). The HERITAGE study showed that the variation in baseline performance, as well as the variance in trainability is larger between families than within families, confirming the role of genetic factors in baseline levels as well as in gain in $\dot{V}O_{2max}$ (Bouchard et al., 1998; Bouchard et al., 1999). Our study used a mixture of sedentary subjects and moderately and vigorous exercisers. $\dot{V}O_{2max}$ in the two latter groups will reflect a mixture of the baseline and trainability components. A possible way to discriminate between the two components is by estimating the heritability of $\dot{V}O_{2max}$ in untrained (persistent sedentary) individuals only. A further limitation is that even though the current study is the largest twin study on measured $\dot{V}O_{2max}$, our sample is still too small to have enough power to analyze sex differences in $\dot{V}O_{2max}$. It might be that the effects of genetic or environmental factors on $\dot{V}O_{2max}$ differ between males and females. A limitation of our meta-analysis is that there was significant heterogeneity across the studies, so that a single estimate therefore does not capture all individual studies adequately. However, recomputation of heritability in a restricted, more homogenous subset led to similar estimates. Finally, it should be noted that maximal exercise tests performed on a cycle ergometer generally yield lower $\dot{V}O_{2max}$ values than maximal exercise tests performed on a treadmill due to a larger excising muscle mass. Comparing the heritability studies of $\dot{V}O_{2max}$ performed on a treadmill and cycle ergometer showed that the two heritability estimates of

treadmill-derived $\dot{V}O_{2max}$ are slightly higher (Klissouras, 1971; Maes et al., 1996), but these estimates are based on small sample sizes consisting of 10-year-olds. Replication of these studies in other age groups is needed to examine the effect of exercise equipment on the heritability of $\dot{V}O_{2max}$.

Twin studies offer a unique opportunity to estimate the importance of genetic and environmental influences on a trait. Estimates of heritability inform us on how much of the variation in a phenotype in a population sample is due to genetic variation and generally define the upper limit of the percentage of variance that is explained by genetics, but does not reveal which and how many genes are involved. Therefore, an important next step is to identify the genetic variants underlying the heritability of $\dot{V}O_{2max}$. Thus far, studies reported case-control candidate gene and linkage studies, mostly characterized by small sample sizes and mixed results (Bouchard et al., 2011a). Two of the most studied polymorphisms is the R577X variation in the *ACTN3* gene and the I/D polymorphism in the *ACE* gene (MacArthur & North, 2011; Skipworth et al., 2011). The preferred approach to identify genetic variants for complex traits (which are known to be influences by multiple genetic factors) is a meta-analysis of genome-wide association (GWA) studies with a large cumulative sample size (Flint, 2013; Visscher et al., 2012). Only one GWA study on $\dot{V}O_{2max}$ has been conducted to date by Bouchard et al. (2011). Strikingly, in spite of the small sample size, this study revealed that 16 Single Nucleotide Polymorphisms (SNPs) accounted for 45% of the variance in gains in $\dot{V}O_{2max}$ after exposure to a standardized 20-weeks exercise program in a sample of 473 sedentary adults (Bouchard et al., 2011b). No GWA studies have yet been performed on $\dot{V}O_{2max}$ in the untrained or baseline state (before training). Such studies will need large samples with both $\dot{V}O_{2max}$ data and genome-wide genotyping. The feasibility of this increases greatly if submaximal exercise tests generate sufficiently valid estimates. Notwithstanding the imperfect correlation between predicted and measured $\dot{V}O_{2max}$, our results can be considered encouraging: The high genetic correlation between measured and predicted $\dot{V}O_{2max}$ in the current study suggests that they largely capture the same latent genetic factors and these genetic factors explained the largest part of the observed correlation between measured and predicted $\dot{V}O_{2max}$. GWA meta-analyses across studies using (graded) submaximal and maximal tests should be able to pick up these shared genetic variants.

To conclude, the results of the current study, together with the results of the meta-analyses, confirm that innate factors determine more than half of the individual differences in the $\dot{V}O_{2max}$ from childhood to young adulthood.

CHAPTER 7

GENETIC MODIFICATION OF THE EFFECTS OF EXERCISE BEHAVIOR ON MENTAL HEALTH

PUBLISHED AS

Schutte NM, Bartels M & de Geus EJC (2014)

Genetic modification of the effects of exercise behavior on mental health

Frontiers in Psychiatry 5:64

ABSTRACT

Regular exercise has been indicated to be effective in reducing anxious and depressive symptoms in clinical samples, suggesting beneficial causal effects of exercising. However, the observed association between exercise and anxious-depressive symptoms might be due to underlying genetic factors that influence both exercise behavior and symptoms of anxiety and depression, mimicking a causal association. Results from population-based twin studies that have tested the nature of the association between a lack of exercise and anxious-depressive symptoms conclude that the association is best explained by underlying genetic effects. In an effort to explain the mechanisms that contribute to the association between exercise activities and mental health in the general population, a model was proposed that accommodates genetic pleiotropic effects, but still allows exercise to causally increase wellbeing in specific subgroups of the population.

Anxiety and depressive disorders are a major contributor to the global disease burden (Ferrari et al., 2013). Although these disorders differ in duration and intensity, they are often chronic and treatment options include medication, psychotherapy, or a combination of both. In addition, regular exercise is argued to be effective in reducing anxious and depressive symptoms. Results from several meta-analyses indicate that exercise has a moderate to large antidepressant effect in clinical populations (Craft & Landers, 1998; Josefsson et al., 2014; Krogh et al., 2011; Lawlor & Hopker, 2001; Stathopoulou et al., 2006). Based on these studies, one might easily conclude that exercise consistently has beneficial causal effects on anxious and depressive symptoms.

The question remains whether this conclusion is also valid with regard to the general population as, despite these beneficial psychological effects, the majority of the population is not engaging in leisure-time exercise activities (Martinez-Gonzalez et al., 2001; Troiano et al., 2008) and population studies on the association between exercise and mental health are scarce. Secondly, there may be mechanisms that only mimic causal effects. The observed association between exercise and anxious-depressive symptoms might be due to underlying factors that influence both exercise behavior and symptoms of anxiety and depression. These factors can reside in the environment or in our genes. Underlying genetic factors might for instance have a detrimental effect on regular exercise behavior while simultaneously increasing the risk for depression, a mechanism known as genetic pleiotropy. The effect of these genetic factors on exercise behavior could even precede their effects on depression, thereby nearly perfectly mimicking a causal association. Only a few research groups have the optimal resources to investigate these possible effects in a genetically informative design, which requires large population-based longitudinal datasets with family data, but preferably twin data.

Results from population-based twin studies that have tested the nature of the association between a lack of exercise and anxious-depressive symptoms conclude that the association is best explained by underlying genetic effects. De Moor et al. (2008) showed that within genetically identical twins, a twin who exercised more did not have fewer symptoms than his or her less exercising co-twin. This suggests that genetic factors independently cause low levels of exercise behavior as well as anxious and depressive symptoms (de Moor et al., 2008). In addition, there is no evidence for causal influences of exercise behavior on feelings of

psychological wellbeing, a phenotype presumably at the other end of the emotional scale i.e. the absence of anxious or depressive symptoms (Bartels et al., 2012b; Stubbe et al., 2007). Taken together, these studies conclude that the association between regular exercise and psychological wellbeing as well as the association between a lack of regular exercise and anxiety and depressive disorders largely reflect the effects of common genetic factors.

In an effort to explain the mechanisms that contribute to the association between exercise activities and mental health in the general population, a model was proposed that accommodates genetic pleiotropic effects, but still allows exercise to causally increase wellbeing in specific subgroups of the population (de Geus & de Moor, 2008). As with any other behavior, for exercise behavior to be repeated regularly, the net appetitive effects of exercise would need to outweigh the net aversive effects. Individuals who experience greater exercise induced mood enhancement are likely to repeat the behavior and become regular lifetime exercisers. This assumption is supported by several studies, which show that a more positive affective response during exercise was associated with greater participation in (voluntary) moderate to vigorous exercise (Schneider & Graham, 2009; Williams et al., 2008) or the intention to engage in voluntary exercise (Kwan & Bryan, 2010). Individual differences in these acute mood effects of exercise could be strongly co-determined by genetic factors.

In addition to differential acute mood effects, there could be a social-psychological mechanism that makes some individuals more attracted to exercise than others. Individuals with higher innate exercise capacities will gain more in exercise performance than others at comparable levels of training. The higher trainability and the superior exercise performance will lead to feelings of competence and mastery. This increased confidence, or self-efficacy, may not only enhance the frequency of exercise in individuals (Dishman, 1990), but will also lead to higher self-esteem and in turn, in feelings of wellbeing. Vice versa, low trainability and lower levels of performance will lead to disappointment and particularly in adolescents to shame and lowered self-esteem. Genetic variation among people influencing exercise ability will therefore become associated with experiencing psychological beneficial effects of exercise activities and, as a consequence, with an increase in the frequency of exercising.

Major future challenges are to test the association between the level of voluntary exercise behavior and the acute and longer term psychological responses to exercise, and to establish

the contribution of shared genetic factors to these associations. This requires a substantial family or twin study with measurements of exercise ability and the acute mood response to exercise. Various experimental design issues should be taken into account in these studies. First, the intensity at which an individual is exercising is an important determinant of the aversive responses to exercise: At intensities that exceed the individuals' ventilatory threshold (VT), when there is a transition from aerobic to anaerobic metabolism, negative changes in exercise induced mood response are observed (Ekkekakis, 2003). Measurements should therefore be standardized for the VT. Second, different types of exercise induced mood responses can be measured: during (immediate response on exercising) or (shortly) after the exercise bout (more complex, long lasting feelings). These responses may differ in origin, but are likely to contribute to the overall balance of appetitive and aversive effects of exercise, therefore, should both be included in measurements. For the assessment of exercise ability it is important to take into account a range of objective determinants like aerobic fitness, balance, flexibility and static and dynamic muscle strength, but also record self-perceived exercise ability, particularly in relation to the relevant peer group.

Acknowledgement of the differential sensitivity to the psychological effects of exercise is of great importance. Some individuals may require a specific exercise program (with respect to intensity of exercise, absence or presence of competitive elements, type of exercise) to create a situation in which the appetitive effects of exercise can predominate. This may ensure that these individuals continue to be engaged in regular exercise while maximizing their psychological benefits in terms of increased feelings of wellbeing and decreased levels of anxiety and depression.

CHAPTER 8

A TWIN STUDY ON THE HERITABLE COMPONENTS OF VOLUNTARY EXERCISE BEHAVIOR IN ADOLESCENCE

ABSTRACT

To improve the success of interventions aimed to increase moderate to vigorous physical activity, we need to better understand the determinants of the extensive individual differences that are found in voluntary exercise activities. Starting in adolescence, genetic effects become a dominant factor in explaining individual differences in voluntary exercise behavior. In the current study we aim to establish the prospective contribution of all potential determinants to the heritability of voluntary exercise behavior in a sample of adolescents and young adult twins. Data on known determinants of exercise behavior were collected using surveys and a laboratory study. Information on personality, perceived barriers & benefits, subjective and objective exercise ability and the affective response to exercise was collected in a set of healthy adolescent twin pairs aged 16 to 18-years old and their non-twin siblings (12 – 25y). Two years later, the subjects were sent an online follow-up survey on their current exercise status. In a multivariate model, the phenotypic variance in these determinants and exercise behavior at *follow-up* were decomposed in sources of genetic (co)variance and environmental (co)variance. Results showed that 66% of the individual differences in exercise behavior at *follow-up* were due to genetic factors. The determinants that showed significant associations with exercise behavior at *follow-up* were extraversion, positive affect after exercise, perceived benefits and barriers (lack of skills, support and/or resources, time constraints, lack of energy, lack of enjoyment, embarrassment), subjective exercise ability, maximal oxygen uptake and flexibility. Multivariate modeling showed that the genetic variation in exercise behavior could be entirely explained by the genetic variation in the twelve determinants measured 2 years earlier. Given their substantial predictive power we can assert that these determinants can be used to develop stratified interventions for adolescent and young adult exercise behavior. In addition, these results provide the first clues on 'where to look' for specific genetic variants for voluntary exercise behavior.

INTRODUCTION

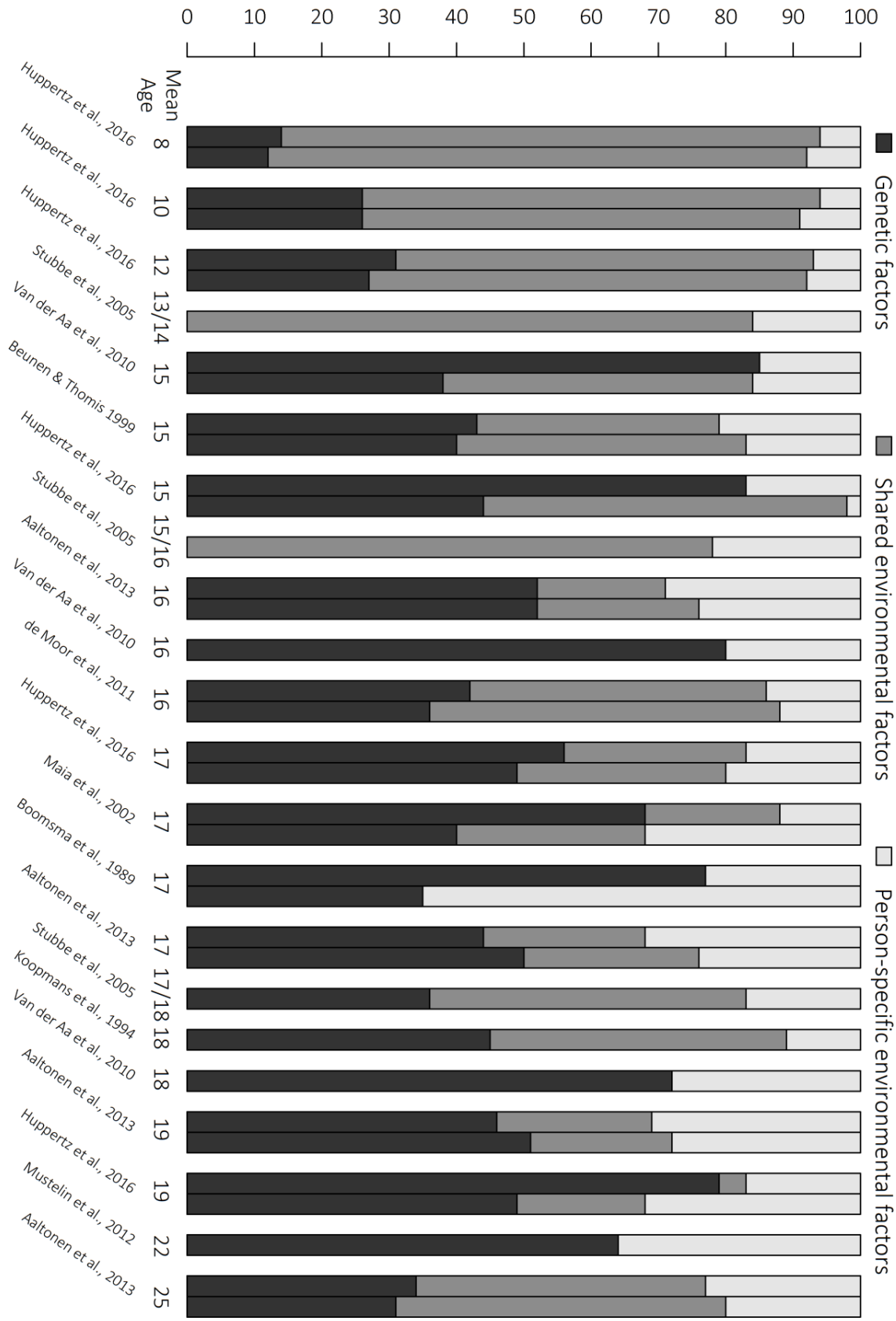
Despite the well-known benefits of physical activity, there is a growing number of adolescents and young adults with a less than optimal physically active lifestyle (Martinez-Gonzalez et al., 2001; Troiano et al., 2008), which puts them at risk for a large number of chronic diseases (Tremblay et al., 2011; Warburton et al., 2006). Prospective cohort studies in adults suggest that increasing regular physical activity, especially moderate to vigorous physical activity, can protect against the onset of chronic disease and mortality (Ekelund et al., 2012; Gebel et al., 2015; Samitz et al., 2011) and it is a reasonable assumption that intervening early on this lifestyle reaps the largest benefits. In response, public health authorities worldwide have launched interventions aimed at physical activity during work/school time and transportation to work and school, and at physical activity in leisure time (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), and the Physical Activity Guidelines for Americans by the U.S. Department of Health and Human Services (2008)).

Because regular exercise in leisure time has become a major source of moderate to vigorous physical activity in industrialized societies (De Geus et al., 2014), increasing voluntary participation in regular exercise and sports activities is an important target for public health interventions, including those aimed at adolescents and young adults. To improve the success of such interventions we need to better understand the determinants of the extensive individual differences that are found in voluntary leisure time exercise activities. Traditionally, research has focused on environmental factors that could either impede or facilitate participation in regular exercise of youngsters. Over the last decades, a growing number of studies have demonstrated that variation in voluntary exercise behavior has a strong heritable component, particularly during adolescence and young adulthood (de Moor et al., 2011; Huppertz et al., 2012; Schutte et al., 2017a). This suggests that additional attention to biological characteristics in the research on determinants of exercise behavior is needed. In sharp contrast to a common misunderstanding, heritable traits can be excellent targets for intervention (Plomin & Haworth, 2010). Biological influences on the motivation to exercise do not impede attempts to increase the mean population level of that motivation, although they can be a cause of maintained variation around the increased post-intervention mean.

Understanding the genetic pathways that lead to differences in voluntary exercise behavior may help identify specific biological and psychological determinants that would be solid targets for intervention. Such knowledge could exploit the genetic influences on exercise behavior in stratified or personalized interventions, rather than fighting an uphill battle against natural differences between individuals by using one-size-fits-all strategies.

The classical twin design, in which the resemblance of identical twins or monozygotic (MZ) and non-identical twins or dizygotic (DZ) is compared, decomposes all phenotypic variance of a trait in sources of genetic influences, shared environmental influences (influences shared with other family members e.g. upbringing) and person-specific influences (influences that are unique to the individual). Figure 8.1 shows the results of previous studies published on the relative influence of these factors on voluntary exercise behavior in children, adolescents and young adults up to 25 years old (Aaltonen et al., 2013; Beunen & Thomis, 1999; Boomsma et al., 1989; de Moor et al., 2011; Huppertz et al., 2016; Koopmans et al., 1994; Maia et al., 2002; Mustelin et al., 2011; Stubbe et al., 2005; van der Aa et al., 2010). In younger children, the shared environmental factors seem to explain a substantial part of the variation in exercise behavior, which can be explained by an important role of the parents; they provide their children with the opportunity to become active by means of transportation to exercise activities, give exercise activities priority over other leisure time activities, and provide motivation and encouragement to exercise. However, the importance of these shared environmental factors seems to decrease in adolescence and young adulthood, where genetic effects become the dominant factor explaining individual differences in voluntary exercise behavior (Huppertz et al., 2016).

Figure 8.1 Summary of previous published studies on the relative influence of genetic factors, shared environmental influences and person-specific environmental influences on voluntary exercise behavior in children, adolescents and young adults. When two bars per studies are displayed, the first bar represents the results for males; the second bar represents the results for females.


0
10
20
30
40
50
60
70
80
90
100
Mean Age
Huppertz et al., 2016 8
Huppertz et al., 2016 10
Huppertz et al., 2016 12
Stubbe et al., 2005 13/14
Van der Aa et al., 2010 15
Beunen & Thomis 1999 15
Huppertz et al., 2016 15
Stubbe et al., 2005 15/16
Aaltonen et al., 2013 16
Van der Aa et al., 2010 16
de Moor et al., 2011 16
Huppertz et al., 2016 17
Maia et al., 2002 17
Boomsma et al., 1989 17
Aaltonen et al., 2013 17
Stubbe et al., 2005 17/18
Koopmans et al., 1994 18
Van der Aa et al., 2010 18
Aaltonen et al., 2013 19
Huppertz et al., 2016 19
Mustelin et al., 2012 22
Aaltonen et al., 2013 25
Genetic factors
Shared environmental factors
Person-specific environmental factors

In this study we use a prospective twin design to test whether the heritability of exercise behavior in adolescents and young adults can be accounted for by potential determinants of exercise behavior from a number of domains that could be used to tailor future interventions: personality, affective response to acute exercise, exercise benefits and barriers, objective exercise ability, and subjective exercise ability. Previous evidence has already shown determinants in these domains to be associated with (voluntary) exercise behavior (Allender et al., 2006; Bonen & Shaw, 1995; Dishman et al., 2005; Rhodes & Smith, 2006; Rhodes & Kates, 2015). Furthermore, of these five domains, determinants in the first four were already proven to be heritable traits (Aaltonen et al., 2016; Bartels et al., 2012a; Distel et al., 2009; Huppertz et al., 2014b; Schutte et al., 2016a; Schutte et al., 2016b; Schutte et al., 2017b). The heritability of subjective exercise ability measures has not yet been reported. However, no previous studies have examined these determinants jointly in a genetically informative design that can established the extent to which the genetic factors that influence these determinants contribute to the heritability of exercise behavior.

A substantial body of evidence confirms *personality* to be a robust correlate of regular exercise behavior. Regular exercisers score lower on neuroticism and higher on extraversion, conscientiousness, and sensation seeking (de Moor et al., 2006; Hoyt et al., 2009; Rhodes & Smith, 2006; Wilkinson et al., 2013; Wilson & Dishman, 2015). Extraversion or sensation seeking are linked to individual differences in the functioning of the reward system, which can be activated in response to appetitive aspects of exercise (Eysenck et al., 1982). Neuroticism may follow a different neurobiological route in that it is associated with higher activity of the punishment system (Gray & McNaughton, 1983; Gray & McNaughton, 2000). This punishment system can be activated in response to physical (pain, fatigue) and social (embarrassment) aversive aspects of exercise activities, and might thereby decrease their attraction on neurotic individuals. Conscientiousness may be an expression of stronger prefrontal connectivity to limbic reward and punishment areas needed for the self-control that is required to pursue regular exercise for its longer term benefits, even when short-term reward value is attenuated e.g. by time pressure due to social or work-related obligations.

To maintain regular exercise participation, the net appetitive effects of exercise activities during and shortly after exertion need to outweigh the net aversive effects (de Geus & de Moor, 2008; de Geus & de Moor, 2011). If the exercise induced affective response is on

balance positive, people are likely to maintain the behavior and become regular exercisers. Vice versa, if the net affective response is not favorable, people are at risk of dropping out and becoming non-exercisers. Strong individual differences are found in the affective responses during and after exercise. Whereas some individuals indeed report an increase in pleasure or no change, others report reduced pleasure or even strong displeasure (Ekkekakis et al., 2005; Ekkekakis et al., 2011; Van Landluyt et al., 2000; Welch et al., 2007). A more favorable affective response during exercise was found to be associated with the intention to engage in voluntary exercise (Kwan & Bryan, 2010; Ruby et al., 2011) as well as greater actual participation in (voluntary) moderate to vigorous exercise (Dunton & Vaughan, 2008; Rhodes & Kates, 2015; Schneider et al., 2009; Williams et al., 2008; Williams et al., 2012). Moreover, the affective response to exercise was found to have significant heritable components (Schutte et al., 2017b).

Although short term appetitive and aversive effects are important, longer term effects also weigh in. Social cognitive models of health behavior have consistently pointed to *perceived benefits and barriers* as a main determinant of the value of exercise behavior to a person (Allender et al., 2006; Hagger et al., 2002; Huppertz et al., 2014b; Rhodes et al., 2009; Trost et al., 2002). A positive attitude towards exercise and, consequently, the likelihood of maintaining exercise behavior increases when an individual perceives that the benefits of exercise outweigh the disadvantages. Huppertz et al. (2014b) demonstrated in a sample of adolescent twins that perceived benefits of and barriers to exercise are heritable and that exercise attitudes may have direct causal effects on exercise behavior.

A further important determinant of voluntary exercise behavior is *subjective exercise ability*. People's beliefs about their capabilities to produce designated levels of exercise performance lead to feelings of competence and mastery and this enhances the frequency of exercise behavior in leisure time. Perceived or subjective exercise ability is an important component of physical self-efficacy and a known determinant of whether someone engages in and adheres to an exercise program (Dishman et al., 2005; McAuley & Blissmer, 2000; Nigg, 2001). The extent to which subjective exercise ability is influenced by genetic variation across individuals is currently unknown.

Subjective exercise ability in part derives from *objective exercise ability*, although the relationship will be imperfect because individuals will base their judgments of their own performance in comparison to the peer groups. In adolescence, objective exercise ability is most directly observable by how individuals rank in competitive performance in specific sports. Performance may be influenced by skills specific to a sport, but a number of general fitness characteristics including strength and endurance are strong predictors of performance across a variety of sports and exercise activities (McArdle, 2009). Both strength and endurance are known to be highly heritable traits (Schutte et al., 2016a; Schutte et al., 2016b) and could therefore contribute to the heritability of voluntary exercise behavior.

To establish the prospective contribution of all these potential determinants to the heritability of voluntary exercise behavior we performed a prospective study in a sample of adolescents and young adult twins. We hypothesized that the genetic factors contributing to aspects of personality, exercise motives and barriers, exercise-induced affective response, and subjective and objective exercise ability correlate with the genetic factors that are responsible for the individual variation seen in exercise behavior in late adolescence/young adulthood.

Personality, motives and barriers, and subjective exercise ability were measured in a sample of 16 to 18 year old twins and their siblings from the Netherlands Twin Register (van Beijsterveldt et al., 2013). The affective state was assessed experimentally, by repeated measurements of the feeling scale during and after graded (sub)maximal exercise tests (Schutte et al., 2017b). Objective exercise ability was also assessed experimentally by tests of muscle strength, balance and flexibility and by $\dot{V}O_{2max}$ testing on a cycle ergometer. Regular voluntary exercise behavior at a 2-year follow-up was characterized in these subjects by an online/telephone interview at age 20.

Multivariate twin designs were used to test the source of the association between the potential determinants and exercise behavior; if there is a common genetic vulnerability to the determinant and exercise behavior, their genetic correlation (r_G) should be significant. Baseline levels of those determinants that had a significant genetic correlation with exercise behavior were then used to test our hypothesis that they would predict exercise behavior at

follow-up. If such prediction proves feasible, these determinants can be used to develop stratified interventions on adolescent and young adult exercise behavior.

METHODS

Subjects

A set of healthy adolescent twin pairs aged between 16 and 18 and their siblings (age range 12 – 25) from the Netherlands Twin Register (van Beijsterveldt et al., 2013) were invited to participate in a study on the determinants of adolescent exercise behavior. In order to be eligible for the study, subjects had to have no history of cardiovascular or respiratory disease, and being physically capable of engaging in exercise activities. Subjects were invited by sending a letter advertising the opportunity to test their fitness in addition to earning a gift voucher. All invitees had to be able and willing to visit the VU University in Amsterdam for lab testing.

All subjects provided written informed consent and if the subjects were under 18 consent was given by both of their parents/guardians. All study procedures submitted to and approved by the Medical Ethics Review Committee of the VU University Medical Center Amsterdam (NL35634.029.10).

Study design

Data used in this study was collected at three time points. Figure 8.2 shows type of data collected, the sample sizes and mean age at every time point. At time point 1, part of the twins and their non-twin siblings received an online self-report survey. The survey contained items about regular exercise behavior, personality, exercise attitudes and subjective exercise ability. The mean age at completion of the survey was 16.9 ± 0.8 (N = 373).

At time point 2, regular exercise behavior was queried by interview during an extended experimental protocol including tests of affective responses to exercise and objective exercise ability. Protocol details of the exercise tests are described elsewhere (Schutte et al., 2017b; Schutte et al., 2016a; Schutte et al., 2016b). Briefly, on arrival at the laboratory, height and weight were measured and a short lifestyle interview was completed, including detailed

questions on current levels of regular exercise. Next, four fitness tests were administered to measure balance, hand grip strength, flexibility and vertical jump height. Thereafter, two exercise test were conducted (in fixed order) on an electromechanically braked Lode cycle ergometer (type Corival) and a Lode treadmill (type Valiant) at fixed loads that are below the intensity of the ventilatory threshold for most adolescents. Both submaximal tests consisted of 4 incremental stages of 5 minutes each followed by a 1-minute cooling-down phase and by a 5-minute recovery period. To ensure that the intensity of every stage was below the intensity of the ventilatory threshold for most adolescents, the ratio of the oxygen consumption and carbon dioxide production ($\dot{V}CO_2/\dot{V}O_2$) was monitored. This respiratory exchange ratio (RER) can be used to estimate the ventilatory threshold. This threshold is passed when exhalation of CO_2 exceeds inhalation of O_2, which is visualized by a RER > 1.00. For each test the load of each stage was adjusted when necessary to keep the intensity below an RER of 1.00. Finally, an incremental maximal exercise test was conducted on a cycle ergometer to establish $\dot{V}O_{2max.}$ The work rate was increased every minute until exhaustion. After cessation of the test, every participant completed a mandatory cool-down phase on the cycle ergometer of 5 minutes on a low, individually chosen work rate.

At time point 3, the original subjects to the exercise tests were sent an online follow-up survey on their current exercise status. When subjects failed to take the survey online, the survey was done by telephone. Five subjects unsubscribed from the Netherlands Twin Register and were therefore not available for the follow-up survey. 59 subjects were lost to follow-up due to missing contact information or did not fill out the questionnaire after several reminders or refused to participate by telephone. This resulted in a response rate of 88%. Complete follow-up data on exercise behavior was available for 423 subjects; 50 MZM pairs (of which 11 participated with a sibling); 26 DZM pairs (of which 1 participated with a sibling); 46 MZF pairs (of which 11 participated with a sibling); 36 DZF pairs (of which 2 participated with a sibling); 28 DOS pairs, 2 (non-twin) sibling pairs and 26 singletons. Mean age at time of the follow-up was 19.7 ± 1.1.

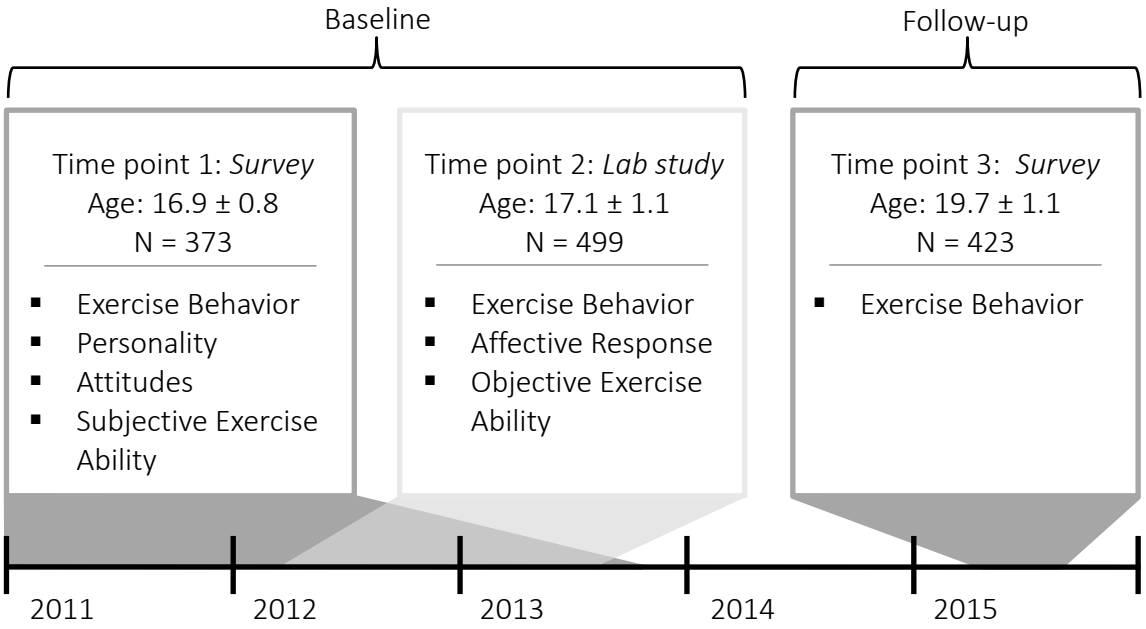


Figure 8.2 Time points and measurements of the cohort.

Measurements

Regular exercise behavior The subjects were asked to indicate what types of regular sports or exercise activities they were involved in. Subjects were asked to indicate for each activity for how many years the subject participated in the activity, for how many months a year, how many times a week, and how many minutes each time. Each activity was recoded into a metabolic equivalent (MET) score, based on the compendium of energy expenditure for youth published by Ainsworth et al. (Ainsworth et al., 2000). By multiplying the MET score, the frequency, and the duration of each exercise activity, weekly MET-hours spent on exercise activities were calculated for each participant. We only included activities that were conducted for at least 3 months a year and since at least half a year (thereby excluding ski holidays, sailing camps, and similar). Exercise behavior was quantified in the same way at all three time points, but surveys were used at time points 1 and 3, whereas an interview was conducted at time point 2. Tracking coefficients (Pearson *r*) were .67 from time points 1 to 2, .57 for time points 2 to 3, and .49 for time points 2 to 3.

Personality Personality traits were measured by the short version of the NEO, reliability and validity of which are well-established (NEO-FFI: Costa & McCrae, 1992). The NEO-FFI consists of 60 items that are rated on a 5-point scale (1–5: "totally disagree", "disagree", "agree", to "totally agree".) and gives a score for the traits neuroticism, agreeableness, conscientiousness, extraversion and openness to experience. For each trait 12 items are summed to obtain a total score.

Affective response Affective responses to exercise were assessed by the Dutch versions of the Feeling Scale (Hardy & Rejeski, 1989) and the Activation-Deactivation Adjective Checklist (Thayer, 1986; Thayer, 1986). The Feeling Scale (FS) is an 11-point bipolar measure of pleasure-displeasure. The scale ranges from -5 "very bad" to +5 "very good" and has been used as in many studies on the affective response to exercise (Ekkekakis et al., 2008; Ekkekakis et al., 2011; Hall et al., 2002; Parfitt et al., 2006; Schneider & Graham, 2009). The Activation-Deactivation Adjective Checklist (AD ACL) is a multidimensional test of transitory arousal states using a four-point self-rating system: "definitely feel" (4), "slightly feel" (3), "cannot decide" (2) or "definitely do not feel" (1). As the subjects experienced some trouble with understanding three of the items "placid" and "wakeful" and "intense", these items were left out the analyses. This questionnaire is scored by averaging five scores for each subscale: Energy, Tiredness, Tension, and Calmness. Only the variables of the FS and AD ACL that showed significant genetic influences and a significant phenotypic correlation with exercise behavior in a previous study (Schutte et al., 2017b) were taken into account here.

Perceived benefits & barriers Perceived benefits of exercise behavior were measured by 10 items with a 4-point response scale, ranging from "strongly disagree" (1), "disagree", "agree", to "strongly agree" (4). Seven items were derived from a questionnaire by Devereaux Melillo et al. (1997). The remaining three items were taken from a questionnaire by Sechrist et al. (1987). Perceived barriers towards exercise behavior were measured by 23 items derived from a questionnaire by Sallis et al. (1989) (van Sluijs et al., 2005). Each item could be answered on a five-point response scale (ranging from "never" (1) to "very often"(5)). All items were combined into six components, according to Huppertz et al. (2014b).

Subjective exercise ability Subjective ability was measured using four items. The first three asked to compare a participant's own sport performance, endurance capacity and muscle strength to their peers. Responses were measured with a 5-point response scale ranging from "I perform much worse than my peers" (1) to "I perform much better than my peers" (5). The final item asked the participant to indicate on a 10-point scale, ranging from "very bad" (1) to "really good" (10) how well they performed at sport activities. This final item was rescaled to a 5-point response scale by dividing the score by two. All four items were combined into one measure (a mean was calculated) for subjective exercise ability (Cronbach's alpha = .78).

Objective exercise ability As detailed elsewhere, tests of muscle strength as well as an maximal exercise test on a cycle ergometer were used to test exercise ability in these subjects (Schutte et al., 2016a; Schutte et al., 2016b). Briefly, explosive strength was measured with a vertical jump test. Subjects were instructed to jump straight up as much as possible and not go sideways Best out of 3 jumps was documented (jumping height in centimeters). To measure handgrip strength, subjects were instructed to hold a dynamometer (Baseline Digital Smedley Hand dynamometer, Fabrication Enterprises Inc., USA) in the dominant hand and when ready, the subject was encouraged to squeeze the dynamometer once with maximum effort (in kg). Flexibility was measured using a standard sit-and-reach box (Baseline Sit-and-reach Trunk Flexibility Box, Fabrication Enterprises Inc., USA). Best out of 3 reaches (in centimeters) was used for analyses. The Balance Error Scoring System (BESS) was used to assess balance. Finally, oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were recorded breath-by-breath by means of a telemetric gas exchange system (Cosmed K4b^2, Cosmed Benelux, Nieuwegein, The Netherlands). To obtain maximal oxygen uptake ($\dot{V}O_{2max}$), only $\dot{V}O_2$ data with a corresponding Respiratory Exchange Ratio of at least 1.10 was selected to ensure good effort above the intensity of the ventilatory threshold. Breath-by-breath $\dot{V}O_2$ data was cut into 20-second blocks. For every 20 second block, the mean $\dot{V}O_2$ was calculated, after discarding deviant breaths. $\dot{V}O_{2max}$ was determined as the highest mean value of $\dot{V}O_2$ of all the 20-second blocks.

Statistical Analysis

Analysis of the data was done in three steps. First, saturated bivariate models including exercise behavior and one potential determinant were fitted in which phenotypic

correlations, MZ and DZ/sibling correlations, as well as cross-trait/cross-twin correlations were estimated to explore their association. To decrease the impact of measurement error and true fluctuation in exercise behavior over time we used the mean exercise behavior for every individual of the first two time points (Figure 8.3). Modeling was done in OpenMx (Boker et al., 2011) under R (R Development Core Team, 2011) with the raw-data ML procedure for estimation of parameters. For all analyses, a threshold of $p < .05$ was considered for statistical significance. Since (non-twin) siblings share, like DZ twins, on average 50% of their segregating genes, parameter estimates were constrained to be equal for DZ twins and siblings. Means were estimated separately for males and females.

Next, total phenotypic variation in these variables was decomposed into additive genetic variance and covariance (A), variance and covariance that can be ascribed to sources that are shared by the twins (e.g. family environment, C) and sources of variance and covariance that are person-specific (unique environment, E). This latter component also includes measurement error. The significance of these components was tested by comparing the bivariate model including these components to a model in which A, C or E is constraint to be equal to zero. These nested submodels were compared by hierarchic χ^2 tests. The χ^2statistic is computed by subtracting log-likelihood (–2LL) for a reduced model from the -2LL for the full model (χ^2= -2LL$_{\text{full model}}$ – -2LL$_{\text{reduced model}}$). This χ^2statistic is distributed with degrees of freedom (*df*) equal to the difference in the number of parameters estimated in the two models (Δdf = $df_{\text{full model}} - df_{\text{reduced model}}$). If the difference test is significant the constraints on the reduced model cause a significant deterioration of the fit of model. By constraining the genetic correlation (r_G) to zero (Figure 8.3), it was tested whether there is substantial overlap in genetic variants underlying the two phenotypes. When the genetic correlation proved to be significantly different from zero, these determinants were selected to be included in further analyses.

A Cholesky decomposition was fitted to the selected determinants *at baseline* (time point 1 of 2) and exercise behavior *at follow-up* (time point 3). Figure 8.4 shows (part of) this Cholesky decomposition, which reveals insight into the etiology of covariances between the determinants and prospective exercise behavior. The path coefficients between the latent

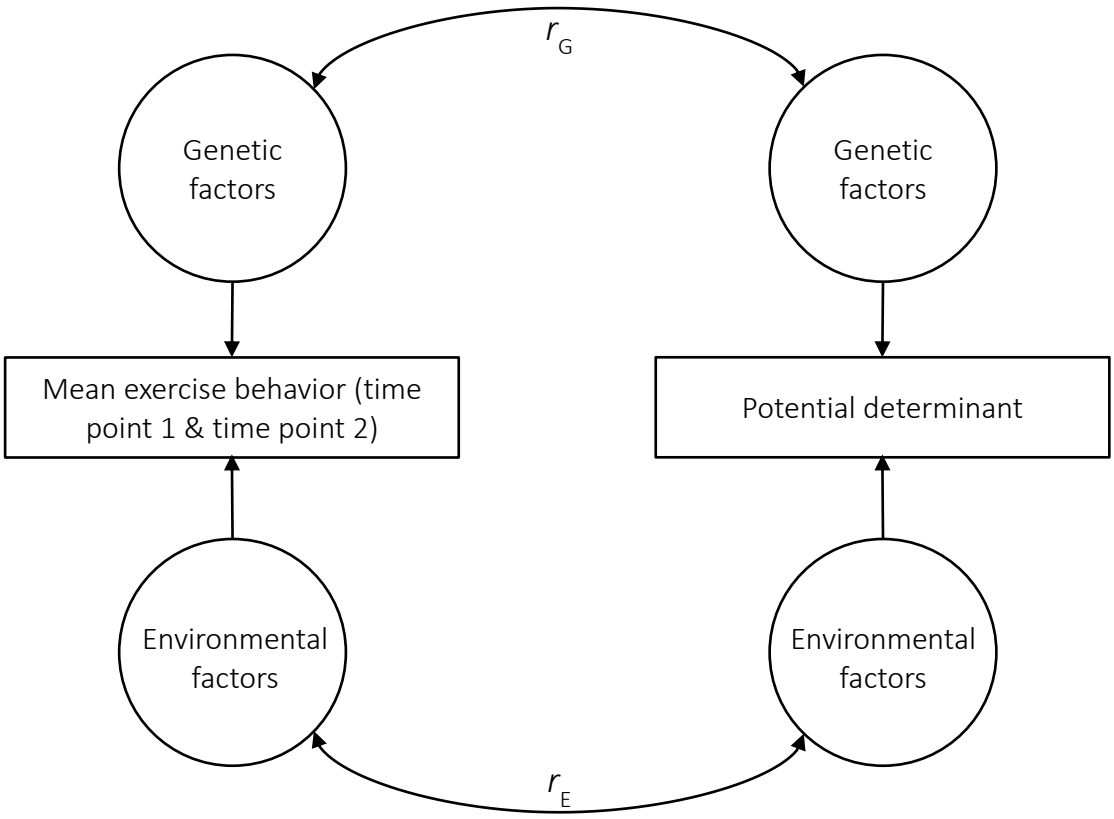


Figure 8.3 Graphic representation of the bivariate models used to test the hypothesis that there is an overlap in genetic factors influencing potential determinants of exercise behavior as well as exercise behavior itself; this predicts a significant genetic correlation (r_G). In the bivariate analyses, the mean of exercise behavior of time point 1 and 2 was used.

genetic (A) and environmental (E) factors and the observed variables (the potential determinants and exercise behavior) can be used to calculate (using path tracing rules) how much of the covariance can be explained by genetics and how much by environmental factors. For example, the total variance in the first determinant is calculated as $a_{1,1} * a_{1,1}$ (the genetic variance, also known as the heritability) + $e_{1,1} * e_{1,1}$ (variance that is explained by environmental factors). The covariance between the first two determinants is computed as $a_{1,1}*a_{2,1}$ (which is the genetic covariance) + $e_{1,1}*e_{2,1}$ (which represents the covariance that is explained by environmental factors). Heritability can be computed by standardizing these coefficients by dividing the summed genetic variance for an observed phenotype by the total

variance. By decomposing the variance and covariance in sources of genetic and environmental factors, it is possible to estimate multivariate heritability and to examine how much of the genetic variance in exercise behavior *at follow-up* is shared with the determinants *at baseline* (path coefficients $a_{12,1}$, $a_{12,2}$ etc. up to $a_{12,11}$) and how much of the genetic variance in exercise behavior *at follow-up* is unique (path coefficient $a_{12,12}$).

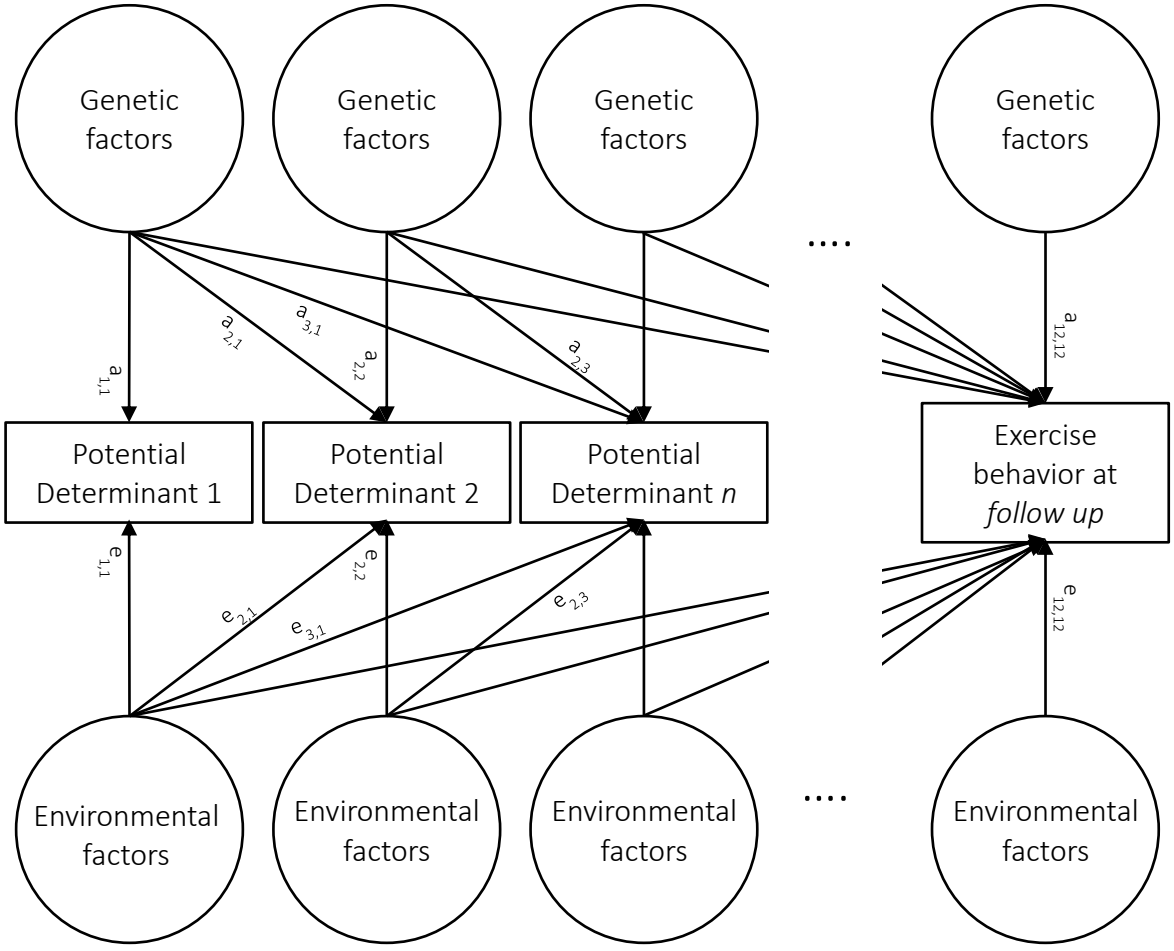


Figure 8.4 Graphic representation of the Cholesky decomposition. Variance and covariance of all twelve determinants and exercise behavior at follow-up were decomposed into genetic (A) and environmental (E) sources. The names next to the arrows represent the unstandardized path coefficients and their 95% confidence intervals. Notations for the other path coefficients follow analogous reasoning.

Finally, a multiple regression analysis was run in Stata/SE (version 14.1, StataCorp LP, USA) to determine how much of the variance in exercise behavior *at follow-up* could be explained by the determinants *at follow-up*. All determinants were forced into the model simultaneously. Family was used as a random effect to take familial relatedness into account.

RESULTS

General descriptives

Table 8.1 shows the means and standard deviations (SDs) for exercise behavior measured at the 3 time points for males and females separately. METs spent on exercise behavior reported by survey at the youngest age were higher than those measured by interview in the lab study and by survey at 2-year follow-up. Females had lower MET scores compared to males at all three time points. Table 8.1 also shows the means and SDs of the potential determinants in the five different domains. Males had lower scores on the Personality subscales Neuroticism and Agreeableness. Females felt more energetic after the submaximal exercise test, whereas males reported higher feelings of tiredness. Regarding the exercise motives and barriers, females scored lower on 'lack of energy' higher on 'embarrassment'. As expected, males score higher on all four measures of objective exercise ability, but no significant differences were seen for subjective exercise ability.

Table 8.2 shows the (univariate) heritability estimates of exercise behavior. In our sample, the heritability was 67% (95% CI: 55% - 76%) for exercise behavior at time point 1, 81% (95% CI: 73% - 86%) at time point 2 and 60% (95% CI: 42% - 72%) for exercise behavior at time point 3.

Bivariate genetic analyses

Table 8.2 displays the heritability estimates of the potential determinants and significant phenotypic correlations to mean exercise behavior across the different time points. In the final column of Table 8.2, the genetic and environmental correlations are listed. The five personality traits showed heritability estimates of 41% to 59%. Extraversion and Conscientiousness showed phenotypic correlations (r = .24 and .12 respectively) with exercise behavior, but only extraversion also showed a significant genetic correlation (r_G = .28).

Heritability estimates for exercise-induced affective responses ranged from 15% to 37%. All measures, except for Calmness measured after the submaximal cycle ergometers test showed low, but significant phenotypic correlations with exercise behavior (.11 < *r* < .14). However, only Calmness measured after the submaximal test on the treadmill showed significant genetic correlation with exercise behavior (r_G =.41).

Heritability estimates for perceived benefits was 47%. For perceived barriers, the estimated ranged from 30% for 'time constraints' to 59% for 'embarrassment'. All phenotypic correlations (*r* = .21 for perceived benefits and -.36 < *r* < -.20 for the remaining perceived barriers) with exercise behavior were moderate. Both perceived benefits and barriers showed significant genetic correlations with exercise behavior.

Subjective exercise ability showed a moderate phenotypic correlation (*r* = .40) with exercise behavior, and high genetic correlation with r_G = .50. Finally, close to half or more of the variation in objective exercise ability quantified by $\dot{V}O_{2max}$, handgrip strength, vertical jump performance, balance and flexibility could be explained by genetics (44% to 80%). Higher $\dot{V}O_{2max}$, better vertical jump performance and flexibility were associated with a higher amount of exercise behavior (*r* = .29 and .30 for $\dot{V}O_{2max}$, *r* = .17 for vertical jump performance, and *r* = .16 for flexibility). Significant genetic correlations were detected between exercise behavior and $\dot{V}O_{2max}$ (r_G = .40 and .46) and between exercise behavior and flexibility (r_G = .15).

Multivariate analyses

In total, 12 of all our potential determinants showed significant phenotypic as well as genetic correlations with mean exercise behavior across all time points: Extraversion, Energy and Calmness measured after the submaximal exercise tests, all six motives and barriers for exercise behavior, subjective ability, $\dot{V}O_{2max}$ measured in mL/min as well as min/mL/kg, and flexibility. We included $\dot{V}O_{2max}$ measured in mL/min/kg in the subsequent analyses, making a total of eleven determinants of interest. These eleven determinants were included in a multivariate Cholesky decomposition, together with exercise behavior at *follow-up* only. Table 8.3 shows the unstandardized estimates of the path coefficients of the Cholesky decomposition. The headings of Table 8.3 correspond to the path coefficients depicted in Figure 8.4. The path coefficients $a_{12,1}$, $a_{12,2}$ etc. up to $a_{12,11}$ larger than the path coefficients $e_{12,1}$, $e_{12,2}$ etc. up to $e_{12,11}$, suggesting that the prospective association between these

determinants and exercise behavior *at follow-up* largely reflect shared genetic factors. Moreover, path coefficient $a_{12,12}$ (Figure 8.4) was only -.03 (*SE* = 2.8, $p > .05$) which was not significant different from zero. Consequently, no additional genetic factor unique to exercise behavior and not shared by any of the eleven determinants was found.

Finally, these eleven determinants were included in a multiple regression analysis (corrected for family structure) to predict exercise behavior *at follow-up.* Table 8.4 shows the regression coefficient estimates and their significance. Nineteen percent of the variance in follow-up exercise behavior could be explained by these determinants. However, we caution that only exercise ability (subjective exercise ability as well as objective exercise ability quantified by $\dot{V}O_{2max}$) was significant, and only at a liberal p-value of 0.05.

Table 8.1 Means and SDs for males and females for cross-sectional exercise behavior and exercise behavior at 3-year follow-up and its potential determinants.

		Males		Females	
		Mean	*SD*	*Mean*	*SD*
Exercise behavior (METs/week)	Time point 1	33.3	28.4	22.9	27.1
	Time point 2	25.2	22.8	18.7	21.0
	Time point 3	23.6	27.7	19.3	22.0
Personality	Neuroticism	28.7	7.1	31.9	7.7
	Extraversion	42.6	6.1	43.7	5.9
	Openness to experience	35.6	4.8	34.9	5.1
	Agreeableness	42.7	4.7	44.9	4.4
	Conscientiousness	42.4	6.7	43.9	5.8
Affective response	*submaximal exercise tests:*				
	Feeling Scale - cycle ergometer	-1.3	3.4	-1.9	3.7
	Energy - cycle ergometer	3.4	0.5	3.3	0.6
	Tiredness - cycle ergometer	1.4	0.5	1.6	0.6
	Calmness - cycle ergometer	2.9	0.7	2.8	0.7
	Calmness - treadmill	2.9	0.8	2.8	0.7
	maximal exercise test:				
	Calmness	3.3	0.6	3.3	0.6
Perceived benefits		31.4	4.9	32.2	4.4
Perceived barriers	Lack of skills, support and/or resources	13.0	4.5	12.9	4.6
	Time constraints	9.1	3.2	9.7	3.2
	Lack of energy	8.3	2.7	9.4	3.2
	Lack of enjoyment	6.6	2.9	6.8	2.9
	Embarrassment	4.5	1.7	5.3	1.9
Subjective exercise ability		3.5	0.6	3.4	0.6
Objective exercise ability	$\dot{V}O_{2max}$ (mL/min)	3134	510	2233	321
	$\dot{V}O_{2max}$ (mL/min/kg)	46.9	6.6	36.6	5.8
	Hand grip	40.1	7.9	29.6	4.6
	Vertical Jump	45.8	6.4	35.4	5.3
	Flexibility	19.7	10.4	28.9	9.9
	Balance	44.9	6.8	46.7	7.1

Table 8.2 Heritability estimates (95% CI) of the potential determinants, phenotypic correlations (95% CI) to exercise behavior and their genetic and environmental correlations (95% CI).

Potential Determinant		Heritability	Phenotypic correlation	r_G	r_E
Exercise behavior (METs/week)	Time point 1	67% (55%, 76%)			
	Time point 2	81% (73%, 86%)			
	Time point 3	60% (42%, 72%)			
Personality	Neuroticism	41% (20%, 59%)	-.07 (-.19, .40)	-.09 (-.33, .16)	-.06 (-.30, .18)
	Extraversion	59% (41%, 71%)	**.24 (.13, .35)**	**.28 (.09, .45)**	.16 (-.08, .38)
	Openness to experience	56% (39%, 68%)	.01 (-.11, .13)	.03 (-.17, .23)	-.03 (-.26, .20)
	Agreeableness	44% (24%, 60%)	.04 (-.08, .16)	.00 (-.23, .23)	.09 (-.14, .32)
	Conscientiousness	57% (38%, 70%)	**.12 (.00, 23)**	.15 (-.05, .36)	.00 (-.24, .24)
Affective response	*submaximal exercise tests:*				
	Feeling Scale - cycle ergometer	19% (0%, 37%)	**.11 (.01, .22)**	.26 (-.06, 1)	.03 (-.17, .23)
	Energy - cycle ergometer	27% (20%, 52%)	**.13 (.02, .23)**	.20 (-.03, .42)	.07 (-.12, .26)
	Tiredness - cycle ergometer	28% (10%, 44%)	**.11 (.00, .21)**	.14 (-.11, .42)	.08 (-.11, .27)
	Calmness - cycle ergometer	29% (10%, 45%)	-.08 (-.18, .03)	.02 (-.23, .31)	-.22 (-.41, -.03)
	Calmness - treadmill	15% (1%, 32%)	**.14 (.04, .24)**	**.41 (.08, 1)**	-.02 (-.20, .17)
	maximal exercise test:				
	Calmness	37% (22%, 54%)	**.12 (.01, .22)**	.20 (-.01, .42)	-.02 (-.22, .17)

Note. r_G = genetic correlation; r_E = environmental correlation.

Table 8.2 Heritability estimates (95% CI) of the potential determinants, phenotypic correlations (95% CI) to exercise behavior and their genetic and environmental correlations (95% CI).

Potential Determinant		Heritability	Phenotypic correlation	r_G	r_E
Perceived benefits		47% (30%, 61%)	**.21 (.09, .31)**	**.27 (.07, .47)**	.11 (-.10, .31)
Perceived barriers	Lack of skills, support and/or resources	50% (34%, 63%)	**-.33 (-.43, -.22)**	**-.43 (-.61, -.25)**	-.16 (-.35, .05)
	Time constraints	30% (11%, 47%)	**-.23 (-.33, -.12)**	**-.38 (-.67, -.13)**	-.12 (-.32, .08)
	Lack of energy	55% (37%, 68%)	**-.31 (-.41, -.20)**	**-.39 (-.57, .21)**	-.16, (-.36, .05)
	Lack of enjoyment	44% (26%, 58%)	**-.36 (-.45, -.26)**	**-.47 (-.67, -.28)**	**-.20 (-.39, -.00)**
	Embarrassment	59% (45%, 70%)	**-.20 (-.31, -.08)**	**-.21 (-.38, -.03)**	-.12 (-.33, .08)
Subjective exercise ability		66% (43%, 75%)	**.40 (.29, .49)**	**.50 (35, .64)**	.08 (-.13, .29)
Objective exercise ability	$\dot{V}O_{2max}$ (mL/min)	58% (45%, 69%)	**.29 (.13, .38)**	**.40 (.24, .55)**	.12 (-.08, .31)
	$\dot{V}O_{2max}$ (mL/min/kg)	54% (41%, 65%)	**.30 (.20, 40)**	**.46 (.29, .61)**	-.00 (-.19, .19)
	Handgrip strength	66% (54%, 75%)	-.01 (-.12, .10)	.03 (-.14, .20)	-.05 (-.24, .15)
	Vertical Jump	50% (33%, .63%)	**.17 (.06, .28)**	.17 (-.03, .35)	**.22 (.02, .40)**
	Flexibility	80% (73%, 86%)	**.16 (.05, .27)**	**.15 (.00, .29)**	.14 (-.05, .33)
	Balance	44% (28%, 59%)	.01 (-.10, .12)	.14 (-.06, .35)	-.19 (-.37, .01)

Note. r_G = genetic correlation; r_E = environmental correlation.

Table 8.3 Unstandardized path coefficients.

	$a_{\ldots,1}$	$a_{\ldots,2}$	$a_{\ldots,3}$	$a_{\ldots,4}$	$a_{\ldots,5}$	$a_{\ldots,6}$	$a_{\ldots,7}$	$a_{\ldots,8}$	$a_{\ldots,9}$	$a_{\ldots,10}$	$a_{\ldots,11}$	$a_{\ldots,12}$
$a_{1,..}$	4.71											
$a_{2,..}$	0.18	-0.31										
$a_{3,..}$	-2.04	0.65	-2.56									
$a_{4,..}$	2.03	0.29	-0.24	2.33								
$a_{5,..}$	0.07	0.84	-1.30	-0.35	1.12							
$a_{6,..}$	-1.07	0.98	-1.41	0.02	0.78	-0.51						
$a_{7,..}$	-1.57	0.60	-0.87	-0.31	-0.17	-0.30	0.61					
$a_{8,..}$	-0.37	0.51	-0.45	-0.50	0.16	-0.81	0.29	0.49				
$a_{9,..}$	0.26	-0.12	0.13	0.06	0.09	-0.03	-0.03	-0.28	-0.12			
$a_{10,..}$	0.18	-0.58	1.56	0.95	-0.37	-0.75	-2.99	-2.48	0.52	-1.19		
$a_{11,..}$	1.79	1.81	0.95	0.49	-1.63	-1.06	-4.15	-1.96	-5.91	3.83	0.00	
$a_{12,..}$	3.62	-5.72	7.41	5.18	0.64	-2.40	-4.06	2.45	-9.58	-13.48	-0.08	-0.03
	$e_{\ldots,1}$	$e_{\ldots,2}$	$e_{\ldots,3}$	$e_{\ldots,4}$	$e_{\ldots,5}$	$e_{\ldots,6}$	$e_{\ldots,7}$	$e_{\ldots,8}$	$e_{\ldots,9}$	$e_{\ldots,10}$	$e_{\ldots,11}$	$e_{\ldots,12}$
$e_{1,..}$	3.74											
$e_{2,..}$	-0.09	0.69										
$e_{3,..}$	-0.90	-0.27	2.90									
$e_{4,..}$	-0.12	0.15	-0.81	3.28								
$e_{5,..}$	-0.36	0.27	1.01	-0.42	2.23							
$e_{6,..}$	-0.51	0.13	0.75	-0.26	0.29	1.73						
$e_{7,..}$	0.03	0.29	0.77	-0.45	0.55	0.53	1.66					
$e_{8,..}$	-0.39	0.03	0.59	0.20	0.00	0.32	-0.01	0.85				
$e_{9,..}$	0.08	-0.02	-0.03	0.08	-0.01	-0.03	-0.02	0.02	0.29			
$e_{10,..}$	-0.23	0.05	0.22	0.18	0.58	-0.24	0.41	-0.55	-0.06	3.96		
$e_{11,..}$	0.08	0.21	0.37	0.77	-0.26	-0.08	-0.32	0.04	-0.74	0.51	4.29	
$e_{12,..}$	1.51	-0.83	0.31	1.51	-0.40	0.78	-2.56	-0.15	0.16	0.25	1.03	14.41

Note. The path coefficients correspond to the path coefficients depicted in Figure 8.4. For clarification: 1 = Extraversion; 2 = Calmness – treadmill; 3 = Perceived benefits; 4 = Lack of skills, support and/or resources; 5 = Time constraints; 6 = Lack of energy; 7= Lack of enjoyment; 8 = Embarrassment; 9 = Subjective ability; 10 = V̇O2max (mL/min/kg); 11 = Flexibility; 12 = Exercise behaviour at *follow-up.*

Table 8.4 Results of the multiple regression analysis. A regression analysis was corrected for family structure.

Determinants		Coefficient	*SE*	*p*
1	Extraversion	0.17	0.30	0.580
2	Calmness - treadmill	-0.09	2.17	0.968
3	Perceived benefits	0.15	0.45	0.731
4	Lack of skills, support and/or resources	-0.35	0.48	0.473
5	Time constraints	-1.01	0.55	0.068
6	Lack of energy	0.06	0.66	0.929
7	Lack of enjoyment	-1.13	0.62	0.069
8	Embarrassment	0.91	0.89	0.308
9	Subjective ability	7.27	3.09	0.020
10	$\dot{V}O_{2max}$ (mL/min/kg)	0.58	0.27	0.036
11	Flexibility	0.02	0.13	0.856

DISCUSSION

The genetic contribution to individual differences in regular voluntary exercise behavior peaks in adolescents and young adults (Figure 8.1). Here, a prospective twin design was used to test whether the heritability of exercise behavior in these age groups could be accounted for by a number of potential determinants of exercise behavior: personality, affective response to acute exercise, exercise motives and barriers, objective exercise ability as well as subjective exercise ability. The first four of these correlates of exercise behavior had been shown to be heritable in previous studies (Aaltonen et al., 2016; Bartels et al., 2012a; de Moor et al., 2012; Distel et al., 2009; Huppertz et al., 2014b) with strongest evidence for objective exercise ability (for meta-analyses see Schutte et al., 2016a and Schutte et al., 2016b). We now also provide evidence for substantial heritability (66%) of subjective exercise ability: one's self-reported ranking of physical fitness compared to peers.

Eleven determinants showed significant genetic overlap with exercise behavior at a three-year follow-up: Extraversion, Calmness measured after the submaximal exercise tests, the perceived benefits and barriers, subjective exercise ability, $\dot{V}O_{2max}$, and flexibility. When including these determinants in a covariance decomposition model, we accounted for all the

genetic variation in exercise behavior at follow-up. Hence, the substantial heritability of exercise behavior (60%) in our sample of young adults aged 19 to 20 is explained by the genetic variation in these eleven determinants measured three years earlier. This finding inevitably reflects some overfitting and independent replication is direly needed. Nonetheless, it is unlikely that these eleven determinants would cease to be of importance. This study therefore provides a valuable glimpse on the factors that give rise to the high heritability estimates reported in late-adolescents and young adults.

Exercise behavior is, like many other behaviors, a complex polygenic trait; whether people perform exercise activities in leisure time and how often are the result from variation within multiple genes (and their interaction with environmental factors). Each of these genetic variants will explain only a very small percentage of the variance (Flint, 2013). To detect them, a major collaborative effort is needed that collects DNA with genome-wide genotyping in a sample large enough to perform genome wide association (GWA) study on regular voluntary exercise behavior, followed by the estimation of molecular genetic correlations between voluntary exercise and the heritable determinants. In parallel, a candidate gene approach could be used based on some of the determinants detected by our analyses. Such an approach could help establish that the traits that we putatively labeled as 'determinants', truly are causes of variation in exercise behavior, rather than a consequence of exercise behavior (reverse causality).

A source of candidate genes repeatedly implicated in voluntary wheel running in animal models is the mesolimbic dopaminergic reward system (Rhodes et al., 2005). However, attempts to link dopaminergic candidate genes to voluntary exercise behavior in humans has showed mixed results (Huppertz et al., 2014a; Jozkow et al., 2013; Simonen et al., 2003). This does not, of course, rule out individual differences in the neurobiology of reward seeking or sensitivity. At least one other reward system, the endocannabinoid system, has long been implicated in exercise (Raichlen et al., 2012; Sparling et al., 2003). In general, genetic variants increasing the balance of appetitive over aversive experiences during and after exercise should be regarded potential candidate genes for exercise behavior. Bryan et al. (2007) reported that a single nucleotide polymorphism (SNP) in the brain-derived neurotrophic factor (*BDNF*) gene (G/A at nucleotide 196; Rs6265) moderated the effect of exercise on positive mood and perceived exertion in a sample of healthy exercisers (Bryan et al., 2007).

BDNF is a peptide with a broad influence on brain function and it has been linked to neural development, cell survival and synaptic plasticity. Karoly et al. (2012) found two SNPs (rs8044769 and rs3751812) in the fat mass and obesity-associated protein gene (*FTO*) gene to be related to positive affect change during exercise (Karoly et al., 2012). The *FTO* gene is strongly linked with body mass index. The risk alleles within this gene seem to act on appetite ratings and satiety, but not on resting energy expenditure or physical activity (Speakman, 2015). The *FTO* gene effect could be related to the 'embarrassment' component of the exercise attitudes linked to voluntary exercise behavior in this study might. Feelings of shame or embarrassment during exercise are known to be larger in overweight adolescents (e.g. Gillison et al., 2006).

Exercise ability was a further determinant of exercise behavior. A number of candidate genes for exercise ability also exist (Sarzynski et al., 2016) although here too caution about potential false positives has been voiced (Pitsiladis et al., 2016). Especially for objective measures of strength and endurance, like maximal oxygen uptake, it is challenging to collect enough data to be well-powered for gene finding studies as measuring these traits involves laboratory equipment and a significant amount of time. This might explain the lack of (well-replicated) findings in this field. Interestingly, we here show an important determinant of regular exercise behavior that should be easy to collect in large samples: people's subjective belief about their capabilities to produce designated levels of exercise performance.

It is essential here to note that the mean levels of most of these determinants, extraversion being the possible exception, are amenable to favorable change by intervention in adolescents and young adults. Our results confirm the usefulness of a strategy that optimizes the acute affective response to exercise, where achieving some fixed level of intensity/performance is made secondary to 'feeling good' while exercising (Ekkekakis, 2009; Parfitt et al., 2006; Williams et al., 2016). This might also improve the low expectation of enjoyment, a (perceived) barrier to regular exercise. While only a few individuals fail to improve their physical fitness from regular exercise activities, by necessity only half of the individuals will end up performing 'better than average', as innate ability plays a big role. The subjective perception of one's relative ability can be a formidable opponent when trying to engage 'the lower half' of the exercise ability distribution, particularly in adolescence. A solution in interventions aiming to increase the adoption and maintenance of exercise

behaviors might be to shift attention from peer-group comparison to a within-person comparison to one's own previous performance. For those lacking high levels of innate exercise ability, the competitive context should be downplayed or, conversely, the social aspects of exercise activities should be increased.

A number of limitations of the present study should be considered. We find no person-specific environmental correlations between exercise behavior and the putative determinants of exercise behavior. Following the logic of De Moor et al. (2008) this could be taken to falsify a true causal effect of these determinants. However, below 5000 twin pairs, the power to detect a significant environmental correlation between exercise behavior and a correlate is very poor (Stubbe & de Geus, 2009) so the non-significance in the current study should not be interpreted.

We did not take into account the possibility that, as part of maturation, different genes were expressed at baseline and follow-up. Modeling longitudinal data on exercise behavior in 7 to 18 year olds by Huppertz et al. (2016) showed that genetic effects on exercise behavior were marked by both transmission (the same genetic effects influence exercise behavior at all ages) as well as innovation (newly emerging genetic effects on exercise behavior at all time points). At the age of 18 this was about fifty-fifty; half of the genetic variants is explained by the same genetic effects influencing exercise behavior at age 16, whereas the other half is explained by newly emerging genetic effects. When new genetic factors come into play only at follow-up they would act to reduce the genetic correlation with determinants measured at an earlier time point. However, in our study, all genetic variation at age of 19.7 was shared with the genetic variance of our selected determinants measured at age 17.1, suggesting far more transmission than innovation at these ages. The age range in our sample is wider than the age ranges in the study by Huppertz et al. (2016), suggesting that genetic factors that come into play during late-adolescence may be already picked up by our study at the first time point.

Finally, a limitation of our experimental protocol, a consequence of logistic and feasibility constraints, was that submaximal and maximal exercise testing was done in a single session on one day. This prevented us from measuring the affective response at a fixed percentage of $\dot{V}O_2max$. In addition, our subjects could anticipate having to pedal until exhaustion during the maximal exercise test. This may have biased our results because individuals who have a low

tolerance for vigorous exercise activities or feel embarrassed when exercising vigorously could be underrepresented in our sample of volunteers.

In conclusion, all five main classes of potential determinants examined showed significant associations with exercise behavior at follow-up: Extraversion, positive affect after exercise, perceived benefits and barriers (lack of skills, support and/or resources, time constraints, lack of energy, lack of enjoyment, embarrassment), subjective exercise ability and objective exercise ability, quantified by maximal oxygen uptake and flexibility. Multivariate modeling showed that the genetic variation in exercise behavior could be entirely explained by the genetic variation in these eleven determinants measured 3 years earlier. Demonstrating such high levels of heritability in determinants of (un)desirable health behaviors can appear intimidating. Heritability sounds like sentence for a life. It is not. Genetic variants are a route to increased future understanding of the actual biological pathways leading to the heritability of exercise behavior. These can provide a rational basis for stratified or personalized interventions on the mean level of exercise behavior.

CHAPTER 9

SUMMARY &

SYNTHESIS

It is not without reason that public health authorities worldwide have launched interventions aimed at physical activity during work/school time and transportation to work and school, and at physical activity in leisure time (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), and the Physical Activity Guidelines for Americans by the U.S. Department of Health and Human Services (2008)). Moderate to vigorous intensity exercise has been shown to have large protective effect on mortality (Samitz et al., 2011). Yet, in spite of these well-motivated attempts, large individual differences remain to be observed in physical activity habits, including the important component of regular exercise behavior in leisure time.

In the knowledge that many twin and family studies have provided evidence that a substantial part of the variation in exercise behavior is determined by genetic predisposition (particularly in late-adolescence and young adulthood as shown in Figure 1.1), the main aim of this thesis was to identify the mechanisms that give rise to this heritability of exercise behavior.

The heritability of adolescent exercise behavior

Chapter 2 provides an overview of published studies on the quantitative genetics and molecular genetic findings for physical activity and exercise behavior. Up to 12 years of age, heritability estimates are low to moderate, whereas in (late-)adolescence, heritability estimates of voluntary exercise behavior are moderate to high. The results from the meta-analyses in three different age groups confirm increasing influence of genetic factors with age on exercise behavior: meta-analytic heritability estimates of 20% (7 to 12 years), 35% (13 to 15 years), and 53% (16 to 18 years) were reported. This changing genetic architecture of voluntary exercise behavior across the life span has been described before (Huppertz et al., 2016; Stubbe et al., 2005; Stubbe & de Geus, 2009). The notion that shared environmental factors play a greater role in childhood than adolescence can be explained by the important role of the parents; they provide the children with the opportunity to become active, by means of transportation to exercise activities, give exercise activities the priority over other leisure time activities and motivation and encouragement to exercise. During adolescence, this parental meddling becomes less prominent, and the influence of genetic factors becomes more important (Huppertz et al., 2016). In spite of the evidence for this increasing

contribution of heritable factors to exercise behavior from twin and family studies, efforts to identify the actual genes contributing to this heritability are limited. The model by De Geus & De Moor (2008) (introduced in the first chapter of this thesis) provided us with testable hypotheses regarding the nature of the genetic factors affecting regular voluntary exercise behavior. In this model it is argued that likelihood of engaging in or maintaining exercise behavior might be increased by the presence of genetic variants that amplify the feelings of pleasure, sense of accomplishment and performance.

A delicate balance: affective response to exercise

The model by De Geus & de Moor (2008) is based on the principles of instrumental conditioning, determined by the positive reinforcement or feelings of punishment. Exercise induced positive affective responses ('feel good' experiences during or shortly after an exercise bout) may be an important contributor to appetitive effects of exercise. Previous studies showed a robust association between a more favorable affective response during exercise and the intention to engage in voluntary exercise (Kwan & Bryan, 2010; Ruby et al., 2011) and greater actual participation in (voluntary) moderate to vigorous exercise (Dunton & Vaughan, 2008; Rhodes & Kates, 2015; Schneider & Graham, 2009; Williams et al., 2008; Williams et al., 2012). Short-term aversive effects may arise during exercise at higher intensities, most strikingly above the level where the supply of energy through oxygen must be supplemented by anaerobic metabolism. Blood lactate begins to accumulate above resting levels because lactate clearance is no longer able to keep up with lactate production and large individual variation in affective responses is seen (Ekkekakis et al., 2005; Ekkekakis et al., 2011; Van Landluyt et al., 2000; Welch et al., 2007). In Chapter 4, we tested the role of exercise induced affective responses in the motivation to exercise, by estimating the heritability of the affective responses during and after exercise and the overlap with the genetic factors influencing regular voluntary exercise behavior. Genetic factors explained 12% to 37% of the individual differences in the affective responses during and after (sub)maximal exercise tests in the cycle ergometer and treadmill. Without exception, more positive affective responses were associated with higher amounts of regular exercise activity ($.15 < r < .21$) and this association was accounted for by an overlap in genetic factors influencing affective responding and exercise behavior.

Other studies that directly test the association between genetic variants and exercise-induced affective responses are scarce. Bryan et al. (2007) showed mediating effects of a single nucleotide polymorphism (SNP) in the brain-derived neurotrophic factor (*BDNF*) gene (G/A at nucleotide 196; Rs6265) on the association between exercise and positive mood, heart rate, and perceived exertion in a sample of healthy exercisers. Karoly et al. (2012) found two SNPs (rs8044769 and rs3751812) in the fat mass and obesity-associated protein gene (*FTO*) gene to be related to positive affect change during exercise (Karoly et al., 2012). Furthermore, a phylogenetically old mechanism that could influence the net balance of positive and negative affective responses during and after a bout of exercise could be an innate drive to be physical active (Swallow et al., 1998). The fulfillment of this 'activity drive' could be intrinsically rewarding, just as relieving hunger or thirst. Mouse lines that were selectively bred for voluntary-wheel running behavior have shed some light on this motivation to exercise. Behavioral pharmacological but also brain imaging studies in these mice by the laboratory of Garland showed that selection for increased voluntary wheel running altered dopamine signaling (Rhodes et al., 2005). Recently, it was shown that the facilitation of dopamine signaling is modulated by glutamate and GABA (Saul et al., 2016), neuromodulators that play a role in brain reward circuitry (Kelley & Berridge, 2002) and the neurotransmitter serotonin (Claghorn et al., 2016; Saul et al., 2016), which is also involved in the brain's rewarding system (Kelley & Berridge, 2002).

Tipping the balance: personality and perceived benefits & barriers

Even more complex factors may influence the balance between aversive and appetitive effects of exercise in humans. A substantial body of evidence confirms personality to be a robust correlate of regular exercise behavior. Regular exercisers score lower on neuroticism and higher on extraversion, conscientiousness, and sensation seeking (de Moor et al., 2006; Rhodes & Smith, 2006; Wilkinson et al., 2013; Wilson & Dishman, 2015). In Chapter 8, we replicate the association of extraversion with voluntary exercise behavior, and show a significant genetic correlation between these traits. Extraverts are argued to be less aroused than introverts, whereas introverts have a higher level of activation to start with. Therefore, introverts might be easily overstimulated and less attracted to social situations and, consequently, exercise activities. The association with exercise behavior might be particularly

prominent in adolescence, when most exercise activities are performed in teams with friends and peers.

In the above, we assume that the genetic correlation between extraversion and exercise behavior results from the causal effects of (heritable) extraversion on exercise behavior. In doing so, we implicitly rule out reverse causality with (heritable) exercise behavior increasing extraversion. Initial support for a causal effect of extraversion on exercise (and not the reverse) comes from twin studies modeling the longitudinal trajectories of both extraversion and exercise behavior over a prolonged time period (De Moor & de Geus, *in press*). Further support could be generated by studies using candidate genes for extraversion to predict exercise behavior, using what is known as the Mendelian randomization approach (Davey Smith & Hemani, 2014). Mendelian randomization entails the utilization of common genetic variants that have a well-characterized biological function to study the effect of a suspected environmental exposure on a disease risk or trait (in this case, exercise behavior). Under the causal hypothesis that extraversion is a determinant of exercise behavior, genetic variants influencing extraversion should also be associated with exercise behavior. Provided sufficient power, failure to find this genetic influence would act to falsify a causal effect of extraversion.

Biological theories suggest that extraversion is linked to the mesolimbic dopamine system as this mechanism is related to individual differences in the functioning of the reward system (Depue & Collins, 1999) and several studies show an association of extraversion with genes involved in the dopaminergic system (Golimbet et al., 2007; Reuter & Hennig, 2005; Smillie et al., 2010). Following the Mendelian Randomization logic these genes should be associated with exercise behavior if extraversion is a causal agent. Simonen et al. (2003) indeed reported an association of a single nucleotide polymorphism (SNP) in the *DRD2* gene with physical activity in families of the Quebec Family Study and HERITAGE Family Study. However, both Jozkow et al. (2013) and Huppertz et al. (2014a) failed to replicate this association. These mixed results could doubt the role of dopaminergic signaling in voluntary exercise behavior. However, as shown by Saul et al. (2016) in mice, the total dopaminergic pathway is complex and part of a large neurobiological framework. For instance, the neurobiology of dopaminergic reward seeking might be linked to another reward system, the endocannabinoid system, which has long been implicated in exercise (Raichlen et al., 2012; Sparling et al., 2003). In addition, we now know that the single genetic variants influencing

complex traits have a very small effect size and that they may not be picked up by samples comprised of 'only' thousands of subjects (Flint, 2013).

Both these problems may be addressed by the more optimal strategy to find genes related to extraversion: a meta-analysis of genome wide association studies (GWAMA) with a large cumulative sample size (Flint, 2013; Visscher et al., 2012). A recent GWAMA for extraversion resulted in only one significant 'hit': a non-coding RNA site (LOC101928162) with unknown function (van den Berg et al., 2016). A clear prediction from our work, following the Mendelian Randomization logic is that this hit should also predict voluntary exercise behavior. As indicated, much larger samples than the one employed here are needed to test this as the effect size of this single variant is very modest. To increase power, a polygenic risk score for extraversion – for which GWA meta-analytic summary statistics are available in the public domain (de Moor et al., 2012) – could further be used to predict voluntary exercise behavior. Under the causal hypothesis that extraversion is a *causal driver* of regular exercise behavior this prediction should be significant. However, finding a significant effect would not rule out a genetic pleiotropic effect: in this case multiple genetic variants influence exercise, but independently also extraversion.

Extraversion may also act through more complex routes, e.g. in attitude formation on exercise behavior. Courneya and Hellsten (1998) showed that extraversion was correlated to exercise motives, such as improvement of fitness and health, social contacts, and sheer enjoyment (Courneya & Hellsten, 1998). In Table 8.3 in Chapter 8, the path coefficients between extraversion and perceived benefits ($a_{3,1}$) and 'lack of skills, support and/or resources' ($a_{4,1}$), 'lack of energy' ($a_{6,1}$), and 'lack of enjoyment' ($a_{7,1}$) were significant ($p < .05$), suggesting that a significant amount of this association is explained by genetic factors. The perceived benefits and barriers of exercise behavior form another set of psychological factors that could influence the net affective response to exercise behavior. Individuals who have come to believe that the (long-term) advantages of exercising outweigh the (short-term) disadvantages are more likely to adopt and maintain exercise activities (Becker, 1974). In line with this, many studies reported robust associations between perceived benefits and exercise behavior whereas perceived barriers are as robustly associated with less engagement in exercise behavior (Allender et al., 2006; Hagger et al., 2002; Rhodes & Smith, 2006; Trost et al., 2002). Perceived benefits of exercise behavior are amongst others fitness and health,

social contacts, and enjoyment, whereas lack of opportunity and support, feelings of embarrassment, the lack of energy, or time constraints are part of the perceived barriers of exercise behavior.

Huppertz et al. (2014b) demonstrated moderate to high heritability estimates for the perceived benefits and barriers in adult twins and siblings, with the highest estimates for 'lack of enjoyment '(44% to 47%) and 'lack of skills, support and resources' (including items such as 'I do not have anybody to exercise with' and 'I do not have the required materials for exercising', 45% to 48%). Aaltonen et al. (2016) reported comparable heritability estimates for motives for engaging in physical activity in leisure time in adult twins, ranking 'Enjoyment' (33% to 53%) and affiliation ('be with friends and/or do activity with others', 35% to 39%) as the motive dimensions with the highest heritability. Both studies also report perceived barriers or motive dimensions related to 'to be fitter and/or look better than others' or the other side of the spectrum 'embarrassment' to be substantial heritable (27 to 49%). Moreover, Huppertz et al. (2014b) revealed that perceived benefits and barriers of exercise may have a causal effect on exercise behavior even in the presence of pleiotropic genetic effects independently influencing exercise motives and barriers and exercise behavior. In Chapter 8, we replicated the heritability estimates for perceived benefits and barriers and showed that they have a substantial genetic overlap with exercise behavior.

Tipping the balance further: exercise ability

Being good at exercise and performing better than others will lead to feelings of competence, whereas lower levels of performance might lead to disappointment or shame. Perceptions of differences in ability will therefore greatly contribute to the affective response to exercise. This might be especially during (late-)adolescence, when the influence of role models in health behaviors is large (DuBois & Silverthorn, 2005; Yancey et al., 2011). Exercise performance level may be influenced by skills specific to a sport, although a number of general fitness characteristics including strength and endurance, are strong predictors of performance across a variety of sports and exercise activities (McArdle, 2009). Chapter 5 and 6 show that both adolescent muscle strength, as well as flexibility, balance and endurance capacity (quantified by $\dot{V}O_{2max}$) are influenced by innate factors. When including the heritability estimates for these traits in meta-analyses, genetic factors explained most of the

variance in vertical jump (62%; *N* = 874), handgrip strength (63%; *N* = 4516) and flexibility (50%; *N* = 1130), $\dot{V}O_{2max}$ (in mL/min 59%; *N* = 1088 and in mL/min/kg 72%; *N* = 1004) in children, adolescents and young adults (age < 30y). However, in our sample we cannot currently determine the exact contribution of the two different components that make up the heritability of physical fitness: genetic factors that contribute to baseline (untrained) physical fitness levels and those related to the extent of the training-induced gains in physical fitness (i.e. genetic factors contributing to 'trainability').

Substantial individual differences exist in trainability, i.e. individuals differ to a great extent in their response to a standardized training protocol. This is in part due to genetic variation. Bouchard demonstrated this effect in families in the HERITAGE Study (Bouchard et al., 1995), by submitting more than 200 families to a 20 week exercise program. Large individual differences in trainability were seen for several performance phenotypes; the training-induced changes in $\dot{V}O_{2max}$, several skeletal muscle phenotypes, resting heart rate, resting blood pressure, and other risk markers for cardiovascular diseases could for a large part be explained by genetics (An et al., 2003; Bouchard et al., 1999; Hong et al., 2000; Perusse et al., 2000; Rice et al., 2002; Rico-Sanz et al., 2003). In our study we could not separately compute heritability for basal ability and trainability as our study used a mixture of sedentary subjects and moderately and vigorous exercisers. Physical fitness in the former will mostly reflect genetics of baseline exercise ability whereas fitness in the two latter groups will reflect a mixture of the genetics of baseline exercise ability and trainability.

So far, identifying the genes involved in either basal exercise ability or trainability has proven difficult (Pitsiladis et al., 2016). Candidate genes studies of muscle strength have focused on insulin-like growth factor- and myostatin-related genes and genes involved in inflammatory factors. Linkage analyses revealed several additional regions of interest in the genome, although individual genes could not be identified as yet (see Thomis and Aerssens, 2012 for a review). One of the most studied polymorphisms is the R577X variation in the *ACTN3* gene. This gene seems to influence the performance of fast skeletal muscle fibers and *ACTN3* XX homozygotes may have modestly lower skeletal muscle strength in comparison with R-allele carriers (Yang et al., 2003). For maximal oxygen uptake, only one GWA study has been conducted to date by Bouchard et al. (2011b). Strikingly, in spite of the small sample size, this study revealed that 16 SNPs accounted for 45% of the variance in gains in $\dot{V}O_{2max}$ after

exposure to a standardized 20-weeks exercise program in a sample of 473 sedentary adults (Bouchard et al., 2011b). No GWA studies have yet been performed on $\dot{V}O_{2max}$ in the untrained or baseline state (before training). It is challenging to collect enough data to be well-powered for gene finding studies as measuring maximal oxygen uptake involves laboratory equipment and a significant amount of time (especially in training studies). This might explain the lack of (well-replicated) findings in this field.

Exercise ability should not only be defined in terms of peak performance capacity but also in terms of being able to withstand potential injuries. A downside of being a fervent exerciser is the increased risk of sports injuries. Of importance is the nature of the injury and the duration of the treatment and discomfort (pain) as well as lost sporting time, school, and working time, and healthcare costs. Environmental risk factors for sports injuries are for example training using incorrect or below-standard sportswear, unmatched opponents in competitive exercise activities, and nutrition (Shanmugam & Maffulli, 2008). However, there is growing evidence that genetic factors are implicated in the susceptibility for sports injuries (Collins & Raleigh, 2009). Especially the *COL1A1* gene, that encodes for the protein type 1 collagen, which is a major component of tendons and ligaments. A SNP upstream of this gene is associated with a decreased risk for acute soft tissue ruptures (Collins et al., 2010). Although the data on sports injuries was not included in this thesis, it might be an interesting addition to 'exercise ability' in the model when explaining the heritability of voluntary exercise behavior.

Expanding the model of De Geus & de Moor (2008)

In Chapter 8, we aimed to establish the prospective contribution of personality, perceived benefits and barriers, exercise-induced affective response, and subjective and objective exercise ability to the heritability of voluntary exercise behavior in a sample of adolescents and young adult twins. We hypothesized that the genetic factors contributing to these determinants correlate with the genetic factors that are responsible for the individual variation seen in exercise behavior in adolescents and young adults. We showed that eleven determinants have genetic overlap with exercise behavior. These determinants included the 'extraversion' dimension of personality, calmness measured by the Activation-Deactivation checklist after a submaximal exercise test, perceived benefits of exercise and perceived barriers to exercise, and subjective and objective exercise ability. When including these

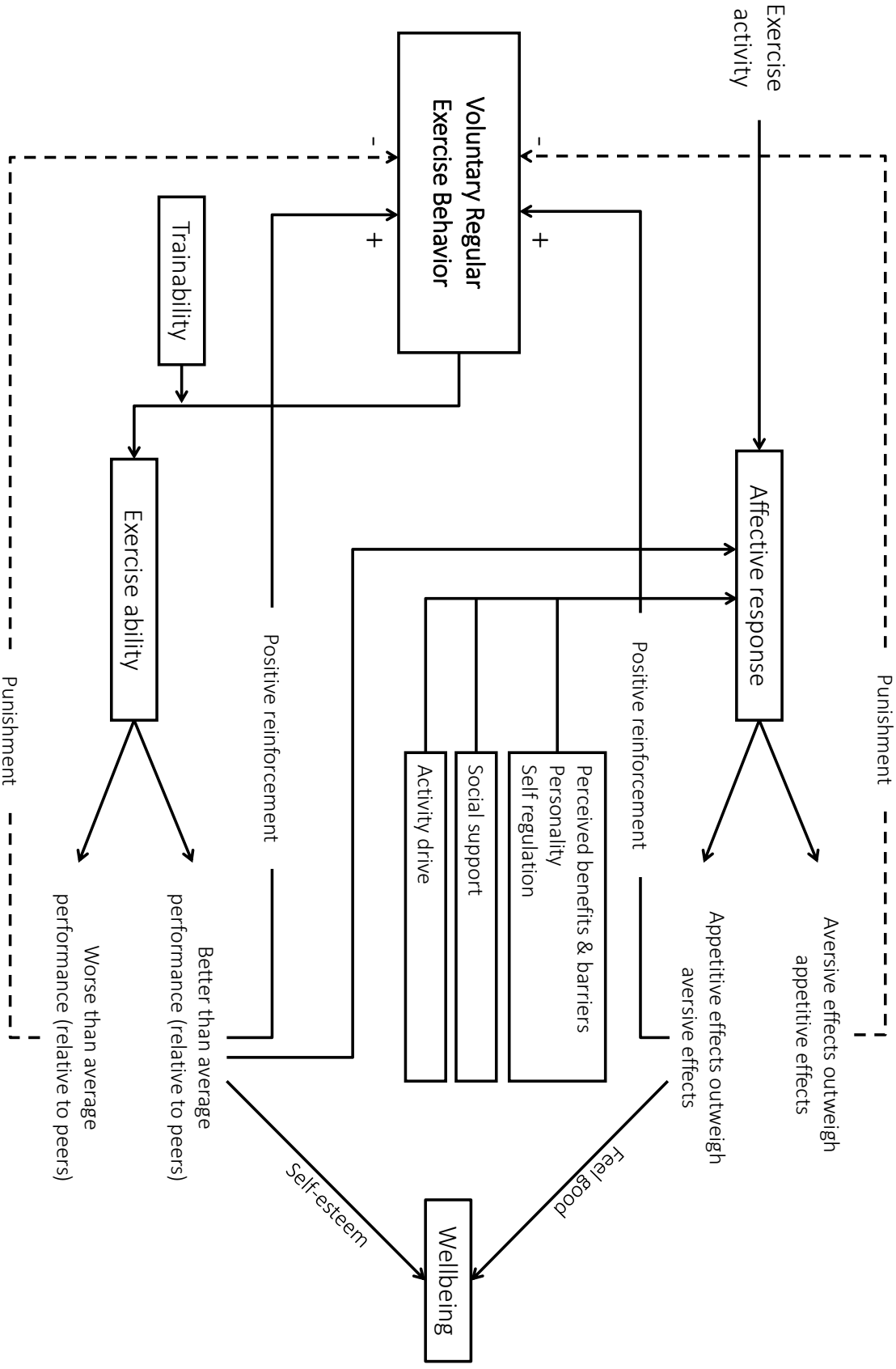


Figure 9.1 An updated model on the heritability of exercise behavior.

determinants in a covariance decomposition model, we showed that all of the covariance of these determinants with exercise behavior at follow-up is due to genetic factors.

These findings allow us to expand the model by De Geus & de Moor (2008). Figure 9.1 shows the expanded model based on the new work in this thesis. The upper part of the extended model consists of the core concept of instrumental conditioning (Hall, 1976) that remains central as it was in the original model. When people engage in regular exercise activities, they are exposed to a combination of acute (during the exercise bout and shortly after) aversive and appetitive effects. The net balance of these effects determines whether the activity will be experienced as rewarding or punishing, and this will strongly contribute to the adoption and maintenance of regular exercise behavior. We add six modulators of the affective response: personality, perceived benefits and barriers, self-regulation, social support, activity drive, and subjective exercise ability. A role for three of these, personality, perceived benefits and barriers and subjective exercise ability, were directly supported by the work in this thesis (Chapter 8).

Self-regulation is the ability to regulate one's emotions, thoughts, and behavior in the face of acute temptations and impulses. It is necessary for regulating one's behavior in order to achieve specific longer term goals (Baumeister et al., 2007). A large body of literature shows that self-regulation, or the related concepts of self-motivation and self-efficacy are correlates of regular exercise behavior (Dishman et al., 2005; MacAuley et al., 1998; Nigg, 2001). According to the model depicted in Figure 9.1, these concepts do so in part by influencing the perception of acute aversive effects. Being able to endure the temporary discomforts of exercise in view of a future reward (e.g. fitness, losing weight, winning the game) or long-term goal (health) is a core characteristic of self-regulation. In addition, the feeling of accomplishment of this self-discipline might tip the balance between aversive and appetite effects even during exercise. The concept of self-regulation is also argued to be shaped by genetic variants (Posner & Rothbart, 2009). Furthermore, including the innate ‘activity drive’ (Rowland, 1998) in the model, as the fulfillment of which is intrinsically rewarding, is based on a large literature of animal studies. Studies with spontaneous wheel-running inbred mice-strains and selective breeding in mice for high voluntary wheel-running activity resulted in numerous genomic regions that were associated with physical activity in mice (Kelly et al., 2010; Lightfoot et al., 2008; Nehrenberg et al., 2010). This suggests a biological mechanism

that propels the motivation to exercise. Finally, social support is a known factor to be of relevance in any behavioral intervention and exercise is no exception (Dishman et al., 1985; Sallis et al., 2000). As reviewed in Chapter 2, we find that in childhood common environmental factors that are shared by siblings of the same family play a much larger role in exercise behavior than genetic factors and this may largely reflect a positive effect of social support by parents and siblings. In part this effect can be purely instrumental; the parents need to enable the children to partake in sports and exercise activities by providing them sportswear, gear, and arranging transportation. However, family social support can also have a component of encouragement which can act to increase the acute appetitive effects of exercise for the child.

A new factor in the model is the importance of subjective exercise ability, measured as the relative ranking of one exercise performance and skills against peers. Although linked to actual exercise ability, it is possible that this subjective ability is even more important than objective ability in modulating the affective response to exercise. In Chapter 8 we demonstrate for the first time that subjective exercise ability, as expected from the underlying objective exercise ability, is a heritable trait (66%) and genetically associated with exercise behavior. Moreover, it may also explain the genetic association between exercise behavior and mental wellbeing that we reported in Chapter 7. People's beliefs about their capabilities to produce designated levels of exercise performance lead to feelings of competence and mastery. The subjective perception of exercise ability may therefore be an important determinant of exercise-induced increases in self-esteem. Together with the often reported ‘feel good’ in those exercisers that are characterized by a favorable balance of appetitive and aversive acute psychological effects, self-esteem can cause the increased wellbeing reported by exercisers.

Many potential and complex interactions between all components of the model have currently been left out. For instance, subjective exercise ability, although based on objective exercise ability, could be influenced by personality and social support. The latter may be particularly relevant in younger children who may find it easier to believe their parents about their performance, in spite of evidence to the contrary, based their actual performance. The biology of the activity drive may overlap with that of the extraverted personality and perceived barriers like ‘lack of time’ may be a function of self-regulatory capacities. While

appreciating these complexities, with the exception of social support, all elements in the model have been shown to be heritable. In keeping with the original model, they remain to be considered part of the intermediate pathways between the genomic level and the behavioral level, and meaningful explanatory variables for the high heritability of regular voluntary exercise behavior.

Remaining tasks

Throughout this thesis, we trustingly labeled the studied traits 'determinants', yet reverse causality cannot simply be ruled out. As previously discussed in relation to extraversion, genetic correlation may arise because the genetic variants influencing exercise behavior could become part of the heritability of the so-called determinant if regular exercise itself affects the determinant. Furthermore, a third scenario is that the same genetic variants independently influence the determinant and the tendency to become a regular exerciser. Mixtures of these three causal scenarios may be at play as well, e.g. there may be bidirectional causality in the presence of pleiotropy. Training studies could help resolve causality, but might suffer from selection bias, as they are typically conducted in sedentary individuals (regular exercisers would not show meaningful changes). Twin studies can resolve causality in unselected population-based samples if the sample size is sufficiently large to detect environmental correlations (de Moor et al., 2008), but might be a challenging undertaking for the relatively involved experimental protocol used here. Below 5000 twin pairs, the power to detect a significant environmental correlation between affective responses and exercise behavior is poor (Stubbe & de Geus, 2009). Mendelian Randomization would be a very good alternative strategy to resolve causality as this technique detects causal effects in an unbiased manner (Davey Smith & Hemani, 2014; Lawlor et al., 2008). Fortunately, for some of our determinants, global genome-wide association analyses initiatives exist and a robust (and replicated) set of genetic variants influencing our intended determinants will become available in time.

Future exploration of the genetic mechanisms underlying exercise behavior should also more prominently model possible gene-environment interplay. The effect of an environmental exposure on an individual may depend on his or her genotype. Vice versa the effects of a specific genetic variant may be dependent on the environment. The effect of genetic variants

can be amplified during or after being exposed to specific environmental factors. New previously 'dormant' genetic variants may become expressed due to exposure to environmental factors, whereas 'active' genetic variants may become suppressed by them. These (heritable) changes in gene expression are also known as epigenetics. Classic twin studies typically assume the gene-environment (GxE) interaction to be negligible, as the design (estimating A, C, and E) cannot discriminate between the main effects of genes and their interaction. When applying the classical twin model, interactions between genetic factors and the shared environment will result in an overestimation of the main effects of genes, whereas interactions between genetic factors and the unique environment will result in an underestimation of the main effects of genes. Fortunately, when (multiple) measures of environmental factors are collected GxE interaction terms can be included in heritability modelling, thereby improving the accuracy of the heritability estimate (Purcell, 2002). Gene-environment interaction can also be incorporated in candidate gene studies (Dick et al., 2015) and even in GWA studies (Thomas, 2010; Winham & Biernacka, 2013).

Implications for intervention on exercise behavior

To encourage adolescents and young adults to adopt a physically active lifestyle, the innate individual differences can be used as a starting point. Acknowledgement of the existence of heritable individual differences in the determinants of exercise behavior can suggest that it may be harder to engage some people in exercise than others, but that in no way means that we should stop trying. As opposed to general beliefs regarding the heritability of behavior, heritable traits can still be worthy targets for intervention (Plomin & Haworth, 2010). Many intervention studies for many traits have shown that genetic influences on the variance in a trait do not hamper attempts to favorably change the mean population level of the trait. Even if genetic factors are still a main cause of remaining variation around the increased post-intervention mean.

Understanding the genetic pathways that lead to differences in voluntary exercise behavior may help identify specific biological and psychological determinants that would be solid targets for intervention. Individuals may experience rather different 'gains' when exercising. Favorable genetic profiles may for instance cause a larger sensitivity to the rewarding or a smaller sensitivity to the punishing effects of broad classes of activities, including exercise. For

some individuals, exercising may be associated with a strong 'feel good' experience and constitute an excellent short-term coping strategy that helps to unwind more rapidly from daily pressures experienced in the school, job or home environment. For others, the aversive effects of exercise, at least in the forms that they tried so far, may greatly overwhelm the rewarding effects, may elicit feelings of punishment and cause the individual to drop-out. The latter individuals might benefit more from an individualized exercise intervention, in which the appetitive aspects for that specific individual should be emphasized and the aversive aspects reduced as much as possible. To optimize the appetitive aspects of exercising that are specific to that individual and generating realistic person-specific goals, different genotypes may require entirely different exercise programs.

Final remarks

The large individual differences in regular voluntary exercise behavior in late-adolescents and young adults are for a large part due to genetic factors. This thesis aimed to unravel the genetic components of this healthy behavior by studying its genetic association with known correlates and determinants. Increased understanding of the individual differences in voluntary exercise behavior is a necessary step to innovate and invigorate public health programs aimed at exercise behavior change. Focusing on the population *variation* and increasing the appetitive aspects of exercising that are specific to an individual by generating realistic person-specific goals will, in the end, increase overall exercise behavior in adolescents and young adults.

REFERENCES

Aadahl M, Kjaer M, Kristensen JH, Mollerup B, & Jorgensen T (2007). Self-reported physical activity compared with maximal oxygen uptake in adults. Eur. J. Cardiovasc. Prev. Rehabil. *14*, 422-428.

Aaltonen S, Kaprio J, Vuoksimaa E, Huppertz C, Kujala UM, & Silventoinen K (2016). Genetic architecture of motives for leisure-time physical activity: a twin study. Scand. J. Med. Sci. Sports.

Aaltonen S, Ortega-Alonso A, Kujala UM, & Kaprio J (2013). Genetic and environmental influences on longitudinal changes in leisure-time physical activity from adolescence to young adulthood. Twin. Res. Hum. Genet. *16*, 535-543.

Ainsworth BE, Haskell WL, Leon AS, Jacobs DR, Jr., Montoye HJ, Sallis JF, & Paffenbarger RS, Jr. (1993). Compendium of physical activities: classification of energy costs of human physical activities. Med. Sci. Sports Exerc. *25*, 71-80.

Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR, Jr., Schmitz KH, Emplaincourt PO, Jacobs DR, Jr., & Leon AS (2000). Compendium of physical activities: an update of activity codes and MET intensities. Med. Sci. Sports Exerc. *32*, S498-S504.

Allender S, Cowburn G, & Foster C (2006). Understanding participation in sport and physical activity among children and adults: a review of qualitative studies. Health Educ. Res. *21*, 826-835.

Ament W & Verkerke GJ (2009). Exercise and fatigue. Sports Med. *39*, 389-422.

An P, Perusse L, Rankinen T, Borecki IB, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Bouchard C, & Rao DC (2003). Familial aggregation of exercise heart rate and blood pressure in response to 20 weeks of endurance training: the HERITAGE family study. Int. J. Sports Med. *24*, 57-62.

Anderson E & Shivakumar G (2013). Effects of exercise and physical activity on anxiety. Front Psychiatry *4*, 27.

Åstrand P & Rhyming I (1960). A Nomogram for Calculation of Aerobic Capacity (Physical Fitness) From Pulse Rate During Submaximal Work. J. Appl. Physiol *7*, 218-221.

Baranowski T, Bouchard C, Bar-Or O, Bricker T, Heath G, Kimm SY, Malina R, Obarzanek E, Pate R, Strong WB, & . (1992). Assessment, prevalence, and cardiovascular benefits of physical activity and fitness in youth. Med. Sci. Sports Exerc. *24*, S237-S247.

Bartels M, van Weegen F, van Beijsterveldt C, Carlier M, Polderman T, Hoekstra R, & Boomsma D (2012a). The five factor model of personality and intelligence: A twin study on the relationship between the two constructs. Pers. Individ. Dif. *53*, 368-373.

Bartels M, de Moor MH, van der Aa N, Boomsma DI, & de Geus EJ (2012b). Regular exercise, subjective wellbeing, and internalizing problems in adolescence: causality or genetic pleiotropy? Front. Genet. *3*, 4.

Bartels M, Van den Berg M, Sluyter F, Boomsma DI, & de Geus EJ (2003). Heritability of cortisol levels: review and simultaneous analysis of twin studies. Psychoneuroendocrinology *28*, 121-137.

Baumeister R, Vohs K, & Tice D (2007). The strength model of self-control. Curr. Dir. Psychol. Sci. *16,* 351-355.

Beaulieu JM & Gainetdinov RR (2011). The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol. Rev. *63*, 182-217.

Becker M (1974). The health belief model and personal health behavior. Health Educ. Monogr. *2*, 324-508.

Bell DR, Guskiewicz KM, Clark MA, & Padua DA (2011). Systematic review of the balance error scoring system. Sports Health *3*, 287-295.

Bergstrom E, Hernell O, & Persson LA (1996). Cardiovascular risk indicators cluster in girls from families of low socio-economic status. Acta Paediatr. *85*, 1083-1090.

Beunen G & Thomis M (1999). Genetic determinants of sports participation and daily physical activity. Int. J. Obes. Relat Metab Disord. *23 Suppl 3*, S55-S63.

Bohannon R, Bubela D, Magasi S, & Gershon R (2011). Relative reliability of three objective tests of limb muscle strength. Isokinet. Exerc. Sci. *19*, 77-81.

Boker S, Neale M, Maes H, Wilde M, Spiegel M, Brick T, Spies J, Estabrook R, Kenny S, Bates T, Mehta P, & Fox J (2011a). OpenMx: An Open Source Extended Structural Equation Modeling Framework. Psychometrika *76*, 306-317.

Bonen A & Shaw SM (1995). Recreational exercise participation and aerobic fitness in men and women: analysis of data from a national survey. J. Sports Sci. *13*, 297-303.

Boomsma DI, de Geus EJ, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TC, Hudziak JJ, Bartels M, & Willemsen G (2006). Netherlands Twin Register: from twins to twin families. Twin. Res. Hum. Genet. *9*, 849-857.

Boomsma DI, van den Bree MB, Orlebeke JF, & Molenaar PC (1989). Resemblances of parents and twins in sports participation and heart rate. Behav. Genet. *19*, 123-141.

Borg G (1970). Perceived exertion as an indicator of somatic stress. Scand. J. Rehabil. Med. *2*, 92-98.

Bouchard C, An P, Rice T, Skinner JS, Wilmore JH, Gagnon J, Perusse L, Leon AS, & Rao DC (1999). Familial aggregation of VO(2max) response to exercise training: results from the HERITAGE Family Study. J. Appl. Physiol. *87*, 1003-1008.

Bouchard C, Daw EW, Rice T, Perusse L, Gagnon J, Province MA, Leon AS, Rao DC, Skinner JS, & Wilmore JH (1998). Familial resemblance for VO2max in the sedentary state: the HERITAGE family study. Med. Sci. Sports Exerc. *30*, 252-258.

Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, & Gagnon J (1995). The HERITAGE family study. Aims, design, and measurement protocol. Med. Sci. Sports Exerc. *27*, 721-729.

Bouchard C, Lesage R, Lortie G, Simoneau JA, Hamel P, Boulay MR, Perusse L, Theriault G, & Leblanc C (1986). Aerobic performance in brothers, dizygotic and monozygotic twins. Med. Sci. Sports Exerc. *18*, 639-646.

Bouchard C & Rankinen T (2001). Individual differences in response to regular physical activity. Med. Sci. Sports Exerc. *33*, S446-S451.

Bouchard C, Rankinen T, & Timmons JA (2011a). Genomics and genetics in the biology of adaptation to exercise. Compr. Physiol *1*, 1603-1648.

Bouchard C, Sarzynski MA, Rice TK, Kraus WE, Church TS, Sung YJ, Rao DC, & Rankinen T (2011b). Genomic predictors of the maximal O(2) uptake response to standardized exercise training programs. J. Appl. Physiol *110*, 1160-1170.

Bryan A, Hutchison KE, Seals DR, & Allen DL (2007). A transdisciplinary model integrating genetic, physiological, and psychological correlates of voluntary exercise. Health Psychol. *26*, 30-39.

Butte NF, Cai G, Cole SA, & Comuzzie AG (2006). Viva la Familia Study: genetic and environmental contributions to childhood obesity and its comorbidities in the Hispanic population. Am. J. Clin. Nutr. *84*, 646-654.

Cai G, Cole SA, Butte N, Bacino C, Diego V, Tan K, Goring HH, O'Rahilly S, Farooqi IS, & Comuzzie AG (2006). A quantitative trait locus on chromosome 18q for physical activity and dietary intake in Hispanic children. Obesity *14*, 1596-1604.

Caldwell Hooper AE, Bryan AD, & Hagger MS (2014). What keeps a body moving? The brain-derived neurotrophic factor val66met polymorphism and intrinsic motivation to exercise in humans. J. Behav. Med. *37*, 1180-1192.

Caspersen CJ, Powell KE, & Christenson GM (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep. *100*, 126-131.

Chatterjee S & Chowdhuri BJ (1991). Comparison of grip strength and isomeric endurance between the right and left hands of men and their relationship with age and other physical parameters. J. Hum. Ergol. *20*, 41-50.

Chatterjee S & Das N (1995). Physical and motor fitness in twins. Jpn. J. Physiol *45*, 519-534.

Chen CY & Bonham AC (2010). Postexercise hypotension: central mechanisms. Exerc. Sport Sci. Rev. *38*, 122-127.

Chinapaw MJ, Mokkink LB, van Poppel MN, van MW, & Terwee CB (2010). Physical activity questionnaires for youth: a systematic review of measurement properties. Sports Med. *40*, 539-563.

Church TS, Earnest CP, Skinner JS, & Blair SN (2007). Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. JAMA *297*, 2081-2091.

Cink RE & Thomas TR (1981). Validity of the Astrand-Ryhming nomogram for predicting maximal oxygen intake. Br. J. Sports Med. *15*, 182-185.

Claghorn GC, Fonseca IA, Thompson Z, Barber C, & Garland T, Jr. (2016). Serotonin-mediated central fatigue underlies increased endurance capacity in mice from lines selectively bred for high voluntary wheel running. Physiol Behav. *161*, 145-154.

Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, & Zondervan KT (2011). Basic statistical analysis in genetic case-control studies. Nat. Protoc. *6*, 121-133.

Cole SA, Butte NF, Voruganti VS, Cai G, Haack K, Kent JW, Jr., Blangero J, Comuzzie AG, McPherson JD, & Gibbs RA (2010). Evidence that multiple genetic variants of MC4R play a functional role in the regulation of energy expenditure and appetite in Hispanic children. Am. J. Clin. Nutr. *91*, 191-199.

Collins M, Posthumus M, & Schwellnus MP (2010). The COL1A1 gene and acute soft tissue ruptures. Br. J. Sports Med. *44*, 1063-1064.

Collins M & Raleigh SM (2009). Genetic risk factors for musculoskeletal soft tissue injuries. Med. Sport Sci. *54*, 136-149.

Courneya K & Hellsten L (1998). Personality correlates of exercise behavior, motives, barriers and preferences: an application of the five-factor model. Pers. Individ. Dif. *24,* 625-633.

Craft L & Landers D (1998). The effect of exercise on clinical depression and depression resulting from mental illness: a meta-analysis. J. Sport Exerc. Psychol. *20*, 339-357.

Davey Smith G & Hemani G (2014). Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum. Mol. Genet. *23*, R89-R98.

Davis JM & Bailey SP (1997). Possible mechanisms of central nervous system fatigue during exercise. Med. Sci. Sports Exerc. *29*, 45-57.

De Chaves RN, Baxter-Jones A, Santos D, Gomes TN, dos Santos FK, de Souza MC, Diego VP, & Maia J (2014). Clustering of body composition, blood pressure and physical activity in Portuguese families. Ann. Hum. Biol. *41*, 159-167.

De Geus EJ, Bartels M, Kaprio J, Lightfoot JT, & Thomis M (2014). Genetics of regular exercise and sedentary behaviors. Twin. Res. Hum. Genet. *17*, 262-271.

De Geus EJ, Boomsma DI, & Snieder H (2003). Genetic correlation of exercise with heart rate and respiratory sinus arrhythmia. Med. Sci. Sports Exerc. *35*, 1287-1295.

De Geus EJ, Willemsen GH, Klaver CH, & Van Doornen LJ (1995). Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. Biol. Psychol. *41*, 205-227.

De Geus EJC & de Moor MHM (2008). A genetic perspective on the association between exercise and mental health. Ment. Health Phys. Act. *1*, 53-61.

De Geus E & de Moor M (2011). Genes, exercise, and psychological factors. In: Genetic and molecular aspects of sport performance, C.Bouchard and E.Hoffman, eds. (Chichester: Blackwell Publishing), pp. 294-305.

De Mars G, Thomis MA, Windelinckx A, Van LM, Maes HH, Blimkie CJ, Claessens AL, Vlietinck R, & Beunen G (2007). Covariance of isometric and dynamic arm contractions: multivariate genetic analysis. Twin. Res. Hum. Genet. *10*, 180-190.

De Moor MH, Beem AL, Stubbe JH, Boomsma DI, & de Geus EJ (2006). Regular exercise, anxiety, depression and personality: a population-based study. Prev. Med. *42*, 273-279.

De Moor MH, Boomsma DI, Stubbe JH, Willemsen G, & de Geus EJ (2008). Testing causality in the association between regular exercise and symptoms of anxiety and depression. Arch. Gen. Psychiatry *65*, 897-905.

De Moor MH, Costa PT, Terracciano A, Krueger RF, de Geus EJ, Toshiko T, Penninx BW, Esko T, Madden PA, Derringer J, Amin N, Willemsen G, Hottenga JJ, Distel MA, Uda M, Sanna S, Spinhoven P, Hartman CA, Sullivan P, Realo A, Allik J, Heath AC, Pergadia ML, Agrawal A, Lin P, Grucza R, Nutile T, Ciullo M, Rujescu D, Giegling I, Konte B, Widen E, Cousminer DL, Eriksson JG, Palotie A, Peltonen L, Luciano M, Tenesa A, Davies G, Lopez LM, Hansell NK, Medland SE, Ferrucci L, Schlessinger D, Montgomery GW, Wright MJ, Aulchenko YS, Janssens AC, Oostra BA, Metspalu A, Abecasis GR, Deary IJ, Raikkonen K, Bierut LJ, Martin NG, van Duijn CM, & Boomsma DI (2012). Meta-analysis of genome-wide association studies for personality. Mol. Psychiatry *17*, 337-349.

De Moor MH, Liu YJ, Boomsma DI, Li J, Hamilton JJ, Hottenga JJ, Levy S, Liu XG, Pei YF, Posthuma D, Recker RR, Sullivan PF, Wang L, Willemsen G, Yan H, de Geus EJ, & Deng HW (2009). Genome-wide association study of exercise behavior in Dutch and American adults. Med. Sci. Sports Exerc. *41*, 1887-1895.

De Moor MH, Posthuma D, Hottenga JJ, Willemsen G, Boomsma DI, & de Geus EJ (2007). Genome-wide linkage scan for exercise participation in Dutch sibling pairs. Eur. J. Hum. Genet. *15*, 1252-1259.

De Moor MH, Willemsen G, Rebollo-Mesa I, Stubbe JH, de Geus EJ, & Boomsma DI (2011). Exercise participation in adolescents and their parents: evidence for genetic and generation specific environmental effects. Behav. Genet. *41*, 211-222.

Den Hoed M., Brage S, Zhao JH, Westgate K, Nessa A, Ekelund U, Spector TD, Wareham NJ, & Loos RJ (2013). Heritability of objectively assessed daily physical activity and sedentary behavior. Am. J. Clin. Nutr. *98*, 1317-1325.

Depue RA & Collins PF (1999). Neurobiology of the structure of personality: dopamine, facilitation of incentive motivation, and extraversion. Behav. Brain Sci. *22*, 491-517.

Dick DM, Agrawal A, Keller MC, Adkins A, Aliev F, Monroe S, Hewitt JK, Kendler KS, & Sher KJ (2015). Candidate gene-environment interaction research: reflections and recommendations. Perspect. Psychol. Sci. *10*, 37-59.

Dishman RK, Jackson AS, & Bray MS (2014). Self-regulation of exercise behavior in the TIGER study. Ann. Behav. Med. *48*, 80-91.

Dishman RK, McIver KL, Dowda M, Saunders RP, & Pate RR (2015). Motivation and Behavioral Regulation of Physical Activity in Middle School Students. Med. Sci. Sports Exerc. *47*, 1913-1921.

Dishman RK, Motl RW, Sallis JF, Dunn AL, Birnbaum AS, Welk GJ, Bedimo-Rung AL, Voorhees CC, & Jobe JB (2005). Self-management strategies mediate self-efficacy and physical activity. Am. J. Prev. Med. *29*, 10-18.

Dishman RK, Sallis JF, & Orenstein DR (1985). The determinants of physical activity and exercise. Public Health Rep. *100*, 158-171.

Dishman R (1990). Determinants of participation in physical activity. In: Exercise, fitness and health: a consensus of current knowledge, C.Bouchard, R.Shephard, T.Stephens, J.Sutton, and B.McPherson, eds. (Champaign, IL, USA: Human Kinetics), pp. 75-101.

Distel MA, Trull TJ, Willemsen G, Vink JM, Derom CA, Lynskey M, Martin NG, & Boomsma DI (2009). The five-factor model of personality and borderline personality disorder: a genetic analysis of comorbidity. Biol. Psychiatry *66*, 1131-1138.

Drenowatz C, Eisenmann JC, Pfeiffer KA, Welk G, Heelan K, Gentile D, & Walsh D (2010). Influence of socio-economic status on habitual physical activity and sedentary behavior in 8- to 11-year old children. BMC Public Health *10*, 214.

DuBois DL & Silverthorn N (2005). Natural mentoring relationships and adolescent health: evidence from a national study. Am. J. Public Health *95*, 518-524.

Duncan GE, Goldberg J, Noonan C, Moudon AV, Hurvitz P, & Buchwald D (2008). Unique environmental effects on physical activity participation: a twin study. PLoS. One. *3*, e2019.

Dunton GF & Vaughan E (2008). Anticipated affective consequences of physical activity adoption and maintenance. Health Psychol. *27*, 703-710.

Ekblom-Bak E, Bjorkman F, Hellenius ML, & Ekblom B (2014). A new submaximal cycle ergometer test for prediction of VO2max. Scand. J. Med. Sci. Sports *24*, 319-326.

Ekelund U, Luan J, Sherar LB, Esliger DW, Griew P, & Cooper A (2012). Moderate to vigorous physical activity and sedentary time and cardiometabolic risk factors in children and adolescents. JAMA *307*, 704-712.

Ekkekakis P (2003). Pleasure and displeasure from the body: Perspectives from exercise. Cognition and Emotion *17*, 213-239.

Ekkekakis P, Hargreaves E, & Parfitt G (2013). Envisioning the next fifty years of research on the exercise-affect relationship. Psychol. Sport Exerc. *14*, 751-758.

Ekkekakis P (2009). Let them roam free? Physiological and psychological evidence for the potential of self-selected exercise intensity in public health. Sports Med. *39*, 857-888.

Ekkekakis P, Hall EE, & Petruzzello SJ (2005). Variation and homogeneity in affective responses to physical activity of varying intensities: an alternative perspective on dose-response based on evolutionary considerations. J. Sports Sci. *23*, 477-500.

Ekkekakis P, Hall EE, & Petruzzello SJ (2008). The relationship between exercise intensity and affective responses demystified: to crack the 40-year-old nut, replace the 40-year-old nutcracker! Ann. Behav. Med. *35*, 136-149.

Ekkekakis P, Parfitt G, & Petruzzello SJ (2011). The pleasure and displeasure people feel when they exercise at different intensities: decennial update and progress towards a tripartite rationale for exercise intensity prescription. Sports Med. *41*, 641-671.

Elmquist JK, Maratos-Flier E, Saper CB, & Flier JS (1998). Unraveling the central nervous system pathways underlying responses to leptin. Nat. Neurosci. *1*, 445-450.

Eysenck HJ, Nias DKB, & Cox DN (1982). Sport and personality. In Advances in behavior research and therapy, S.Rachman and T.Wilson, eds. (Oxford: Pergamon Press), pp. 1-56.

Fagard R, Bielen E, & Amery A (1991). Heritability of aerobic power and anaerobic energy generation during exercise. J. Appl. Physiol *70*, 357-362.

Ferrari AJ, Charlson FJ, Norman RE, Patten SB, Freedman G, Murray CJ, Vos T, & Whiteford HA (2013). Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. PLoS. Med. *10*, e1001547.

Fisher A, van Jaarsveld CH, Llewellyn CH, & Wardle J (2010). Environmental influences on children's physical activity: quantitative estimates using a twin design. PLoS. One. *5*, e10110.

Flint J (2013). GWAS. Curr. Biol. *23*, R265-R266.

Franks PW, Ravussin E, Hanson RL, Harper IT, Allison DB, Knowler WC, Tataranni PA, & Salbe AD (2005). Habitual physical activity in children: the role of genes and the environment. Am. J. Clin. Nutr. *82*, 901-908.

Garcia-Artero E, Ortega FB, Ruiz JR, Mesa JL, Delgado M, Gonzalez-Gross M, Garcia-Fuentes M, Vicente-Rodriguez G, Gutierrez A, & Castillo MJ (2007). [Lipid and metabolic profiles in adolescents are affected more by physical fitness than physical activity (AVENA study)]. Rev. Esp. Cardiol. *60*, 581-588.

Gebel K, Ding D, Chey T, Stamatakis E, Brown WJ, & Bauman AE (2015). Effect of Moderate to Vigorous Physical Activity on All-Cause Mortality in Middle-aged and Older Australians. JAMA Intern. Med. *175*, 970-977.

Gleim GW & McHugh MP (1997). Flexibility and its effects on sports injury and performance. Sports Med. *24*, 289-299.

Golimbet VE, Alfimova MV, Gritsenko IK, & Ebstein RP (2007). Relationship between dopamine system genes and extraversion and novelty seeking. Neurosci. Behav. Physiol *37*, 601-606.

Grant S, Corbett K, Amjad AM, Wilson J, & Aitchison T (1995). A comparison of methods of predicting maximum oxygen uptake. Br. J. Sports Med. *29*, 147-152.

Gray JA & McNaughton N (1983). Comparison between the behavioural effects of septal and hippocampal lesions: a review. Neurosci. Biobehav. Rev. *7*, 119-188.

Gray JA & McNaughton N (2000). The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-hippocampal System. (Oxford: Oxford University Press).

Haase A, Steptoe A, Sallis JF, & Wardle J (2004). Leisure-time physical activity in university students from 23 countries: associations with health beliefs, risk awareness, and national economic development. Prev. Med. *39*, 182-190.

Hagger M, Chatzisarantis N, & Biddle S (2002). A meta-analytic review of the theories of reasoned action and planned behavior in physical activity: predictive validity and the contribution of additional variables. Journal of Sport and Exercise Psychology *24*, 3-32.

Hakanen M, Raitakari OT, Lehtimaki T, Peltonen N, Pahkala K, Sillanmaki L, Lagstrom H, Viikari J, Simell O, & Ronnemaa T (2009). FTO genotype is associated with body mass index after the age of seven years but not with energy intake or leisure-time physical activity. J. Clin. Endocrinol. Metab *94*, 1281-1287.

Hall EE, Ekkekakis P, & Petruzzello SJ (2002). The affective beneficence of vigorous exercise revisited. Br. J. Health Psychol. *7*, 47-66.

Hall J (1976). Classical conditioning and instrumental learning: A contemporary approach. (Philadelphia: Lippincott).

Hardy C & Rejeski W (1989). Not what, but how one feels: the measurement of affect during exercise. J. Sport. Exerc. Psychol. *11*, 304-317.

Heller RF, O'Connell DL, Roberts DC, Allen JR, Knapp JC, Steele PL, & Silove D (1988). Lifestyle factors in monozygotic and dizygotic twins. Genet. Epidemiol. *5*, 311-321.

Higgins JP & Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. Stat. Med. *21*, 1539-1558.

Hong Y, Rice T, Gagnon J, Perusse L, Province M, Bouchard C, Leon AS, Skinner JS, Wilmore JH, Rao DC, & Despres JP (2000). Familiality of triglyceride and LPL response to exercise training: the HERITAGE study. Med. Sci. Sports Exerc. *32*, 1438-1444.

Hoyt A, Rhodes R, Hausenblas H, & Giacobbi P (2009). Integrating five-factor model facet-level traits with the theory of planned behavior and exercise. Psychol. Sport Exerc. *10*, 565-572.

Hrysomallis C (2011). Balance ability and athletic performance. Sports Med. *41*, 221-232.

Hsu YC, Tsai SF, Yu L, Chuang JI, Wu FS, Jen CJ, & Kuo YM (2015). Long-term moderate exercise accelerates the recovery of stress-evoked cardiovascular responses. Stress. 1-27.

Huppertz C, Bartels M, de Zeeuw EL, van Beijsterveldt CE, Hudziak JJ, Willemsen G, Boomsma DI, & de Geus EJ (2016). Individual Differences in Exercise Behavior: Stability and Change in Genetic and Environmental Determinants From Age 7 to 18. Behav. Genet. *46*, 665-679.

Huppertz C, Bartels M, Groen-Blokhuis MM, Dolan CV, de Moor MH, Abdellaoui A, van Beijsterveldt CE, Ehli EA, Hottenga JJ, Willemsen G, Xiao X, Scheet P, Davies GE, Boomsma DI, Hudziak JJ, & de Geus EJ (2014a). The dopaminergic reward system and leisure time exercise behavior: a candidate allele study. Biomed. Res. Int. *2014*, 591717.

Huppertz C, Bartels M, Jansen IE, Boomsma DI, Willemsen G, de Moor MH, & de Geus EJ (2014b). A twin-sibling study on the relationship between exercise attitudes and exercise behavior. Behav. Genet. *44*, 45-55.

Huppertz C, Bartels M, van Beijsterveldt CE, Boomsma DI, Hudziak JJ, & de Geus EJ (2012). Effect of shared environmental factors on exercise behavior from age 7 to 12 years. Med. Sci. Sports Exerc. *44*, 2025-2032.

Janssen I & Leblanc AG (2010). Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. Int. J. Behav. Nutr. Phys. Act. *7*, 40-55.

Jette M (1979). A comparison between predicted VO2 max from the Astrand procedure and the Canadian Home Fitness Test. Can. J. Appl. Sport Sci. *4*, 214-218.

Josefsson T, Lindwall M, & Archer T (2014). Physical exercise intervention in depressive disorders: meta-analysis and systematic review. Scand. J. Med. Sci. Sports *24*, 259-272.

Jozkow P, Slowinska-Lisowska M, Laczmanski L, & Medras M (2013). DRD2 C313T and DRD4 48-bp VNTR polymorphisms and physical activity of healthy men in Lower Silesia, Poland (HALS study). Ann. Hum. Biol. *40*, 186-190.

Kaprio J, Koskenvuo M, & Sarna S (1981). Cigarette smoking, use of alcohol, and leisure-time physical activity among same-sexed adult male twins. Prog. Clin. Biol. Res. *69 Pt C*, 37-46.

Karoly HC, Stevens CJ, Magnan RE, Harlaar N, Hutchison KE, & Bryan AD (2012). Genetic Influences on Physiological and Subjective Responses to an Aerobic Exercise Session among Sedentary Adults. J. Cancer Epidemiol. *2012*, 540563.

Kasch F (1984). The validity of the Astrand and Sjöstrand submaximal tests. Phys. Sportsmed. *12*, 47-51.

Kelley AE & Berridge KC (2002). The neuroscience of natural rewards: relevance to addictive drugs. J. Neurosci. *22*, 3306-3311.

Kelly SA, Nehrenberg DL, Peirce JL, Hua K, Steffy BM, Wiltshire T, Pardo-Manuel d, V, Garland T, Jr., & Pomp D (2010). Genetic architecture of voluntary exercise in an advanced intercross line of mice. Physiol. Genomics *42*, 190-200.

Kenney W, Wilmore J, & Costill D (2012). Physiology of Sport and Exercise. (Champaign, IL, USA: Human Kinetics).

Kirby KN & Marakovic NN (1996). Delay-discounting probabilistic rewards: Rates decrease as amounts increase. Psychon. Bull. Rev. *3*, 100-104.

Kirby KN, Petry NM & Bickel WK (1999). Heroin addicts have higher discount rates for delayed rewards than non-drug-using controls. J Exp Psychol Gen *128*, 78-87.

Klein RJ (2007). Power analysis for genome-wide association studies. BMC. Genet. *8*, 58.

Klissouras V (1971). Heritability of adaptive variation. J. Appl. Physiol *31*, 338-344.

Klissouras V, Pirnay F, & Petit JM (1973). Adaptation to maximal effort: genetics and age. J. Appl. Physiol *35*, 288-293.

Knab AM & Lightfoot JT (2010). Does the difference between physically active and couch potato lie in the dopamine system? Int. J. Biol. Sci. *6*, 133-150.

Koopmans J, van Doornen L, & Boomsma D (1994). Smoking and sports participation. In Genetic Factors in Coronary Heart Disease, U.Goldbourt, U.de Faire, and K.Berg, eds. (Dordrecht, The Netherlands: Springer Science+Business Media), pp. 217-235.

Kovar R (1976). Genetic analysis of motor performance. J. Sports Med. Phys. Fitness *16*, 205-208.

Krogh J, Nordentoft M, Sterne JA, & Lawlor DA (2011). The effect of exercise in clinically depressed adults: systematic review and meta-analysis of randomized controlled trials. J. Clin. Psychiatry *72*, 529-538.

Kwan BM & Bryan A (2010). In-task and post-task affective response to exercise: translating exercise intentions into behaviour. Br. J. Health Psychol. *15*, 115-131.

Lawlor DA, Harbord RM, Sterne JA, Timpson N, & Davey SG (2008). Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat. Med. *27*, 1133-1163.

Lawlor DA & Hopker SW (2001). The effectiveness of exercise as an intervention in the management of depression: systematic review and meta-regression analysis of randomised controlled trials. BMJ *322*, 763-767.

Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, & Katzmarzyk PT (2012). Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. Lancet *380*, 219-229.

Legge BJ & Banister EW (1986). The Astrand-Ryhming nomogram revisited. J. Appl. Physiol *61*, 1203-1209.

Lerman I, Harrison BC, Freeman K, Hewett TE, Allen DL, Robbins J, & Leinwand LA (2002). Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains. J. Appl. Physiol *92*, 2245-2255.

Lesage R, Simoneau JA, Jobin J, Leblanc J, & Bouchard C (1985). Familial resemblance in maximal heart rate, blood lactate and aerobic power. Hum. Hered. *35*, 182-189.

Li MD, Cheng R, Ma JZ, & Swan GE (2003). A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. Addiction *98*, 23-31.

Liang KY & Beaty TH (2000). Statistical designs for familial aggregation. Stat. Methods Med. Res. *9*, 543-562.

Lightfoot JT, Turner MJ, Daves M, Vordermark A, & Kleeberger SR (2004). Genetic influence on daily wheel running activity level. Physiol Genomics *19*, 270-276.

Lightfoot JT, Turner MJ, Pomp D, Kleeberger SR, & Leamy LJ (2008). Quantitative trait loci for physical activity traits in mice. Physiol. Genomics *32*, 401-408.

Liu G, Zhu H, Lagou V, Gutin B, Stallmann-Jorgensen IS, Treiber FA, Dong Y, & Snieder H (2010). FTO variant rs9939609 is associated with body mass index and waist circumference, but not with energy intake or physical activity in European- and African-American youth. BMC. Med. Genet. *11*, 57.

Lorentzon M, Lorentzon R, Lerner UH, & Nordstrom P (2001). Calcium sensing receptor gene polymorphism, circulating calcium concentrations and bone mineral density in healthy adolescent girls. Eur. J. Endocrinol. *144*, 257-261.

Lortie G, Bouchard C, Leblanc C, Tremblay A, Simoneau JA, Theriault G, & Savoie JP (1982). Familial similarity in aerobic power. Hum. Biol. *54*, 801-812.

MacArthur DG & North KN (2011). The ACTN3 Gene and Human Performance. In Genetic and Molecular Aspects of Sport Performance, C.Bouchard and E.Hoffman, eds. (West Sussex, UK: Wiley-Blackwell), pp. 204-214.

MacAuley D, Mc Crum EE, Stott G, Evans AE, Gamble RP, Mc RB, Boreham CA, Trinick TR, & Sweeney K (1998). Levels of physical activity, physical fitness and their relationship in the Northern Ireland Health and Activity Survey. Int. J. Sports Med. *19*, 503-511.

Maes HH, Beunen GP, Vlietinck RF, Neale MC, Thomis M, Vanden Eynde B, Lysens R, Simons J, Derom C, & Derom R (1996). Inheritance of physical fitness in 10-yr-old twins and their parents. Med. Sci. Sports Exerc. *28*, 1479-1491.

Maia JA, Thomis M, & Beunen G (2002). Genetic factors in physical activity levels: a twin study. Am. J. Prev. Med. *23*, 87-91.

Martinez-Gonzalez MA, Martinez JA, Hu FB, Gibney MJ, & Kearney J (1999). Physical inactivity, sedentary lifestyle and obesity in the European Union. Int. J. Obes. Relat Metab Disord. *23*, 1192-1201.

Martinez-Gonzalez MA, Varo JJ, Santos JL, De IJ, Gibney M, Kearney J, & Martinez JA (2001). Prevalence of physical activity during leisure time in the European Union. Med. Sci. Sports Exerc. *33*, 1142-1146.

Matson-Koffman DM, Brownstein JN, Neiner JA, & Greaney ML (2005). A site-specific literature review of policy and environmental interventions that promote physical activity and nutrition for cardiovascular health: what works? Am. J. Health Promot. *19*, 167-193.

Mays RJ, Boer NF, Mealey LM, Kim KH, & Goss FL (2010). A comparison of practical assessment methods to determine treadmill, cycle, and elliptical ergometer VO2 peak. J. Strength. Cond. Res. *24*, 1325-1331.

McArdle WD & Katch FI (2009). Exercise Physiology. Nutricion, Energy, and Human Performance. (Philadelphia, USA: Lippincott Williams & Wilkins).

McAuley E & Blissmer B (2000). Self-efficacy determinants and consequences of physical activity. Exerc. Sport Sci. Rev. *28*, 85-88.

McCaffery JM, Papandonatos GD, Bond DS, Lyons MJ, & Wing RR (2009). Gene X environment interaction of vigorous exercise and body mass index among male Vietnam-era twins. Am. J. Clin. Nutr. *89*, 1011-1018.

Montoye HJ & Gayle R (1978). Familial relationships in maximal oxygen uptake. Hum. Biol. *50*, 241-249.

Mustelin L, Joutsi J, Latvala A, Pietilainen KH, Rissanen A, & Kaprio J (2012). Genetic influences on physical activity in young adults: a twin study. Med. Sci. Sports Exerc. *44*, 1293-1301.

Mustelin L, Latvala A, Pietilainen KH, Piirila P, Sovijarvi AR, Kujala UM, Rissanen A, & Kaprio J (2011). Associations between sports participation, cardiorespiratory fitness, and adiposity in young adult twins. J. Appl. Physiol. *110*, 681-686.

Neale M & Cardon L (1992). Methodology for Genetic Studies of Twins and Families. (Dordrecht, The Netherlands: Kluwer Academic Publishers).

Nehrenberg DL, Wang S, Hannon RM, Garland T, Jr., & Pomp D (2010). QTL underlying voluntary exercise in mice: interactions with the "mini muscle" locus and sex. J. Hered. *101*, 42-53.

Neyeloff JL, Fuchs SC, & Moreira LB (2012). Meta-analyses and Forest plots using a microsoft excel spreadsheet: step-by-step guide focusing on descriptive data analysis. BMC. Res. Notes *5*, 52.

Nigg CR (2001). Explaining adolescent exercise behavior change: a longitudinal application of the transtheoretical model. Ann. Behav. Med. *23*, 11-20.

Okuda E, Horii D, & Kano T (2005). Genetic and environmental effects on physical fitness and motor performance. IJSHS *3*, 1-9.

Ortega FB, Ruiz JR, Castillo MJ, & Sjostrom M (2008). Physical fitness in childhood and adolescence: a powerful marker of health. Int. J. Obes. *32*, 1-11.

Parfitt G, Rose EA, & Burgess WM (2006). The psychological and physiological responses of sedentary individuals to prescribed and preferred intensity exercise. Br. J. Health Psychol. *11*, 39-53.

Payne N, Jones F, & Harris PR (2005). The impact of job strain on the predictive validity of the theory of planned behaviour: an investigation of exercise and healthy eating. Br. J. Health Psychol. *10*, 115-131.

Payne VG & Morrow JR, Jr. (1993). Exercise and VO2 max in children: a meta-analysis. Res. Q. Exerc. Sport *64*, 305-313.

Perusse L, Rice T, Province MA, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, & Bouchard C (2000). Familial aggregation of amount and distribution of subcutaneous fat and their responses to exercise training in the HERITAGE family study. Obes. Res. *8*, 140-150.

Perusse L, Tremblay A, Leblanc C, & Bouchard C (1989). Genetic and environmental influences on level of habitual physical activity and exercise participation. Am. J. Epidemiol. *129*, 1012-1022.

Pitsiladis YP, Tanaka M, Eynon N, Bouchard C, North KN, Williams AG, Collins M, Moran CN, Britton SL, Fuku N, Ashley EA, Klissouras V, Lucia A, Ahmetov II, de GE, & Alsayrafi M (2016). Athlome Project Consortium: a concerted effort to discover genomic and other "omic" markers of athletic performance. Physiol. Genomics *48*, 183-190.

Plomin R & Haworth CM (2010). Genetics and Intervention Research. Perspect. Psychol. Sci. *5*, 557-563.

Polderman TJ, Benyamin B, de Leeuw CA, Sullivan PF, van BA, Visscher PM, & Posthuma D (2015). Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat. Genet. *47*, 702-709.

Posner MI & Rothbart MK (2009). Toward a physical basis of attention and self regulation. Phys. Life Rev. *6*, 103-120.

Posthuma D & Boomsma DI (2000). A note on the statistical power in extended twin designs. Behav. Genet. *30*, 147-158.

Purcell S (2002). Variance components models for gene-environment interaction in twin analysis. Twin. Res. *5*, 554-571.

Raichlen DA, Foster AD, Gerdeman GL, Seillier A, & Giuffrida A (2012). Wired to run: exercise-induced endocannabinoid signaling in humans and cursorial mammals with implications for the 'runner's high'. J. Exp. Biol. *215*, 1331-1336.

Reuter M & Hennig J (2005). Association of the functional catechol-O-methyltransferase VAL158MET polymorphism with the personality trait of extraversion. Neuroreport *16*, 1135-1138.

Rhodes JS, Gammie SC, & Garland T, Jr. (2005). Neurobiology of Mice Selected for High Voluntary Wheel-running Activity. Integr. Comp Biol. *45*, 438-455.

Rhodes RE, Fiala B, & Conner M (2009). A review and meta-analysis of affective judgments and physical activity in adult populations. Ann. Behav. Med. *38*, 180-204.

Rhodes RE & Kates A (2015). Can the Affective Response to Exercise Predict Future Motives and Physical Activity Behavior? A Systematic Review of Published Evidence. Ann. Behav. Med. *49*, 715-731.

Rhodes RE, Martin AD, Taunton JE, Rhodes EC, Donnelly M, & Elliot J (1999). Factors associated with exercise adherence among older adults. An individual perspective. Sports Med. *28*, 397-411.

Rhodes RE & Smith NE (2006). Personality correlates of physical activity: a review and meta-analysis. Br. J. Sports Med. *40*, 958-965.

Rice T, Despres JP, Perusse L, Hong Y, Province MA, Bergeron J, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Bouchard C, & Rao DC (2002). Familial aggregation of blood lipid response to exercise training in the health, risk factors, exercise training, and genetics (HERITAGE) Family Study. Circulation *105*, 1904-1908.

Richert L, Chevalley T, Manen D, Bonjour JP, Rizzoli R, & Ferrari S (2007). Bone mass in prepubertal boys is associated with a Gln223Arg amino acid substitution in the leptin receptor. J. Clin. Endocrinol. Metab *92*, 4380-4386.

Rico-Sanz J, Rankinen T, Joanisse DR, Leon AS, Skinner JS, Wilmore JH, Rao DC, & Bouchard C (2003). Familial resemblance for muscle phenotypes in the HERITAGE Family Study. Med. Sci. Sports Exerc. *35*, 1360-1366.

Rijsdijk FV & Sham PC (2002). Analytic approaches to twin data using structural equation models. Brief. Bioinform. *3*, 119-133.

Rowland TW (1996). Developmental Exercise Physiology. (Champaign, IL, USA: Human Kinetics).

Rowland TW (1998). The biological basis of physical activity. Med. Sci. Sports Exerc. *30*, 392-399.

Ruby MB, Dunn EW, Perrino A, Gillis R, & Viel S (2011). The invisible benefits of exercise. Health Psychol. *30*, 67-74.

Ruiz J, Ortega F, Gutierrez A, Meusel D, Sjöström M, & Castillo M (2006). Health-related fitness assessment in childhood and adolescence: a European approach based on the AVENA, EYHS and HELENA studies. Journal of Public Health *14*, 269-277.

Sallis JF, Prochaska JJ, & Taylor WC (2000). A review of correlates of physical activity of children and adolescents. Med. Sci. Sports Exerc. *32*, 963-975.

Samitz G, Egger M, & Zwahlen M (2011). Domains of physical activity and all-cause mortality: systematic review and dose-response meta-analysis of cohort studies. Int. J. Epidemiol. *40*, 1382-1400.

Sarzynski MA, Loos RJ, Lucia A, Perusse L, Roth SM, Wolfarth B, Rankinen T, & Bouchard C (2016). Advances in Exercise, Fitness, and Performance Genomics in 2015. Med. Sci. Sports Exerc. *48*, 1906-1916.

Saudino KJ & Zapfe JA (2008). Genetic influences on activity level in early childhood: do situations matter? Child Dev. *79*, 930-943.

Saul M, Majdak P, Perez S, Reilly M, Garland T, Jr., & Rhodes JS (2016). High motivation for exercise is associated with altered chromatin regulators of monoamine receptor gene expression in the striatum of selectively bred mice. Genes Brain Behav. *16(3),* 328-341.

Schneider M, Dunn A, & Cooper D (2009). Affective, exercise, and physical activity among healthy adolescents. J. Sport Exerc. Psychol. *31*, 706-723.

Schneider ML & Graham DJ (2009). Personality, physical fitness, and affective response to exercise among adolescents. Med. Sci. Sports Exerc. *41*, 947-955.

Schonbeck Y, Talma H, van DP, Bakker B, Buitendijk SE, Hirasing RA, & Van BS (2011). Increase in prevalence of overweight in Dutch children and adolescents: a comparison of nationwide growth studies in 1980, 1997 and 2009. PLoS. One. *6*, e27608.

Schutte NM, Bartels B, & de Geus EJC (2017a). Genetics of Physical Activity and Physical Fitness. In Oxford Textbook of Children's Exercise and Medicine, N.Armstrong and W.van Mechelen, eds. (Oxford: Oxford University Press).

Schutte N, Nederend I, Hudziak J, Bartels M, & de Geus E (2017b). Heritability of the affective response to exercise and its correlation to exercise behavior. Psychol. Sport Exerc.*31,* 139-148.

Schutte NM, Bartels M, & de Geus EJ (2014). Genetic modification of the effects of exercise behavior on mental health. Front. Psychiatry *5*, 64.

Schutte NM, Nederend I, Hudziak JJ, Bartels M, & de Geus EJ (2016a). Twin-sibling study and meta-analysis on the heritability of maximal oxygen consumption. Physiol. Genomics *48*, 210-219.

Schutte NM, Nederend I, Hudziak JJ, de Geus EJ, & Bartels M (2016b). Differences in Adolescent Physical Fitness: A Multivariate Approach and Meta-analysis. Behav. Genet. *46*, 217-227.

Seabra AF, Mendonca DM, Goring HH, Thomis MA, & Maia JA (2014). Genetic influences of sports participation in Portuguese families. Eur. J. Sport Sci. *14*, 510-517.

Shanmugam C & Maffulli N (2008). Sports injuries in children. Br. Med. Bull. *86*, 33-57.

Siconolfi SF, Cullinane EM, Carleton RA, & Thompson PD (1982). Assessing VO2max in epidemiologic studies: modification of the Astrand-Rhyming test. Med. Sci. Sports Exerc. *14*, 335-338.

Siconolfi SF, Lasater TM, Snow RC, & Carleton RA (1985). Self-reported physical activity compared with maximal oxygen uptake. Am. J. Epidemiol. *122*, 101-105.

Silventoinen K, Magnusson PK, Tynelius P, Kaprio J, & Rasmussen F (2008). Heritability of body size and muscle strength in young adulthood: a study of one million Swedish men. Genet. Epidemiol. *32*, 341-349.

Simonen RL, Rankinen T, Perusse L, Leon AS, Skinner JS, Wilmore JH, Rao DC, & Bouchard C (2003). A dopamine D2 receptor gene polymorphism and physical activity in two family studies. Physiol Behav. *78*, 751-757.

Simons J, Beunen G, Ostyn M, Renson R, Swalus P, Van Gerven D, & Willems E (1969). Construction d'une batterie de tests d'aptitude motrice pour garçons de 12à 19 ans, par la méthode de l'analyse factorielle. Kinanthropologie *1*, 323-362.

Skelton DA & Beyer N (2003). Exercise and injury prevention in older people. Scand. J. Med. Sci. Sports *13*, 77-85.

Skinner JS, Jaskolski A, Jaskolska A, Krasnoff J, Gagnon J, Leon AS, Rao DC, Wilmore JH, & Bouchard C (2001). Age, sex, race, initial fitness, and response to training: the HERITAGE Family Study. J. Appl. Physiol *90*, 1770-1776.

Skipworth JRA, Puhucheary ZA, Rawal J, & Montgomery HE (2011). The ACE Gene and Performance. In Genetic and Molecular Aspects of Sport Performance, C.Bouchard and E.Hoffman, eds. (West Sussex, UK: Wiley-Blackwell), pp. 195-203.

Smillie LD, Cooper AJ, Proitsi P, Powell JF, & Pickering AD (2010). Variation in DRD2 dopamine gene predicts Extraverted personality. Neurosci. Lett. *468*, 234-237.

Solberg G, Robstad B, Skjonsberg OH, & Borchsenius F (2005). Respiratory gas exchange indices for estimating the anaerobic threshold. J. Sports. Sci. Med. *4*, 29-36.

Solinas M, Yasar S, & Goldberg SR (2007). Endocannabinoid system involvement in brain reward processes related to drug abuse. Pharmacol. Res. *56*, 393-405.

Sparling PB, Giuffrida A, Piomelli D, Rosskopf L, & Dietrich A (2003). Exercise activates the endocannabinoid system. Neuroreport *14*, 2209-2211.

Speakman JR (2015). The 'Fat Mass and Obesity Related' (FTO) gene: Mechanisms of Impact on Obesity and Energy Balance. Curr. Obes. Rep. *4*, 73-91.

Stathopoulou G, Powers M, Berry A, Smits J, & Otto M (2006). Exercise intervenions for mental health: a quantitative and qualitative review. Clin. Psychol. Sci. Pract. *13*, 179-193.

Stubbe JH, Boomsma DI, & de Geus EJ (2005). Sports participation during adolescence: a shift from environmental to genetic factors. Med. Sci. Sports Exerc. *37*, 563-570.

Stubbe JH, Boomsma DI, Vink JM, Cornes BK, Martin NG, Skytthe A, Kyvik KO, Rose RJ, Kujala UM, Kaprio J, Harris JR, Pedersen NL, Hunkin J, Spector TD, & de Geus EJ (2006). Genetic influences on exercise participation in 37,051 twin pairs from seven countries. PLoS. One. *1*, e22.

Stubbe JH, de Moor MH, Boomsma DI, & de Geus EJ (2007). The association between exercise participation and well-being: a co-twin study. Prev. Med. *44*, 148-152.

Stubbe J & de Geus E (2009). Genetics of Exercise Behavior. In Handbook of Behavior Genetics, Y.-K.Kim, ed. (New York, NY, USA: Springer Science+Business Media), pp. 343-358.

Sundet J, Magnus P, & Tambs K (1994). The heritability of maximal aerobic power: a study of Norwegian twins. Scand. J. Med. Sci. Sports *4*, 181-185.

Swallow JG, Carter PA, & Garland T, Jr. (1998). Artificial selection for increased wheel-running behavior in house mice. Behav. Genet. *28*, 227-237.

Talbot LA, Metter EJ, & Fleg JL (2000). Leisure-time physical activities and their relationship to cardiorespiratory fitness in healthy men and women 18-95 years old. Med. Sci. Sports Exerc. *32*, 417-425.

Tanaka H, Monahan KD, & Seals DR (2001). Age-predicted maximal heart rate revisited. J. Am. Coll. Cardiol. *37*, 153-156.

Thayer RE (1986). Activation-Deactivation Adjective Check List: Current overview and structural analysis. Psychol. Rep. *58*, 607-614.

Thomas D (2010). Methods for investigating gene-environment interactions in candidate pathway and genome-wide association studies. Annu. Rev. Public Health *31*, 21-36.

Thomis MA & Aerssens J. (2012). Genetic variation in human muscle strength—opportunities for therapeutic interventions? Curr. Opin. Pharmacol *12*, 355-362.

Tiainen K, Sipila S, Alen M, Heikkinen E, Kaprio J, Koskenvuo M, Tolvanen A, Pajala S, & Rantanen T (2004). Heritability of maximal isometric muscle strength in older female twins. J. Appl. Physiol. *96*, 173-180.

Tremblay MS, LeBlanc AG, Kho ME, Saunders TJ, Larouche R, Colley RC, Goldfield G, & Connor GS (2011). Systematic review of sedentary behaviour and health indicators in school-aged children and youth. Int. J. Behav. Nutr. Phys. Act. *8*, 98.

Tresch MC (2007). A balanced view of motor control. Nat. Neurosci. *10*, 1227-1228.

Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, & McDowell M (2008). Physical activity in the United States measured by accelerometer. Med. Sci. Sports Exerc. *40*, 181-188.

Trost SG, Owen N, Bauman AE, Sallis JF, & Brown W (2002). Correlates of adults' participation in physical activity: review and update. Med. Sci. Sports Exerc. *34*, 1996-2001.

Van Beijsterveldt CE, Groen-Blokhuis M, Hottenga JJ, Franic S, Hudziak JJ, Lamb D, Huppertz C, de ZE, Nivard M, Schutte N, Swagerman S, Glasner T, van FM, Brouwer C, Stroet T, Nowotny D, Ehli EA, Davies GE, Scheet P, Orlebeke JF, Kan KJ, Smit D, Dolan CV, Middeldorp CM, de Geus EJ, Bartels M, & Boomsma DI (2013). The Young Netherlands Twin Register (YNTR): longitudinal twin and family studies in over 70,000 children. Twin. Res. Hum. Genet. *16*, 252-267.

Van den Berg SM, de Moor MH, Verweij KJ, Krueger RF, Luciano M, Arias VA, Matteson LK, Derringer J, Esko T, Amin N, Gordon SD, Hansell NK, Hart AB, Seppala I, Huffman JE, Konte B, Lahti J, Lee M, Miller M, Nutile T, Tanaka T, Teumer A, Viktorin A, Wedenoja J, Abdellaoui A, Abecasis GR, Adkins DE, Agrawal A, Allik J, Appel K, Bigdeli TB, Busonero F, Campbell H, Costa PT, Smith GD, Davies G, de WH, Ding J, Engelhardt BE, Eriksson JG, Fedko IO, Ferrucci L, Franke B, Giegling I, Grucza R, Hartmann AM, Heath AC, Heinonen K, Henders AK, Homuth G, Hottenga JJ, Iacono WG, Janzing J, Jokela M, Karlsson R, Kemp JP, Kirkpatrick MG, Latvala A, Lehtimaki T, Liewald DC, Madden PA, Magri C, Magnusson PK, Marten J, Maschio A, Mbarek H, Medland SE, Mihailov E, Milaneschi Y, Montgomery GW, Nauck M, Nivard MG, Ouwens KG, Palotie A, Pettersson E, Polasek O, Qian Y, Pulkki-Raback L, Raitakari OT, Realo A, Rose RJ, Ruggiero D, Schmidt CO, Slutske WS, Sorice R, Starr JM, St PB, Sutin AR, Timpson NJ, Trochet H, Vermeulen S, Vuoksimaa E, Widen E, Wouda J, Wright MJ, Zgaga L, Porteous D, Minelli A, Palmer AA, Rujescu D, Ciullo M, Hayward C, Rudan I, Metspalu A, Kaprio J, Deary IJ, Raikkonen K, Wilson JF, Keltikangas-Jarvinen L, Bierut LJ, Hettema JM, Grabe HJ, Penninx BW, van Duijn CM, Evans DM, Schlessinger D, Pedersen NL, Terracciano A, McGue M, Martin NG, & Boomsma DI (2016). Meta-analysis of Genome-Wide Association Studies for Extraversion: Findings from the Genetics of Personality Consortium. Behav. Genet. *46*, 170-182.

Van der Aa N, de Geus EJ, van Beijsterveldt TC, Boomsma DI, & Bartels M (2010). Genetic Influences on Individual Differences in Exercise Behavior during Adolescence. Int. J. Pediatr. *2010*, 138345.

Van Landluyt L, Ekkekakis P, Hall E, & Petruzzello S (2000). Throwing mountains into the lakes: On the perils of nomothetic conceptions of the exercise-affect relationship. J. Sport Exerc. Psychol. *22*, 208-234.

Vandenberg SG (1962). The Hereditary Abilities Study: Hereditary Components in a Psychological Test Battery. Am. J. Hum. Genet. *14*, 220-237.

Varo JJ, Martinez-Gonzalez MA, De Irala-Estevez J, Kearney J, Gibney M, & Martinez JA (2003). Distribution and determinants of sedentary lifestyles in the European Union. Int. J. Epidemiol. *32*, 138-146.

Vartanian LR & Shaprow JG (2008). Effects of weight stigma on exercise motivation and behavior: a preliminary investigation among college-aged females. J. Health Psychol. *13*, 131-138.

Venerando A & Milani-Comparetti M (1970). Twin studies in sport and physical performance. Acta Genet. Med. Gemellol. *19*, 80-82.

Visscher PM, Brown MA, McCarthy MI, & Yang J (2012). Five years of GWAS discovery. Am. J. Hum. Genet. *90*, 7-24.

Warburton DE, Nicol CW, & Bredin SS (2006). Health benefits of physical activity: the evidence. CMAJ. *174*, 801-809.

Wedderkopp N, Froberg K, Hansen H, Riddoch C, & Andersen L (2003). Cardiovascular Risk Factors Cluster in Children and Adolescents With Low Physical Fitness: The European Youth Heart Study (EYHS). Pediatr. Exerc. Sci. *15*, 419-429.

Welch A, Hulley A, Ferguson C, & Beauchamp M (2007). Affective responses of inactive women to a maximal incremental execise test: A test of the dual-mode model. Psychol. Sport Exerc. *8*, 401-423.

White E, Slane JD, Klump KL, Burt SA, & Pivarnik J (2014). Sex differences in genetic and environmental influences on percent body fatness and physical activity. J. Phys. Act. Health *11*, 1187-1193.

Wilkinson AV, Gabriel KP, Wang J, Bondy ML, Dong Q, Wu X, Shete S, & Spitz MR (2013). Sensation-seeking genes and physical activity in youth. Genes Brain Behav. *12*, 181-188.

Willemsen G, Vink JM, Abdellaoui A, den BA, van Beek JH, Draisma HH, van DJ, van 't ED, Geels LM, van LR, Ligthart L, Kattenberg M, Mbarek H, de Moor MH, Neijts M, Pool R, Stroo N, Kluft C, Suchiman HE, Slagboom PE, de Geus EJ, & Boomsma DI (2013). The Adult Netherlands Twin Register: twenty-five years of survey and biological data collection. Twin. Res. Hum. Genet. *16*, 271-281.

Williams DM, Dunsiger S, Ciccolo JT, Lewis BA, Albrecht AE, & Marcus BH (2008). Acute Affective Response to a Moderate-intensity Exercise Stimulus Predicts Physical Activity Participation 6 and 12 Months Later. Psychol. Sport Exerc. *9*, 231-245.

Williams DM, Dunsiger S, Emerson JA, Gwaltney CJ, Monti PM, & Miranda R, Jr. (2016). Self-Paced Exercise, Affective Response, and Exercise Adherence: A Preliminary Investigation Using Ecological Momentary Assessment. J. Sport Exerc. Psychol. *38*, 282-291.

Williams DM, Dunsiger S, Jennings EG, & Marcus BH (2012). Does affective valence during and immediately following a 10-min walk predict concurrent and future physical activity? Ann. Behav. Med. *44*, 43-51.

Williams LR & Gross JB (1980). Heritability of motor skill. Acta Genet. Med. Gemellol. *29*, 127-136.

Wilmore JH, Green JS, Stanforth PR, Gagnon J, Rankinen T, Leon AS, Rao DC, Skinner JS, & Bouchard C (2001). Relationship of changes in maximal and submaximal aerobic fitness to changes in cardiovascular disease and non-insulin-dependent diabetes mellitus risk factors with endurance training: the HERITAGE Family Study. Metabolism *50*, 1255-1263.

Wilson K & Dishman R (2015). Personality and physical activity: A systematic review and meta-analysis. Pers. Individ. Dif. *72*, 230-242.

Winham SJ & Biernacka JM (2013). Gene-environment interactions in genome-wide association studies: current approaches and new directions. J. Child Psychol. Psychiatry *54*, 1120-1134.

Wood AC, Rijsdijk F, Saudino KJ, Asherson P, & Kuntsi J (2008). High heritability for a composite index of children's activity level measures. Behav. Genet. *38*, 266-276.

Yancey AK, Grant D, Kurosky S, Kravitz-Wirtz N, & Mistry R (2011). Role modeling, risk, and resilience in California adolescents. J. Adolesc. Health *48*, 36-43.

Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S, & North K (2003). ACTN3 genotype is associated with human elite athletic performance. Am. J. Hum. Genet. *73*, 627-631.

DUTCH SUMMARY

NEDERLANDSE SAMENVATTING

Het is niet zonder reden dat autoriteiten wereldwijd aandacht besteden aan programma's en interventies om mensen meer te laten bewegen. Sportactiviteiten van gemiddelde tot zware inspanning dragen bij aan de gezondheid. Desondanks zijn er nog steeds grote individuele verschillen in regelmatig sportgedrag. Studies in families en tweelingen hebben aangetoond dat sportgedrag een erfelijke eigenschap is. In hoofdstuk 2 laat ik in drie grote meta-analyses zien dat in een leeftijdsgroep met een gemiddelde leeftijd van 7 tot 12 jaar, de genen nog niet zo'n belangrijke rol spelen: maar 20% van de verschillen in sportgedrag wordt verklaard door genetische invloeden. Waarschijnlijk is hier de rol van ouders belangrijker: zij bieden hun kinderen de kans om te gaan sporten door hen te motiveren, door sport de voorkeur te geven boven andere vrijetijdsbestedingen, hen van en naar de sportfaciliteiten te brengen etc. In de vroege adolescentie (leeftijd 13 tot 15 jaar) gaan genen steeds meer een rol spelen (35%). In de leeftijd van 16 tot 18 jaar wordt meer dan helft van de verschillen in sportgedrag verklaard door genen.

Ondanks deze hoge erfelijkheid tijdens de late adolescentie zijn er tot nu toe nog weinig genetische varianten geïdentificeerd die deze erfelijkheid kunnen verklaren. Een model geïntroduceerd door de Geus & de Moor (2008) kan meer inzicht bieden in de erfelijke componenten van sportgedrag. Dit model gaat er vanuit dat de waarschijnlijkheid dat iemand regelmatig gaat sporten afhangt van genetische varianten die plezierige, positieve gevoelens tijdens en na het sporten versterken en die het goed kunnen presteren in sport beïnvloeden. Dit model voorziet in toetsbare hypothesen om meer te weten te komen over de oorsprong van de hoge erfelijkheid van sportgedrag tijdens de late adolescentie.

Hiertoe heb ik een onderzoek naar de individuele verschillen in sportgedrag opgezet en uitgevoerd, waaraan tweelingen tussen de 16 en de 18 jaar oud (en hun zusje of broertje) die stonden ingeschreven bij het Nederlands Tweelingen Register werden gevraagd deel te nemen. Het onderzoek bestond uit het afnemen van fitheidstesten, zoals spierkrachtmetingen en balans, een leefstijlinterview, twee submaximale inspanningstesten (op de fietsergometer en op de loopband), en een maximale inspanningstest. Bijna 500 personen namen deel aan het onderzoek. Het protocol staat uitgebreid beschreven in hoofdstuk 3. Tijdens de testen werden hartslag en zuurstofopname continu gemeten. Bijna 3 jaar later (op een gemiddelde leeftijd van ongeveer 20 jaar) werden zij nogmaals gevraagd aan te geven of en hoeveel zij op dat moment nog regelmatig sportten.

Eerdere studies lieten zien dat er een verband bestaat tussen positieve gevoelens tijdens het sporten en (de intentie om meer te gaan) sporten maanden later. Een hele hoge mate van inspanning, boven de lactaatdrempel, lokt voor iedereen gevoelens van misnoegen uit. Juist onder die drempel, waarbij het fysieke systeem nog steeds flink wordt uitgedaagd, is er een grote variabiliteit in hoe mensen zich voelen in reactie op het sporten. In hoofdstuk 4 worden de gevoelens tijdens en vlak na de submaximale inspanningstesten gemeten. De individuele verschillen in gevoel (gemeten met verschillende vragenlijsten) werden voor 12 tot 37% verklaard door genetische verschillen. Meer positieve gevoelens tijdens en meer gevoel van kalmte en energie na de inspanning waren geassocieerd met meer regelmatig sportgedrag.

Bekwaamheid in sport of het snel boeken van resultaten bij trainingen kunnen de balans tussen positief en negatief gevoel tijdens en na sporten beïnvloeden. Betere sportprestaties (dan anderen) dragen bij aan gevoelens van bekwaamheid, terwijl minder goede sportprestaties kunnen leiden tot schaamte of teleurstelling. Juist tijdens de adolescentie, waarin competitief gedrag een grote rol speelt, kan meer of minder bekwaamheid in sport leiden tot overheersing van een positieve of juiste negatieve gevoelens tijdens of vlak na het sporten. Hoofdstuk 5 en 6 laten zien dat de belangrijkste indicator van bekwaamheid voor sport, de fysieke fitheid, erg erfelijk is in de leeftijd van 16 tot 18 jaar. In een meta-analyse in kinderen, adolescenten en jongvolwassenen tot 30 jaar zien we dat meer dan de helft van de verschillen in elementen van fitheid wordt verklaard door genetische factoren: verticale sprongkracht 62%, handknijpkracht 63%, flexibiliteit 50%, maximale zuurstofopname 59-72%.

In hoofdstuk 8 kijk ik naar de voorspellende waarde van de gevoelens tijdens sporten en de fysieke fitheid op het toekomstig sportgedrag. Ons onderzoek laat zien dat verticale sprongkracht, flexibiliteit en vooral maximale zuurstofopname genetisch samenhangt met sportgedrag 3 jaar later. Ook subjectieve bekwaamheid (hoe goed je bent ten opzichte van leeftijdsgenoten) in sport hangt (genetisch) samen met het sportgedrag in de toekomst. Twee andere factoren die in eerder onderzoek van het Nederlands Tweelingen Register een belangrijke bijdrage bleken te leveren aan verschillen in sportgedrag, persoonlijkheid en sportattitudes, werden eveneens getest. Persoonlijkheid van een individu kan de balans tussen positief en negatief gevoel tijdens en na sporten kan beïnvloeden. Eerdere studies toonden al een verband aan tussen sportgedrag en lagere scores op neuroticisme en hogere scores op extraversie, zorgvuldigheid en openstaan voor nieuwe ervaringen. In hoofdstuk 8

zien we een erfelijk verband tussen extraversie tijdens de adolescentie en sportgedrag op latere leeftijd. In de literatuur wordt extraversie in verband gebracht met dopaminerge genen. Studies die de relatie tussen dopamine en sportgedrag in mensen onderzoeken laten wisselende resultaten zien, maar het dopaminerge systeem maakt onderdeel uit van een complex neurobiologisch netwerk, waarin ook het endocannabinoide systeem (welke al langer in verband wordt gebracht met sport) en serotonerge systeem samenwerken. Persoonlijkheid kan ook een rol spelen in bij sportattitudes, dat wil zeggen de voordelen van sport die mensen zien en de barrières die ze noemen die hun sportgedrag in de weg kunnen zitten. Voorbeelden van voordelen zijn fitter worden en interactie met vrienden/anderen. Als barrières worden tijdsgebrek, gebrek aan sociale ondersteuning, schaamte en futloosheid genoemd. Ook deze zelfervaren voordelen en barrières laten een substantiële erfelijkheid zien (30%-59%) en ze hangen (genetisch) samen met sportgedrag op latere leeftijd.

Al deze bevindingen maken het mogelijk het model van De Geus & de Moor uit te breiden, zoals weergegeven hoofdstuk 9 (Figuur 9.1). Centraal in het bovenste gedeelte van het model staat het principe van instrumentale conditionering. Wanneer mensen gaan sporten, worden ze blootgesteld aan een combinatie van positief en negatief gevoelens. De netto balans van deze effecten zal uiteindelijk de doorslag geven of de sportactiviteit wordt gezien als belonend of juist als ‘straf’ en dit draagt sterk bij aan de voorzetting of beëindiging van de regelmatige sportactiviteiten. Aan het model hebben we zes modulatoren van deze gevoelens toegevoegd: (subjectieve) bekwaamheid, persoonlijkheid, zelfervaren voordelen & barrières, zelfregulatie, sociale ondersteuning en intrinsieke behoefte tot bewegen. De rol van eerste drie worden bevestigd in dit proefschrift, de overige drie komen uit voorgaande studies maar behoeven nog nader onderzoek.

In dit proefschrift ga ik overwegend uit van een oorzakelijk verband tussen de componenten in het model en sportgedrag. Maar een omgekeerd causaal verband kunnen we niet zomer uitsluiten. Daarnaast kan een genetische samenhang ook wijzen op genetische pleiotropie, waarbij dezelfde genetische varianten onafhankelijk van elkaar twee verschillende eigenschappen beïnvloeden. Tweelingstudies kunnen een rol spelen in het oplossen van dit probleem, maar de steekproefgrootte moet daarvoor wel groot genoeg zijn. Een alternatieve strategie is Mendeliaanse randomisatie, waarbij gebruik wordt gemaakt van genetische varianten waarvan we weten welk effect ze hebben op een eigenschap (in dit geval

sportgedrag). Genetische varianten die geassocieerd met componenten uit het model zouden ook geassocieerd moeten zijn met sportgedrag, terwijl het omgekeerd niet waar hoeft te zijn. Mits de steekproef groot genoeg is (en er genoeg statistische power is) dan zou het uitblijven van deze genetische associatie een causaal effect falsificeren. Alhoewel het een uitdaging is om bij heel veel mensen zowel DNA als (objectieve) gegevens over de componenten in het model te verzamelen, zijn er voor sommige van de componenten (bijvoorbeeld voor extraversie) al resultaten van genoomwijde associatiestudies gepubliceerd en zullen betrouwbare sets van (gerepliceerde) genen spoedig beschikbaar zijn.

Het ontrafelen van de genetische mechanismen onderliggend aan sportgedrag draagt bij aan het identificeren van biologische en psychologische determinanten van sportgedrag, waar interventies op kunnen worden gericht. In een geïndividualiseerd sportprogramma kan de focus worden gelegd op het vergroten van positieve gevoelens en minimaliseren van negatieve gevoelens. Verschillende genotypen zullen verschillende sportprogramma's behoeven, zodat voor elk individu specifieke doelen kunnen worden gesteld. Het *focussen op individuele verschillen* en het vergroten van persoon-specifieke aantrekkelijke aspecten van sportgedrag zullen uiteindelijke bijdragen aan het verhogen van het gemiddelde sportgedag in adolescenten en jongvolwassenen.

APPENDICES

- Appendix A – Information Brochure
- Appendix B – Lifestyle interview
- Appendix C – Delay discounting questionnaire
- Appendix D – Feeling Scale
- Appendix E – Activation Deactivation Adjective Checklist
- Appendix F – RPE (Borg Scale)
- Appendix G – Example of a report for the subjects
- Appendix H – Instruction folder DNA collection
- Appendix I – Follow-up survey

gerelateerde eigenschappen en de reactie op lichamelijke inspanning. Afname van DNA gebeurt door een monduitstrijkje, waarbij een wattenstaafje langs de binnenkant van de wang wordt geschraapt. De uitslag van de zygositeitstest wordt na het onderzoek thuis gestuurd.

> PRIVACY

Alle gegevens die met dit onderzoek worden verzameld, worden vertrouwelijk behandeld. De gegevens worden geregistreerd en verwerkt onder een nummer en dus niet onder een naam of andere persoonlijke gegevens. Ook in publicaties zijn namen niet terug te vinden.

> PRAKTISCHE INFORMATIE

> Je bent geheel vrij in het al dan niet deelnemen aan het onderzoek. We adviseren je voldoende tijd te nemen om erover na te denken of je wilt meedoen. Je kunt altijd zonder opgaaf van redenen stoppen met deelname aan het onderzoek. Dit zal geen nadelige gevolgen voor je verdere relatie met het Nederlands Tweelingenregister of de VU.

> Mochten er tijdens het onderzoek bevindingen zijn, waarvoor medisch handelen noodzakelijk wordt gevonden, dan wordt je hiervan uiteraard op de hoogte gesteld.

> De gehele testprocedure op de VU duurt ongeveer twee-en-half uur en start om 10:00, 14:00 of 16:00.

> Voor iedereen die meedoet aan dit onderzoek is een verzekering afgesloten. Dit geldt voor schade door het onderzoek die tijdens het onderzoek of binnen vier jaar na het einde van het onderzoek naar boven komt. Je moet de schade ook binnen die vier jaar aan de verzekeraar hebben gemeld.

> De reiskosten die gemaakt zijn, zullen worden vergoed. Als dank voor je deelname krijg je een VVV bon van €10.

> Het is de bedoeling dat de tweeling (plus evt. broertjes/zusjes) op dezelfde dag langskomen om te worden getest.

> De belangrijkste resultaten van het onderzoek krijg je direct na afloop mee naar huis!

> Neem sportkleding mee! Na afloop kun je op de VU douchen.

> MEER INFORMATIE?

Het Nederlands Tweelingen Register heeft een website. Ga naar: www.tweelingenregister.org en lees meer over het NTR. Alle publicaties met de uitkomsten die het NTR onderzoek al heeft opgeleverd kun je hier vinden. Voor meer informatie over dit onderzoek kun je contact opnemen met de uitvoerend onderzoeker aan de VU: Nienke Schutte, Tel. 020-5988766, Email: ntr.sportonderzoek@vu.nl

Indien jij of je ouders er prijs op stelt informatie over dit onderzoek in te winnen bij een onafhankelijke arts die niet bij de uitvoering van het onderzoek betrokken is, dan is dr. Petra Zwijnenburg, klinisch geneticus in het VUmc, bereid jullie vragen te beantwoorden. Zij is te bereiken op 020-4440150 / 020-4443389.

Wij hopen je binnenkort te zien op de VU!

Informatie

Nederlands Tweelingen Register

FAMILIEONDERZOEK NAAR INDIVIDUELE VERSCHILLEN IN DE REACTIE OP LICHAMELIJKE INSPANNING

ALGEMENE INFORMATIE OVER TWEELINGONDERZOEK

Tweelingonderzoek is belangrijk voor medisch en wetenschappelijk onderzoek. Met onderzoek bij tweelingen kunnen wetenschappers erachter komen in hoeverre verschillen in gedrag of gezondheid worden beïnvloed door verschillen in erfelijke aanleg (de genen) of door leefomgeving.

Om goed onderzoek te kunnen doen, zijn vaak gegevens nodig van veel tweelingen. Daarom is in 1987 aan de Vrije Universiteit (VU) in Amsterdam het Nederlands Tweelingen Register (NTR) opgericht. Bij het NTR staan nu zo'n 110.000 jonge en volwassen tweelingen ingeschreven. De meeste gegevens van deze tweelingen worden verkregen met vragenlijsten die door tweelingen zelf, hun broers of zussen, door hun ouders of leerkracht worden ingevuld. Soms worden tweelingen en hun gezinsleden uitgenodigd om naar de VU te komen voor onderzoek. Sommige tweelingen doen al meer dan 10 jaar mee aan deze onderzoeken en zijn een aantal keren in Amsterdam geweest.

Verschillende wetenschappers maken gebruik van deze gegevens om de invloed van erfelijke aanleg en omgeving op de ontwikkeling van gedrag, leefgewoonten en de gezondheid te onderzoeken. De belangeloze medewerking van tweelingen en hun gezinsleden is hierbij van onschatbare waarde!

INFORMATIE OVER HET HUIDIGE ONDERZOEK

Met behulp van onderzoeksgegevens van het NTR hebben we kunnen vaststellen dat jongeren erg kunnen verschillen in de hoeveelheid sport en regelmatige lichaamsbeweging die ze doen. Omdat regelmatige lichaamsbeweging een belangrijke voorwaarde is voor een gezond gewicht en een goede gezondheid in de volwassenheid willen we graag beter begrijpen waarom sommige jongeren veel aan sport doen en anderen juist liever niet. De reacties op lichamelijke inspanning zoals die bijvoorbeeld optreden bij fietsen, hardlopen of sporten, lijken een grote rol te spelen. Deze reacties hangen mogelijk samen met erfelijke aanleg of juist met hoe goed je conditie is. In het huidige onderzoek gaan we op zoek naar de oorzaken van verschillen tussen personen in de reacties op lichamelijke inspanning.

> WIE DOEN ER MEE?

Voor het onderzoek worden 16 tot 20-jarige tweelingen en evt. hun broers en zussen uitgenodigd die staan ingeschreven bij het NTR. Je hoeft zelf niet aan sport te doen, iedereen kan deze testen gemakkelijk uitvoeren.

> HET ONDERZOEK OP DE VU

Jij en je broers of zussen worden op de afgesproken dag samen op de VU verwacht. Dit kan zijn om 10:00, 14:00 of 16:30. Na een korte rondleiding en uitleg begint het onderzoek dat 2,5 uur zal duren. Het onderzoek begint met een korte vragenlijst over je sportgedrag. Vervolgens testen we je balans, flexibiliteit, arm- en beenspierkracht. Daarna brengen we een aantal plakkers (elektroden) aan op je huid. Deze plakkers zijn verbonden met een apparaatje, waar gedurende het onderzoek jouw hartslag en ademhaling mee wordt gemeten. Tijdens dit experiment adem je in en uit door een masker waarmee jouw maximale zuurstofopname wordt gemeten.

Het sportonderzoek wordt gestart met een fietstest op een hometrainer waar je gedurende 20 minuten op een steeds zwaardere maar goed vol te houden belasting zal fietsen. Dan mag je even rusten, voor we verder gaan met een looptest op een loopband waar je gedurende 20 minuten op een steeds zwaardere, maar goed vol te houden belasting zal lopen/joggen. Dan mag je weer even rusten. Gedurende de inspanningstesten en de rust zullen we je regelmatig vragen om op een vragenlijst aan te geven wat je gevoel is bij de verschillende mate van lichamelijk inspanning. Het laatste onderdeel is een test van je uithoudingsvermogen. Deze test duurt 9 tot 12 minuten, waarbij je in de laatste minuten op je maximale kracht probeert te fietsen. Na de testen kun je op de VU douchen.

> VOORAFGAANDE AAN HET ONDERZOEK

Voor het onderzoek is het van essentieel belang dat we zeker weten of een tweeling eeneiig of twee-eiig is. DNA is de drager van ons erfelijk materiaal. Met behulp van het DNA kunnen we vaststellen of jullie een- of twee-eiige tweeling zijn. Het DNA kan ons ook helpen te begrijpen welke genetische varianten een rol spelen bij de individuele verschillen in sportgedrag, daaraan

APPENDIX B – LIFESTYLE INTERVIEW

Leefstijlinterview

Inventarisatie dag / beroep / school of studie

1. Wat voor dag is het?

- ☐ Vrije dag
- ☐ Werkdag (ook indien je huisvrouw / -man bent)
- ☐ Je hebt momenteel geen werk *(door naar 5)*
- ☐ Een schooldag *(door naar 5)*
- ☐ Anders, namelijk …….

Werk/beroep

2. Wat voor soort werk doe je momenteel?

- ☐ student/schoolgaand *(door naar 5)*
- ☐ fulltime betaald werk: meer dan 32 uur per week, namelijk ……. uur
- ☐ parttime betaald werk: 12-32 uur per week, namelijk ……. uur
- ☐ parttime betaald werk: minder dan 12 uur per week, namelijk ……. uur
- ☐ werkeloos, sinds ……. (jaartal) *(door naar 4)*
- ☐ huisman / huisvrouw, sinds ……. (jaartal) *(door naar 4)*
- ☐ arbeidsongeschikt ……. (jaartal) *(door naar 4)*
- ☐ anders, namelijk ……. *(door naar 4)*

3. Wat is jouw beroep? (Gedetailleerd weergeven, ook: leidinggevende functie of niet?)

……

……

4. Wat is jouw hoogst genoten opleiding welke je met een diploma hebt afgerond?

- ☐ Lager onderwijs
- ☐ Middelbaar onderwijs (mavo, lbo, vmbo)
- ☐ Hoger middelbaar onderwijs (havo, vwo, mbo)
- ☐ Hoger onderwijs (universiteit, hoger niet-universitair, hbo, BaMa)

☐ Anders, namelijk: ……..

School / studie

5. In welk jaar van de opleiding zit je momenteel?

☐ middelbare school afgerond

☐ ……. Jaar middelbaar onderwijs

Niveau:

☐ VMBO (basisberoepsgerichte leerweg - kaderberoepsgerichte leerweg - gemengde leerweg - theoretische leerweg)

☐ HAVO

☐ VWO (atheneum - gymnasium)

☐ middelbaar beroepsonderwijs (MBO)

☐ hoger beroepsonderwijs (HBO)

☐ universiteit of post-hbo onderwijs (WO)

☐ anders, namelijk: ……..

Ben je ooit blijven zitten? ……. Zo ja, in welke klas? ……. Klas van het basis / middelbaar onderwijs

Gezondheid

6. Hoe is in het algemeen jouw gezondheid?

☐ slecht

☐ matig

☐ redelijk *(door naar 8)*

☐ goed *(door naar 8)*

☐ uitstekend *(door naar 8)*

7. Kun je aangeven waarom je je matig / slecht gezond voelt?

……

……

Sport en beweging

8. Doe je regelmatig aan sport of lichaamsbeweging?

☐ Nee *(door naar 10)*

☐ Ja, alleen gymnastieklessen op school, namelijk …….uur *(door naar 10)*

☐ Ja, geen gymnastieklessen op school

☐ Ja, plus gymnastieklessen op school, namelijk …….uur

9a. Zo ja, welke sport(en) beoefen je?

Sport	Aantal jaren	Aantal maanden per jaar	Aantal keren per week	Aantal minuten per keer	Opmerkingen

9b. Heb je wel eens een blessure gehad?

☐ Ja, namelijk ……

☐ Nee

10. Hoeveel fiets je in een normale week? ……. uur en ……. minuten per week

11. Hoeveel wandel je in een normale week? ……. uur en ……. minuten per week

12. Heb je je gisteren fysiek ingespannen? (In tuin werken, sportwedstrijd, verhuizing)

☐ Ja, namelijk ……

☐ Nee

Roken

13. Heb je ooit gerookt?

- ☐ Nee *> sectie roken kan worden overgeslagen*
- ☐ Een paar keer om te proberen *> sectie roken kan worden overgeslagen*
- ☐ Ja

14. Hoeveel jaar rook of rookte je in totaal? ……. jaar

15. Hoe vaak rook je nu?

- ☐ Ik ben gestopt met roken sinds …….. (mm/jjjj)
- ☐ Ik rook 1 keer per week of minder
- ☐ Ik rook meerdere keren per week, niet elke dag
- ☐ Ik rook 1 of meerdere malen per dag

16. Hoeveel keer heb je serieus geprobeerd met roken te stoppen? ……. keer

17. Wat rook of rookte je?

- ☐ Sigaretten en/of shag, eventueel samen met sigaren, pijptabak etc.
- ☐ Uitsluitend sigaren of pijptabak *> sectie roken kan verder worden overgeslagen*
- ☐ Softdrugs (marihuana)

VOOR ROKERS EN EX-ROKERS VAN SIGARETTEN

18. Hoeveel sigaretten rook(te) je gemiddeld per dag? ……. sigaretten per dag

<u>Alcohol</u>

19. Hoeveel glazen alcohol drink je gemiddeld per week (inclusief weekend)?

- ☐ Minder dan 1 glas
- ☐ 1-5 glazen per week
- ☐ 6-10 glazen per week
- ☐ 11-15 glazen per week
- ☐ 16-20 glazen per week
- ☐ 21-40 glazen per week
- ☐ Meer dan 40 glazen per week

<u>Nachtrust</u>

20. Hoe laat ben je gisteravond naar bed gegaan? ……. uur ……. minuten

21. Hoe laat ben je vanochtend opgestaan? ……. uur ……. minuten

(totaal = ……. uur)

22. Slaap je normaal ook ongeveer zo lang?

☐ Ja

☐ Nee, normaal slaap ik ……. uur …….. minuten

Medicatie

23. Welke medicijnen gebruik je?

Merknaam	Substantienaam	Dosis / hoe vaak	Reden
……	……	……	……
……	……	……	……
……	……	……	……

ALLEEN VOOR VROUWEN

Menstruatie

24. Enkele vragen met betrekking tot de menstruele cyclus.

Is jouw menstruele cyclus regelmatig?

☐ Ja

☐ Nee onregelmatig

☐ Menopauze

☐ Anders, nl. ……

25. Wat is gemiddeld het aantal dagen tussen twee menstruaties? ……. dagen

26. Wat was de eerste dag van jouw laatste menstruatie? …… (dd/mm/jjjj)

27. Gebruik je anticonceptie?

☐ Ja, nl. …… (pil / spiraaltje / pessarium / injecties / etc.)

van het merk: ……..

☐ Nee

VOOR IEDEREEN

28. Als je jouw leven een cijfer moest geven, waarbij 10 betekent: het beste leven dat je je kunt voorstellen en de 1 het slechtste leven dat je je kunt voorstellen, welk cijfer zou dat dan zijn? ……

APPENDIX C – DELAY DISCOUNTING QUESTIONNAIRE

*In elke rij zie je twee opties: keuze A of keuze B. Kies de optie die jij het liefst zou willen hebben als dit **echte** keuzes zouden zijn. Dit is geen test met goede of foute antwoorden. Kies waar jij voor zou gaan als dit echte keuzes waren!*

A)	€ 19 vandaag	of	B)	€ 25 over 53 dagen
A)	€ 55 vandaag	of	B)	€ 75 over 61 dagen
A)	€ 54 vandaag	of	B)	€ 55 over 117 dagen
A)	€ 31 vandaag	of	B)	€ 85 over 7 dagen
A)	€ 14 vandaag	of	B)	€ 25 over 19 dagen
A)	€ 47 vandaag	of	B)	€ 50 over 160 dagen
A)	€ 15 vandaag	of	B)	€ 35 over 13 dagen
A)	€ 25 vandaag	of	B)	€ 60 over 14 dagen
A)	€ 78 vandaag	of	B)	€ 80 over 162 dagen
A)	€ 40 vandaag	of	B)	€ 55 over 62 dagen
A)	€ 11 vandaag	of	B)	€ 30 over 7 dagen
A)	€ 67 vandaag	of	B)	€ 75 over 119 dagen
A)	€ 34 vandaag	of	B)	€ 35 over 186 dagen
A)	€ 27 vandaag	of	B)	€ 50 over 21 dagen
A)	€ 69 vandaag	of	B)	€ 85 over 91 dagen
A)	€ 49 vandaag	of	B)	€ 60 over 89 dagen
A)	€ 80 vandaag	of	B)	€ 85 over 157 dagen
A)	€ 24 vandaag	of	B)	€ 35 over 29 dagen
A)	€ 33 vandaag	of	B)	€ 80 over 14 dagen
A)	€ 28 vandaag	of	B)	€ 30 over 179 dagen
A)	€ 34 vandaag	of	B)	€ 50 over 30 dagen
A)	€ 25 vandaag	of	B)	€ 30 over 80 dagen
A)	€ 41 vandaag	of	B)	€ 75 over 20 dagen
A)	€ 54 vandaag	of	B)	€ 60 over 111 dagen
A)	€ 54 vandaag	of	B)	€ 80 over 30 dagen
A)	€ 22 vandaag	of	B)	€ 25 over 136 dagen
A)	€ 20 vandaag	of	B)	€ 55 over 7 dagen

APPENDIX D – FEELING SCALE

Hoe voel jij je op dit moment?

+5	Erg goed
+4	
+3	Goed
+2	
+1	Beetje goed
0	Neutraal
-1	Beetje slecht
-2	
-3	Slecht
-4	
-5	Erg slecht

APPENDIX E – ACTIVATION DEACTIVATION ADJECTIVE CHECKLIST

De woorden hieronder beschrijven hoe jij je op dit moment voelt. Geef aan hoe jij je voelt

	Zeker	*Een beetje*	*Weet niet*	*Nee*	
Actief	++	+	?		Nee
Onbezorgd	++	+	?		Nee
Slaperig	++	+	?		Nee
Gejaagd	++	+	?		Nee
Energiek	++	+	?		Nee
Intens/Sterk	++	+	?		Nee
Kalm	++	+	?		Nee
Moe	++	+	?		Nee
Krachtig	++	+	?		Nee
In rust	++	+	?		Nee
Druilerig/Slaperig	++	+	?		Nee
Angstig	++	+	?		Nee
Levendig	++	+	?		Nee
Stil/Kalm	++	+	?		Nee
Uitgeslapen	++	+	?		Nee
Beklemd	++	+	?		Nee
Rustig	++	+	?		Nee
Vol van energie	++	+	?		Nee
Gespannen	++	+	?		Nee
Waakzaam	++	+	?		Nee

APPENDIX F – RPE (BORG'S RATING OF PERCEIVED EXERTION)

Geef aan hoe zwaar je de belasting vindt

6	
7	zeer zeer licht
8	
9	zeer licht
10	
11	tamelijk licht
12	
13	redelijk zwaar
14	
15	zwaar
16	
17	zeer zwaar
18	
19	zeer zeer zwaar
20	

APPENDIX G – EXAMPLE OF A REPORT FOR THE SUBJECTS

Nederlands Tweelingen Register

Resultaten Sportonderzoek

Naam:	xxx
Datum:	14 augustus 2013
Bloeddruk in rust:	112 / 65
Hartslag in rust:	75 hartslagen/min
Maximale spronghoogte:	43 cm
Flexibiliteit:	13.5 cm
Maximale handknijpkracht:	Rechts: 41.2 kg
	Links: 31.7 kg
Maximale zuurstofopname:	45.4 ml/min/kg
Maximaal gefietste wattage:	250 W
Maximale hartslag:	197 hartslagen/min

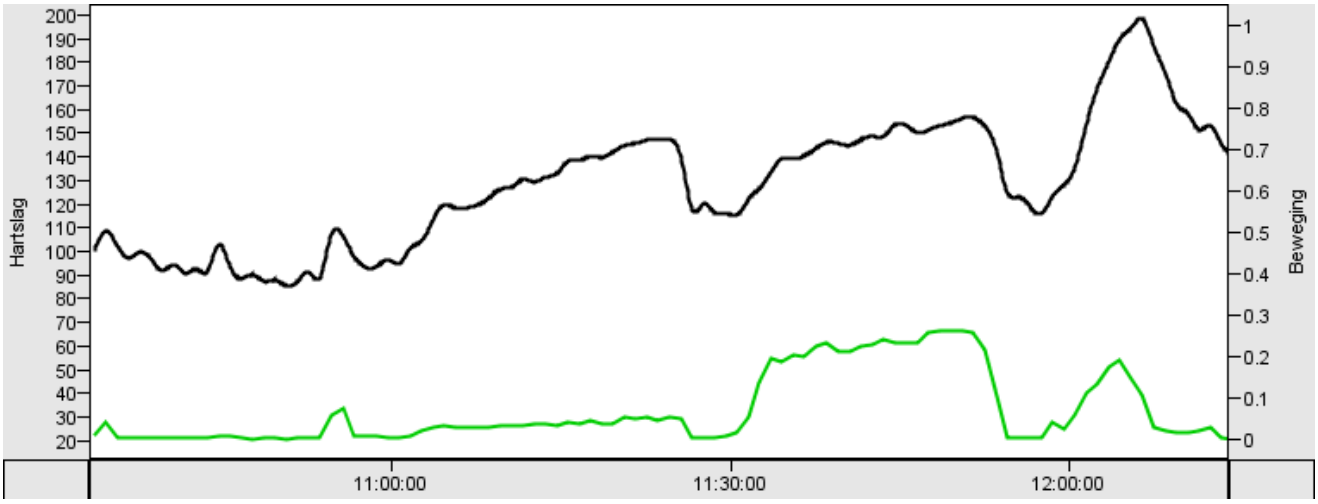
Bekijk hier je foto!

Persoonlijke link naar foto: http://www.tweelingenregister.org/03d70ca9-1ec0-4cf1-b97f-6d9221d30f4c

Spronghoogte, flexibiliteit en handknijpkracht

Bij het sportonderzoek van het Nederlands Tweelingen Register hebben we al bij ruim 100 tweelingparen tussen de 16 en 18 jaar spronghoogte, flexibiliteit en handknijpkracht gemeten.
De gemiddelde *spronghoogte* voor jongens was 46 cm, voor meisjes was dit 36 cm. De gemiddelde *flexibiliteit* voor jongens was 20 cm, voor meisjes was dit 28 cm. De gemiddelde *handknijpkracht* voor jongens was 40 kg, voor meisjes was dit 29 kg.

Hieronder zie je een grafiek waarin je hartslag en beweging kunt zien. De groene lijn is beweging en de zwarte lijn is hartslag. Een standaardregel om je maximale hartslag te schatten is 220 minus je leeftijd. Dus als je 16 jaar oud bent is je geschatte maximale hartslag (220 – 16 =) 204. Dit is dus een schatting; je werkelijke maximale hartslag kan dus hoger of lager zijn. Je echte maximale hartslag weet je alleen na een maximaaltest die jij zojuist hebt gedaan!



Grafieken

Met behulp van het masker dat je tijdens het onderzoek moest dragen hebben we een aantal grafieken gemaakt. Een korte uitleg van de grafieken op de volgende pagina:
VE = Hoeveelheid uitgeademde lucht in liters per minuut.
VO2 = de hoeveelheid zuurstof (in milliliters) die je lichaam per minuut verbruikt. Zodra je gaat sporten, verbruikt je lichaam meer zuurstof dan wanneer je rustig zit. Met behulp van deze zuurstof maakt je lichaam energie om te kunnen bewegen. Je **maximale zuurstofopname** (VO_{2max}) is de beste maat voor uithoudingsvermogen. Deze waarde vind je ook terug op je certificaat. Gebruikelijk voor jouw leeftijd is deze waarde 32 tot 51 ml/min/kg (vrouwen) of 38 tot 62 ml/min/kg (mannen).
VCO2 = de hoeveelheid koolstofdioxide (in milliliters) die je lichaam per minuut produceert. Deze waarde behoort in rust en tijdens lage inspanning ietsje lager dan VO2 te zijn en tijdens maximale inspanning iets hoger dan VO2.
R = de ratio tussen VCO2 en VO2; dus VCO2 gedeeld door VO2. In rust is dit ongeveer 0.80 (dus je verbruikt meer zuurstof dan je koolstofdioxide produceert). Als je gaat sporten gaat deze waarde omhoog (zoals je in je grafiek kunt zien). Dit ratio heeft alles te maken met het substraat dat je lichaam verbrandt om aan energie te komen; vet of koolhydraten. In rust verbrand je vooral vetten (''langzame energie''). Zodra R boven de 1 komt, is VCO2 groter dan VO2. Dit betekent dat je lichaam op een andere soort energie is overgegaan om energie te krijgen: je bent nu vooral koolhydraten aan het verbranden (''snelle energie'').
T = tijd in minuten. Op het tijdstip 00:00 zijn we de testen begonnen.

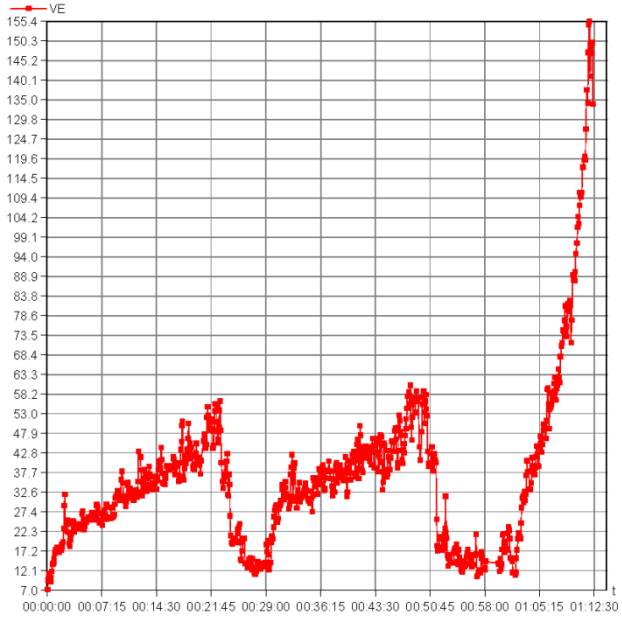
Voor meer informatie over dit rapport kunt u bellen naar 020-5988766 of een email sturen naar ntr.sportonderzoek@vu.nl

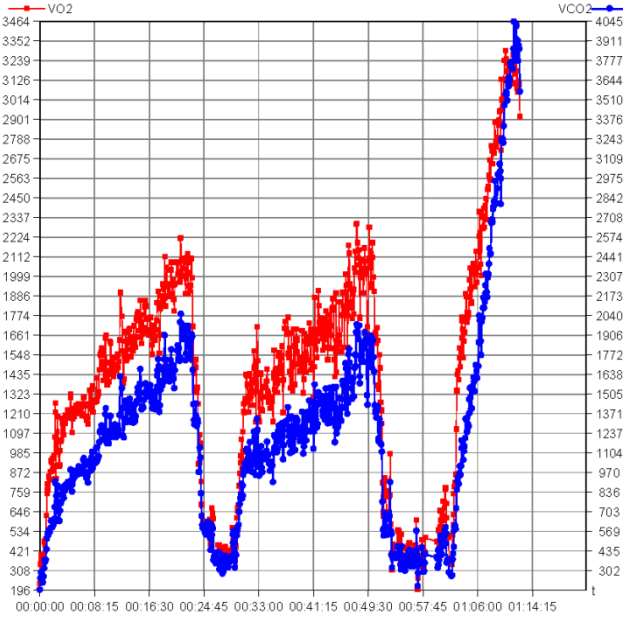
Nederlands Tweelingen Register

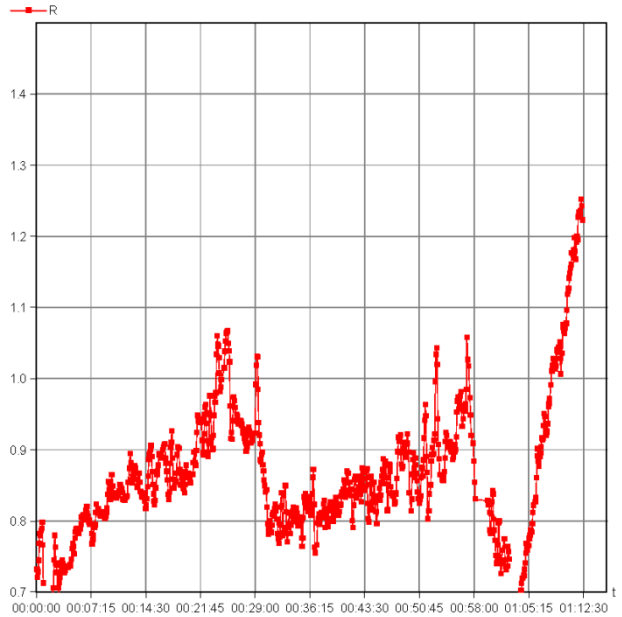
Afdeling Biologische Psychologie
Vrije Universiteit Amsterdam
Van der Boechorststraat 1
1081 BT Amsterdam

[redacted]

[redacted]				Barometric press. (mmHg):	770
Sex:	M	[redacted]		Temperature (degrees C):	23
Age:	17			STPD:	0.838
Height (cm):	175.0	N. of steps:	807	BTPS insp:	1.100
Weight (Kg):	72.7	Duration (hh:mm:ss):	01:12:25	BTPS exp:	1.020
HR max (bpm):	203	BSA (m^2):	1.9	BMI (Kg/m^2):	23.7
Last turbine calibration:	14-8-2013	Last Gas calibration:	14-8-2013		







APPENDIX H – INSTRUCTION FOLDER DNA COLLECTION

Nederlands Tweelingen Register

Vrije Universiteit Amsterdam
Biologische Psychologie
Van der Boechorststraat 1
1081 BT Amsterdam

telefoon: 020-59 88766
e-mail: ntr.sportonderzoek@vu.nl

VRIJE UNIVERSITEIT AMSTERDAM

INSTRUCTIE

HET MONDUITSTRIJKJE:
hoe verzamelt u DNA
met een wattenstaafje

vrije Universiteit *Amsterdam*
Biologische psychologie

VRIJE UNIVERSITEIT AMSTERDAM

Waarom monduitstrijkjes?
Met behulp van tweelingen en hun families onderzoekt het Nederlands Tweelingen Register de invloed van erfelijke factoren en omgevingsfactoren op individuele verschillen in gedrag, leefstijl en gezondheid.
Door middel van vragenlijst- en laboratoriumonderzoek kan worden nagegaan hoe groot deze erfelijke en omgevingsinvloeden zijn. Als er erfelijke invloeden zijn, kan met deze methoden echter niet bepaald worden welke genen verantwoordelijk zijn voor verschillen in gedrag, leefstijl en gezondheid. Daarvoor is erfelijk materiaal nodig, het zogenaamde DNA.

Met een monduitstrijkje kan op een eenvoudige en pijnloze manier erfelijk materiaal worden verzameld. Een monduitstrijkje wordt gemaakt door met een wattenstaafje zachtjes langs de binnenkant van de mond te wrijven. De cellen van het wangslijmvlies worden zeer vaak vernieuwd. Daarom zijn deze cellen bij uitstek geschikt voor verzameling van erfelijk materiaal. De cellen kunnen ook worden gebruikt om te bepalen of een tweeling een- of twee-eiig is (de 'zygositeit'). De tweelingen die aan dit onderzoek meedoen krijgen deze uitslag te zijner tijd thuisgestuurd.

Wat zit er in de envelop?
U heeft (per persoon) 5 buisjes ontvangen: een wat dikkere buis met 16 wattenstaafjes en 4 dunne buisjes met alleen een beetje vloeistof.

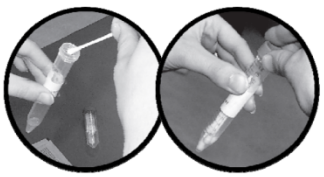
Wat te doen met het materiaal?
De monduitstrijkjes moeten 4 keer worden afgenomen, verspreid over twee dagen. Op dag 1 doet u het 2 keer; voor de ochtend- en avondmaaltijden. De volgende dag herhaalt u deze handelingen. Niet van te voren de mond spoelen, tanden poetsen of eten!
Per afname gebruikt u 4 wattenstaafjes:

- 1 staafje voor de binnenkant van de bovenlip en het tandvlees van de bovenkaak.
- 1 staafje voor de binnenkant van de onderlip en het tandvlees van de onderkaak.
- 1 staafje voor de binnenkant van de linkerwang.
- 1 staafje voor de binnenkant van de rechterwang.

VRIJE UNIVERSITEIT AMSTERDAM

U wrijft per wattenstaafje ongeveer 10-20 seconden zorgvuldig en met enige druk (het hoeft niet hard; het mag geen pijn doen).

Na het wrijven deponeert u het staafje, met het watje naar beneden, in de vloeistof in één van de dunne buisjes. Doe alle 4 staafjes in ditzelfde dunne buisje. De andere dunne buisjes zijn voor de volgende monduitstrijkjes. Wilt u het deksel van het buisje goed dichtdraaien? (Niet te hard om barsten van de buis te voorkomen).



Als u na twee dagen alle vier de afnames heeft gedaan, heeft u de dikke buis niet meer nodig. Deze kunt u dus weggooien. Wilt u de vier dunne buisjes met de monduitstrijkjes hierna direct terug in de envelop doen?

Met het verzamelen van monduitstrijkjes kunt u op ieder moment beginnen. U kunt de buisjes rechtop bewaren, op een koele droge plaats (niet in de koelkast of vriezer). Vervolgens kunt u deze meenemen op de dag van het onderzoek. Voor meer informatie over de wijze van omgang met gegevens kunt u vinden op de website: www.tweelingenregister.org.

Nadat het erfelijk materiaal is geanalyseerd zullen eventuele restanten gedurende 15 jaar worden bewaard voor onderzoeksdoeleinden. Het DNA kan ons ook helpen genen voor de erfelijkheid van sportgedrag, daaraan gerelateerde eigenschappen en de reactie op lichamelijk inspanning te onderzoeken. Wanneer je dit niet wilt, stel ons dan daarvan s. v. p. op de hoogte. In dat geval zullen wij het overgebleven materiaal vernietigen.
Indien u nog vragen heeft, kunt u contact met ons opnemen. Onze gegevens staan op de achterzijde van deze brochure.

VRIJE UNIVERSITEIT AMSTERDAM

APPENDIX I – FOLLOW-UP SURVEY

Follow-up vragenlijst

Sport

1a. Doe je regelmatig aan sport of lichaamsbeweging?

- ☐ Nee *(door naar 3)*
- ☐ Ja

1b. Zo ja, welke sport(en) beoefen je?

Sport	Aantal jaren	Aantal maanden per jaar	Aantal keren per week	Aantal minuten per keer	Plezier	Competitie	Selectie	Regionaal	Nationaal

Blessures

2a. Heb je wel eens een sportblessure gehad?

(Een sportblessure is een letsel dat direct of indirect is ontstaan door deelname aan sport en ertoe heeft geleid dat je minstens één dag niet hebt kunnen sporten)

- ☐ Nee
- ☐ Ja

2b. Hoeveel sportblessures heb je gehad? …….

2c. Wat was de blessure die je het meest heeft beperkt in sporten?

- ☐ Schaafwond
- ☐ Verstuiking/gescheurde banden
- ☐ Verrekking/gescheurde spier
- ☐ Open wond/snee
- ☐ Kneuzing
- ☐ Zwelling/ontsteking
- ☐ Breuk (incl. verdenking op)
- ☐ Dislocatie/subluxatie (ontwrichting)

- ☐ Overbelasting van spier of pees(-aanhechting)
- ☐ Blaren
- ☐ Hersenschudding
- ☐ Anders, namelijk……

2d. Welk lichaamsdeel was geblesseerd?

- ☐ Hoofd/gezicht
- ☐ Nek/hals
- ☐ Schouder
- ☐ Bovenarm
- ☐ Elleboog
- ☐ Onderarm
- ☐ Pols/hand
- ☐ Vinger
- ☐ Borstkas incl. organen
- ☐ Buik incl. organen
- ☐ Bovenrug (borst)
- ☐ Onderrug (lumbaal)
- ☐ Bekken
- ☐ Heup/lies
- ☐ Bovenbeen
- ☐ Knie
- ☐ Onderbeen
- ☐ Enkel
- ☐ Voet/teen
- ☐ Borst
- ☐ Anders, namelijk……

2e. Hoeveel dagen heb je door deze blessure niet kunnen sporten? ……..

2f. Ben je voor deze blessure medisch behandeld?

- ☐ Nee
- ☐ Ja

Subjectieve bekwaamheid

	Veel minder goed	Minder goed	Ongeveer even goed	Beter	Veel beter
3a. Hoe goed ben je in sport ten opzichte van je leeftijdsgenoten?					
3b. Hoe goed is je uithoudingsvermogen ten opzichte van je leegtijdsgenoten?					

3c. Hoe goed is je spierkracht ten opzicht van je leeftijdsgenoten?					

3d. Op schaal van 1 tot 10, hoe goed ben je in sport? ……

Beweeggedrag

4a. Hoeveel fiets je gemiddeld per week (inclusief het weekend?) …… uur …… minuten

4b. Hoeveel wandel je gemiddeld per week (inclusief het weekend?) …… uur …… minuten

4c. Heb je gymnastieklessen op school?

- ☐ Nee
- ☐ Ja, namelijk: ……..minuten

4d. Hoeveel dans je gemiddeld per week tijdens het uitgaan? …… uur …… minuten

Zitgedrag

	Doordeweekse dag	Dag in het weekend
5a. Hoe lang ben je aan het zitten terwijl je onderweg bent? (met de auto of in het OV)	…… uur …… minuten	…… uur …… minuten
5b. Hoe lang ben je aan het zitten terwijl je aan het werk bent of op school bent?	…… uur …… minuten	…… uur …… minuten
5c. Hoe lang ben je aan het zitten terwijl je TV aan het kijken bent?	…… uur …… minuten	…… uur …… minuten
5d. Hoe lang ben je aan het zitten terwijl je thuis achter je computer zit?	…… uur …… minuten	…… uur …… minuten
5e. Hoe lang ben je aan het zitten terwijl je andere dingen aan het doen bent? (Bij vrienden op bezoek, bioscoop, uit eten)	…… uur …… minuten	…… uur …… minuten

ABOUT THE AUTHOR

Nienke Maria Schutte (1987) was born in Apeldoorn, the Netherlands. She graduated from high school (Christelijk Lyceum in Apeldoorn) in 2005 with a profile in Culture & Society. Determined to get an academic degree in science, she moved to Amsterdam and started studying at the Vrije Universiteit, where she completed her BSc Psychology with a specialization in biological psychology in 2009, and the research master Neuroscience, with a specialization in psychophysiology, in 2011. During her studies, she committed herself to the academic community by holding positions such as board member of the student association VSPVU (2006-2007), member of the university student council (2007-2008), student assistant at the Amsterdam University College (2008-2009) and member of the educational committee of the neuroscience master (2010).

In 2011, she started a PhD project supervised by prof.dr. Eco de Geus and prof.dr. Meike Bartels at the Netherlands Twin Register (NTR, maintained by the Biological Psychology department at the Faculty of Behavioral and Movement Sciences at the Vrije Universiteit Amsterdam). This project aimed to unravel the genetic components of adolescent exercise behavior. During her PhD, she presented her work at several international conferences and organized workshops for researchers, families enrolled at the Netherlands Twin Register and others. In addition, she was president of the board ProVU, the association for PhD candidates and postdoctoral researchers at the Vrije Universiteit and VU University medical center (2013-2015). The results of her PhD project are presented in this thesis.

List of publications:

ACKNOWLEDGEMENTS

First of all, I would like to thank all the twins and their siblings who participated in my study. Without your commitment to support scientific research and the enthusiasm to exercise to the point of exhaustion it would not have been possible to collect the data needed to answer the questions we had regarding the heritability of adolescent exercise behavior. Thank you very much.

My sincere thanks goes to my supervisors, Eco and Meike. As a dedicated non-exerciser, I was positively surprised that you asked me to work on this exercise project. However, I am really glad you did as this project was not only very interesting and challenging, but perhaps it even provided me with a good reason for my exercise-loathing. Eco, when I was a second year BSc student I was, despite my immature and distracted teenage brain, fascinated by your Biological Psychology lectures on the physiological basis of behavior. It is not without reason that my BSc thesis (on cardiac electrophysiology), my MSc thesis (on EEG) as well as my PhD project (on exercise behavior) came about under your supervision. Thank you for all the discussions, ideas you shared and your infectious science-enthusiasm. Meike, thank you for the time and effort you put into critically reviewing my (manu)scripts. When I made things too complicated you helped me stay grounded with a no-nonsense attitude and a smile. Exactly what I needed.

Members of the Doctoral Examination Committee: prof.dr. Willem van Mechelen, prof.dr. Harold Schnieder, prof.dr. Maria Hopman, dr. Marcel den Hoed and dr. Jos de Koning, thank you for devoting a significant amount of time reviewing this thesis.

A special thanks goes to my paranymphs; Ineke, Charlotte and, if it was possible to be accompanied by 3 paranypmhs, Eveline for all their support, academically but also socially. It was amazing to share an office for over four years, to spend hours in the lab, at a conference or road tripping together. I would like to extend my thanks and appreciation to all the colleagues at the Biological Psychology department and the guys from tech support for all their help in setting up my study, solving statistical problems, discussions over lunch and drinks and all the fun times. It's hard to leave after so many years!

I would also like to thank my friends for being there when I left my office or lab at the end of the day (or night). Thank you for your patience, pleasant distractions, motivating discussions and best Friday nights ever.

Last but not least, I would like to thank Sophie and Steven, my parents, and Pim for supporting me throughout writing this thesis and in my life in general.

Nienke