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PHYSICAL ACTIVITY, SEDENTARY BEHAVIOUR AND PHYSICAL FITNESS

ASSOCIATIONS WITH CARDIOMETABOLIC HEALTH OUTCOMES

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FYSIEKE ACTIVITEIT, SEDENTAIR GEDRAG EN FYSIEKE FITHEID

ASSOCIATIES MET CARDIOMETABOLE GEZONDHEID

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Table of content

List of abbreviations

CMRS	Clustered cardio-metabolic risk score
CRF	Cardiorespiratory fitness
FPACQ	Flemish Physical Activity Computerized Questionnaire
GI	Gini index
IDF	International Diabetes Foundation
LPA	Light physical activity
METs	Metabolic equivalent units
MPA	Moderate physical activity
MVPA	Moderate-to-vigorous physical activity
PAL	Physical activity level
VPA	Vigorous physical activity

GENERAL INTRODUCTION AND OUTLINE

General introduction and outline

Hippocrates wrote: "eating alone will not keep a man well; he must also take exercise. For food and exercise … work together to produce health" [1]. He discussed extensively the benefits of exercise for a variety of diseases, including both physical and mental illnesses. Hippocrates inspired other great health authorities that exercise could be considered medicine. In 1873 Edward Stanley, Earl of Derby, said that "Those who think they have not time for bodily exercise will sooner or later have to find time for illness". However, after centuries of including exercise in health prescription, physical activity disappears to the background, resulting in frequently prescribing bed rest in the beginning of the 20th century [1]. In the 1960s exercise returns to the medical field with the work of Morris and Paffenbarger [2, 3]. For the past half century, epidemiologists and physiologists have validated the perceptions of the ancient scholars by demonstrating that persons who perform **physical activity** on a regular basis manifest an excess of physiological benefits and experience reduced risk of chronic disease and premature mortality [4-6]. In 2009, with the publication of *ACSM's Exercise is Medicine: A Clinician's Guide to Exercise Prescription* [7] and the recommendations from the European Society of Cardiology [8], we are back to the point where prescribing lifestyle modifications for health is generally accepted. Over the last decades more attention has also been given to different aspects of **sedentary behaviour** and **physical fitness**.

1 Defining cardiometabolic health, physical activity, sedentary behaviour and physical fitness

1.1 Cardiometabolic health

Non-communicable diseases, also known as chronic diseases that are not passed from person to person, were responsible for 38 million (68%) of the world's deaths in 2012 [9]. Non-communicable diseases are generally classified in four main types: 1) cardiovascular diseases (such as heart attacks and stroke); 2) diabetes mellitus type 2; 3) cancers and 4) chronic respiratory diseases (such as chronic obstructed pulmonary disease and asthma). A combination of the first two types (cardiovascular diseases and diabetes) can be categorised as cardiometabolic diseases, where major **risk factors** include **visceral obesity**, **hypertension**,

hyperglycaemia, and **atherogenic dislipidemia** [10]. Worldwide more than 25% of the adult population has a clustering of three or more cardiometabolic risk factors [11] and over the last few decades, the prevalence of these cardiometabolic risk factors has increased steadily [12].

Clustering of cardiometabolic risk factors in the same person appears to confer a substantial additional risk for cardiovascular diseases, diabetes mellitus type 2, and all-cause mortality over and above the sum of the risk associated with each risk factor [10, 13]. Therefore, when evaluating cardiometabolic risk not only the separate risk factors are assessed, but also clustering of these risk factors. There are two frequently used approaches to assess clustered cardiometabolic risk. First is the **metabolic syndrome**, a dichotomous approach, and defined by the International Diabetes Foundation as: "Central obesity plus any two of the following four factors: raised triglyceride levels, reduced HDL-cholesterol, raised blood pressure and/or raised fasting plasma glucose" [10]. Second is a **continuous cardiometabolic risk score** that includes the same five risk factors as the metabolic syndrome. This is a more appropriate approach for epidemiological studies [14], firstly because cardiovascular and diabetes risk increase progressively with an increasing number of risk factors. Secondly, these risk factors are continuous and less favourable scores on these risk factors will gradually lead to a less favourable cardiometabolic health profile. Lastly, a continuous score provides better statistical power than a dichotomous score. For these reasons a continuous clustered cardiometabolic risk score (CMRS) was calculated for all studies included in this thesis.

1.2 Physical activity

Physical activity is a commonly used term and has multiple different meanings and interpretations. In research physical activity is most often defined as "Bodily movement via skeletal muscles, which results in **energy expenditure**. This energy expenditure varies continuously from low to high and is positively correlated with physical fitness" [15]. It differs from exercise because physical activity does not have to be planned, structured and/or a repetitive bodily movement. Furthermore, the ultimate objective can, but does not have to be, to improve or maintain physical fitness components [15]. Consequently, physical activity is a very broad term that includes exercise, but also household work, active transportation and occupational activities. A more objective cut-off for defining physical activity is 1.5 **Metabolic Equivalent of Task** (METs). MET is a physiological measure expressing the energy cost of physical activities, where one MET is the energy cost of resting quietly. MET can also be defined in terms of oxygen uptake, where each MET represents an energy consumption of 3.5 mL.kg-

1.min-1 [16, 17]. Hence, in congruence with the definition by Caspersen, every activity above 1.5 METs (assuming there is minimum of bodily movement) is defined as physical activity, regardless of the intention or reason for this activity.

Because physical activity contains various **behaviours** and **intensities**, it is often more specified. One possible way of categorizing physical activity is by intensity. **Light physical activity** (LPA) contains all behaviours with intensities between 1.5 and 3 METs. **Moderate physical activity** (MPA) and **vigorous physical activity** (VPA) are activities with intensities between 3 and 6 METs, and more than 6 METs, respectively [17]. These last two categories are often joint to one overarching category, namely **moderate-to-vigorous physical activity** (MVPA), consequently containing all activities with an intensity above 3 METs. Intensities can be objectively measured with accelorometry or approximated according to the Compendium of Ainsworth [18].

The World Health Organisation (WHO) constructed **global recommendations** on physical activity for health that are relevant to all healthy adults [19]. Worldwide only 59% of adults are physically active, defined as meeting the international guidelines of 150 minutes or more of MPA a week, 75 minutes or more of VPA a week or an equivalent combination of MPA and VPA [20]. Activity declines with age, is decreased in high-income countries and is lower in women than men [20]. In Flanders, a different guideline is adopted: minimum 30 minutes of MVPA every day of the week. Overall, 40% of the Flemish adults met this standard in 2013 [21]. Comparable to international data almost twice as many men were sufficiently active in comparison to Flemish women (52% vs 28%, respectively) and there is a strong decline with age. Additionally, adult physical activity levels were stable during the past 25 years [22]. However, there is a shift in the type of physical activity that is performed. Worldwide **leisure-time physical activity** in adults is increasing over time, whereas **work-related physical activity** decreased. Similarly in Flanders, there is no apparent trend in total physical activity levels over the past 15 years [21].

1.3 Sedentary behaviour and sedentary time

Sedentary behaviour is a more recent term than physical activity. In older research it was defined as the absence of physical activity, however, recently a more specific definition was proposed by the Sedentary Behaviour Research Network: "Any waking behaviour characterized by an energy expenditure ≤ 1.5 METs while in a sitting or reclining posture" [16, 23]. This definition implies that sedentary behaviour does not include sleep or standing, however both activities below 1.5 METs. A person may be described as sedentary or 'having a sedentary lifestyle' if they engage in a large amount of sedentary behaviour [23]. However, people who perform insufficient amounts of MVPA (i.e., not meeting specified physical activity guidelines) are not necessary sedentary. Therefore, the absence of physical activity is more appropriately defined as 'being inactive' [23]. Furthermore, it is possible for an individual to accumulate large amounts of both sedentary behaviour and MVPA in the course of a day. Besides the total accumulation of sedentary time, researchers have been investigating the detrimental effects of prolonged, uninterrupted bouts of sedentary behaviour [24].

Just as **physical inactivity**, sedentary behaviour is very prevalent, although recently it did not increase in the European region [25]. The Flemish guideline is to constrain long bouts of sedentary time and to interrupt these bouts every 20 to 30 minutes [26]. In a European study on a population aged 15 years and over, the mean sitting time was almost five hours a day [25]. However, in Flemish adults the mean sitting time is estimated at 8.3 hours a day of which most is during working hours [26]. This is a concern because during working hours sedentary behaviour is only rarely interrupted, leading to long bouts of sedentary behaviour. Another concern is how people compose their sedentary behaviour. For example, screen time is associated with lesser health, possibly because screen time is associated with higher energy intake, particularly induced by snacking while watching TV [27]. During weekdays, 50% of the Flemish adults watches more than 2 hours of TV, and on weekend days this proportion rises to 70% of the Flemish adults [26].

A differentiation between sedentary behaviour and sedentary time is necessary. On the one hand, <u>sedentary behaviour</u>, just as physical activity, is an overarching term. However, as it includes the word behaviour, sedentary behaviour can also be used when focussing on the specific behaviours. Therefore, this is often self-reported and measured by a questionnaire or physical activity diary [28]. On the other hand, <u>sedentary time</u> is the time spent in those sedentary behaviours. Consequently, it focusses more on the actual time spent sedentary. To get the most accurate assessment, it is best to objectively measure sedentary time in combination with an activity diary to assess the specific sedentary behaviour. Nevertheless, sedentary time can also be approximated by questionnaires that query time in sedentary behaviour.

The differentiation between both terms is important as both terms have their benefits. For example, different sedentary behaviours with the same duration, may have various implications on our health [29]. Stamatakis et al. found that associations between sedentary behaviour and cardiometabolic risk factors are more consistent when sedentary behaviour is measured by self-report that includes TV viewing, in comparison to objective measurement including all sedentary behaviours. Furthermore, a longitudinal French study, observed that increased TV viewing over six years was associated with increased body mass index and percent body fat, while associations between reading and the same cardiometabolic risk factors were less consistent [30]. Moreover, results for the association between occupational sitting and cardiometabolic health have been equivocal [31], however, occupational sitting may be associated more with musculoskeletal injuries. In conclusion, objective measurement of sedentary time is more precise and can accurately measure breaks in **bouts of sedentary time**. This is important because studies have shown that the manner of sitting time accumulation can be important. Specifically, breaks in sitting time have been shown to be associated with cardiometabolic health, independent of total sitting time and exercise levels [24].

1.4 Physical fitness

Physical fitness is a multidimensional concept and has numerous subcategories. It can be related to skill-related components such as agility, power and balance; or physiologic components such as bone integrity and status of metabolic systems [32]. However, physical fitness can also be related to specific health-related components. Because associations with health are the main objective of this thesis, the comprehensive concept of ***health-related fitness*** is used as exposure [32] and metabolic systems will be included as outcomes. Health-related fitness is defined as: "The ability to perform daily activities with vigor, and the possession of traits and capacities that are associated with a low risk of premature development of hypokinetic diseases (e.g. those associated with physical inactivity)" [32]. Health-related fitness consists of four components: 1) **cardiorespiratory fitness** (CRF); 2) **muscular strength and endurance**; 3) **flexibility** and 4) **body composition** [32].

1.5 Interrelation between physical activity, sedentary behaviour and health-related fitness

Although physical activity, sedentary behaviour and health-related fitness are three distinct concepts they are very much interwoven (Figure 1). Firstly, physical activity and sedentary behaviour are behaviours that can be changed, whereas health-related fitness reflects a combination of genetic potential, training and functional health of various organ systems [33]. As a result, health-related fitness improves mostly when physical activity increases and sedentary behaviour decreases [34-36]. However, all three exposures are affected by heredity, other lifestyles, the environment and personal attributes (Figure 1) [37]. Furthermore, health-related fitness is a potential mediator in all significant associations between physical activity, sedentary behaviour and cardiometabolic health. In a study by Sassen et al. up to 78% of the association between average physical activity and cardiovascular disease was mediated through CRF [38]. Moreover, a more recent study by Celis-Morales et al. found that also muscular fitness moderates the association of physical activity and mortality and cardiovascular disease [39]. Furthermore, high levels of CRF eliminated the increased odds of having clustered cardiometabolic risk with high sedentary behaviour [40, 41].

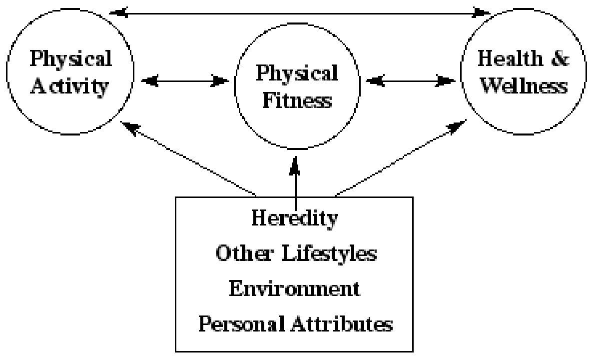


Figure 1. Complex relationships among physical activity, physical fitness, health, wellness and other factors (Adapted from Bouchard et al.,

Secondly, the total daily time of one person is fixed (24h), hence participating in one activity means not participating in another activity. Consequently, although being sedentary and being inactive are two separate concepts, people who reported being very sedentary accumulated less physical activity than people who report being less sedentary [42]. However, participants who comply with the physical activity guidelines have a similar amount of sedentary behaviour compared to those who do not comply with the guidelines [42]. Lately, **isotemporal substitution** analyses are trying to account for the fact that a day has only 24h [43]. The isotemporal substitution model, by definition, estimates the effect of replacing one physical activity type with another physical activity type for the same amount of time [43].

2 Assessment of physical activity, sedentary behaviour and health-related fitness

Physical activity, sedentary time and fitness can be assessed with various techniques. Most important difference in physical activity and sedentary behaviour assessment is objective or subjective measurement. Objective measurement relies on accelerometry and physiological parameters, whereas subjective measurement depends on self-report, usually assessed by questionnaires. Furthermore physical fitness parameters can be approximated or measured directly, with for example a maximal exercise test. Each technique has several strengths and limitations and therefore different techniques will be used in certain situations. The differences between techniques and their aims are shortly discussed below.

2.1 Objective measurement: SenseWear Pro 3 Armband

Objective measurement is the optimal method to achieve the most accurate assessment of the amount of time spent in specific physical activity intensities [44]. In the following chapters the **multisensor body monitor** ***SenseWear Pro 3 Armband*** was used to objectively measure physical activity and sedentary time. We opted for this device, because compared to most physical activity monitors, it integrates measurement of acceleration and other physiological responses. Furthermore, it has been validated at length in multiple contexts and is a valid and reliably measurement tool [45-47]. For example, Johanssen et al. found that average total energy expenditure of the SenseWear Pro 3 Armband was within 112 kcal.d-1 of the criterion value, which was determined by doubly labeled water. The armband was worn over the triceps of the right arm during the whole day (24h), excluding water-based activities, for seven consecutive days. Multiple sensors in the armband continuously measure various physiological and movement parameters, including a two-axis accelerometer and sensors measuring heat flux, galvanic skin response, skin temperature and near body ambient temperature [48]. To estimate minute-by-minute energy expenditure, physical activity intensity, sleep and number of steps, data of these sensors are combined with sex, age, body weight and height, using algorithms developed by the manufacturer (SenseWear Professional software version 6.1).

Strengths of objective measurement include the precision of measurement of intensities [44]. The combination of **accelerometry** and **physiological parameters** generates a detailed description of a person's physical activity intensity at all times, while subjective measurement approximates intensities by physical activity type. Furthermore, objective measurement does not require recalling how active you were during a certain time period and does not require interpretation of intensities. Consequently, objective measurement circumvent reporting errors created by translation, misinterpretation, and social desirability [44]. Moreover, due to the minute-by-minute measurement over a 24h period, the SenseWear Armband generates the most holistic view including sleep and sedentary time. A limitation of this type of assessment is that data are without context and do not include behaviours. Furthermore, it does not give information about posture, therefore some standing time will be included in sedentary time. Finally, specific to the studies included in this thesis, is that there was no objective measurement included at baseline (2002-2004) and therefore cannot be included in any longitudinal analyses *(See 5. Description of the sample and study design)*.

2.2 Qualitative measurement: Flemish Physical Activity Computerized Questionnaire

Over time multiple questionnaires, both single-item measures and composite measures, assessing sedentary behaviour and physical activity have been developed [49, 50]. The **Flemish Physical Activity Computerized Questionnaire** (FPACQ) assess sedentary behaviour and physical activity in Flemish adults and collects information about behaviour at work, for transportation and during leisure time throughout a usual week [51]. Because this questionnaire is especially developed for a Flemish population and generates estimates with high reliability and reasonable criterion validity it was utilised in this thesis [51, 52]. MVPA was estimated as a combination of sports participation and active transportation, only including activities with an intensity ≥ three METs, according to the Ainsworth Compendium [18]. Sedentary behaviour is estimated by asking participants to report the amount of time they spent in screen time and passive transportation [51].

Major strengths of questionnaires are the ease of administration and distribution, low cost, and low participant burden, which is definitely so for computerized questionnaires [51]. Furthermore, questionnaires mostly assess behaviour and therefore give an idea of the context in which a certain physical activity intensity was obtained [44]. This is important because not every behaviour, although at a similar intensity, has the same potential health benefits [29]. A specific strength of the FPACQ is the reasonably high reliability and validity in comparison to similar questionnaires and single-item questionnaires [1] [49]. Moreover, in the studies included in this thesis, this questionnaire was administered at both baseline and follow-up, and therefore

[1] For example: 'In the past week, on how many days have you done a total of 30 minutes of more of physical activity, which was enough to raise your breathing rate?

allows for longitudinal analyses *(See 5. Description of the sample and study design)*. Limitations of questionnaires are recall bias and the accuracy of measurement. Furthermore, some behaviours are classified as sport or exercise by some, but are not considered exercise by other people or cultures. Hence, due to disparities in definition, some will include certain behaviours, while others will not.

2.3 Maximal-exercise assessment: Health-related fitness

Health-related fitness was as far as possible measured with a **maximal exercise test**. Major strengths of maximal measurements are the reliability, validity and sensitivity to within-participant changes over time [32]. They do, however, have the disadvantage of requiring participants to exercise to the point of volitional fatigue and might require medical supervision [32]. Firstly, CRF was measured with a maximal exercise test on an electrically braked Lode Excalibur **cycle ergometer** (Lode, Groningen, The Netherlands) with directly measured breath-by-breath **respiratory gas exchange analysis**, using a Cortex MetaLyzer 3B analyser [53, 54]. Secondly, muscular fitness was also maximally assessed with a calibrated Biodex System Pro 3® **dynamometer** (Biodex Medical Systems, Shirley, NY) [55]. Both maximal tests have been proven valid and reliable [54, 55], with reliability over .95 for the Cortex MetLayzer and over .99 for the Biodex. Thirdly, total body flexibility cannot be tested with one single test. Because of the importance of hamstring flexibility for activities of daily living and sport performance, flexibility of the hamstring muscles was tested [32]. **Sit and Reach** is a commonly used test for hamstring flexibility as well as lower back and hip-joint flexibility [32]. Lastly, percentage of body fat (Fat%) was evaluated by means of **bioelectrical impedance analysis** with a tetrapolar BIA analyser (Bodystat 1500, Bodystat Ltd, Isle of Man, UK). Bioelectrical impedance analysis is a relatively simple, quick and non-invasive technique, to measure body composition and is proven valid for large epidemiological studies in one specific ethnic group [56].

3 Physical activity, sedentary behaviour, physical fitness and cardiometabolic health

3.1 Physical activity, sedentary behaviour and cardiometabolic health

Starting from the early 1950s with the ground-breaking work of Morris et al., physical activity has continuously been linked to health and mortality [3]. Recently, the WHO ranked physical inactivity as the fourth leading risk factor for global mortality, accounting for approximately 3.2 million deaths annually [57]. A review stated that for cardiometabolic diseases active individuals have a risk reduction of 31 to 40% compared to inactive individuals [58]. Furthermore, evidence suggests that physical activity volumes half of the one recommended by the international guideline, could already lead to marked health benefits [59]. Additionally, specifically examining clustered cardiometabolic risk and its risk factors, regular physical activity of various intensities presents beneficial outcomes [6, 60-65]. In conclusion, there is no doubt about the positive effects of regular physical activity on non-communicable diseases and cardiometabolic risk. There is however still uncertainty and disagreement about the most beneficial amount, intensity, duration, etc. of physical activity.

Besides physical activity, sedentary behaviour has also been linked numerous times to health, and more particularly cardiometabolic health [66-70]. Most studies agree that sedentary behaviour is a risk factor distinct from physical activity [67, 71-73], however some disagree [74, 75]. Although cardiometabolic health benefits obtained by decreasing sedentary time, might be smaller than those obtained by increasing physical activity [76], a combination of high sedentary time and physical inactivity is probably most detrimental [77]. However, physical activity represents only 1.5% of a total week, or 3% of our awake time, while sedentary time can be up to 40% of a total week and even 55% of our awake time. This illustrates the large potential for changes in behaviour and consequently people's health.

3.2 Physical fitness and cardiometabolic health

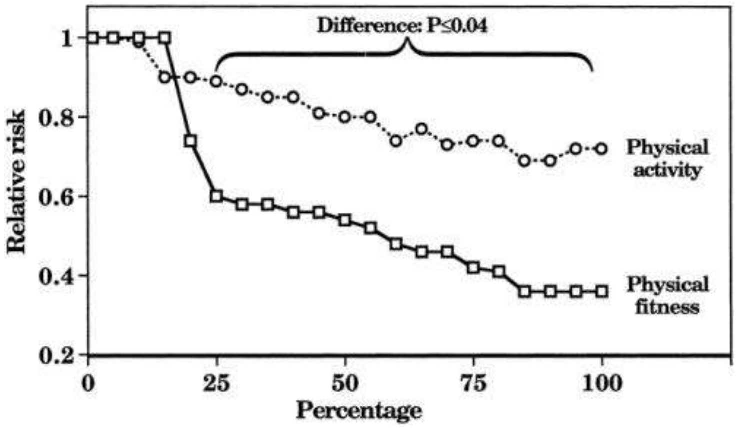


Figure 2. Estimated dose-response curve for the relative risk of both coronary heart disease and cardiovascular disease by sample percentages of fitness and physical activity

CRF has been recognized as a vital sign and powerful predictor of mortality and morbidity, beyond classical cardiovascular disease risk factors such as smoking, cholesterol and hypertension [78, 79]. The incidence of the metabolic syndrome in low-fit individuals is remarkably higher than in individuals with high fitness [80]. The risk of cardiovascular mortality in the moderate-fitness group is less than half the risk in the low-fitness group [81] and small increments in CRF in low-fit individuals are associated with disproportionately larger decreases in risk [80]. In other words, the largest health benefits can be accomplished in the least fit individuals (Figure 2). Moreover, high CRF also appears to be associated with lower risk of mortality in people with conditions such as diabetes, metabolic syndrome, hypertension and overweight, even if the comorbidity is not reversed [81].

Similarly to CRF, muscular fitness is related to mortality and morbidity in healthy individuals and clinical populations with chronic disease [82, 83], and mortality rates are lower for individuals with moderate or high muscular fitness in comparison to low muscular fitness [84]. The prevalence of the metabolic syndrome is significantly lower in adults who lift weights compared to adults who do not lift weights [85]. Furthermore, weight training has been associated with reduced coronary heart disease incidence independently of total physical activity, running or walking [86]. However, in comparison to CRF, cardiometabolic health benefits are smaller [87] and when investigating contributions towards cardiometabolic health of CRF and muscular fitness, independent of one another, the impact of muscular fitness appears to be low or even non-existent [88-91]. Nevertheless, muscular fitness may be more strongly associated to physical function, the risk of falling and other morbidities such as sarcopenia, cognitive function and osteopenia [82, 83].

3.3 Physical activity, sedentary behaviour, physical fitness and cardiometabolic health

Physical activity and sedentary behaviour are the most important adjustable determinants of physical fitness [34-36]. However, physical fitness is often observed as an independent, yet overlapping, disease risk factor [75, 92-97]. Furthermore, the relative risk reduction is significantly greater for physical fitness than physical activity [92, 98]. Additionally, inverse associations between physical activity and cardiometabolic health are much steeper in unfit individuals than in fit individuals (Figure 3) [99]. Therefore, improving physical fitness should be encouraged in unfit individuals to reduce cardiometabolic risk. Measurement of physical fitness can indicate which individuals need to be targeted and can benefit most from physical activity and sedentary behaviour interventions.

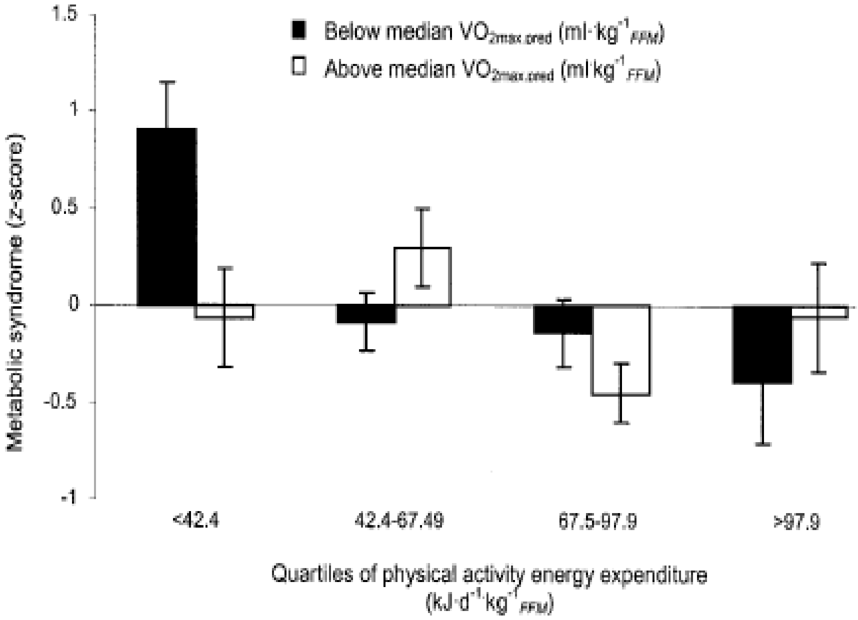


Figure 3. The interaction of physical activity energy expenditure and cardiorespiratory fitness on cardio-metabolic risk. (Adapted from Franks et al., 2004)

Only few studies included physical activity, sedentary behaviour and CRF together when examining associations with cardiometabolic health [75, 94-96]. Adjusting for CRF might provide a more precise representation of the associations between MVPA or sedentary behaviour and cardiometabolic health. Furthermore, because CRF is an integral component of overall physiologic health and function [33], controlling for CRF might address some of the concerns that the association between excessive sitting and cardiometabolic health is a consequence of impaired health rather than the cause of it [95]. In other words, controlling for physical fitness can help detect reverse and reciprocal causality. Results of studies including all three exposures (MVPA, sedentary behaviour and CRF) are contradictory, with results indicating that the associations of sedentary behaviour with cardiometabolic health were independent of CRF [94, 96], markedly less pronounced after correction for CRF [95] or not existent [75]. Based on these results and the temporal associations of sedentary behaviour and MVPA with CRF [34, 35], it is possible that CRF partly mediates associations between sedentary behaviour, MVPA, and clustered cardiometabolic risk [38]. In a study by Sassen et al. up to 78% of the association between average physical activity and cardiovascular disease was mediated through CRF [38]. To the best of our knowledge a similar analysis has not been done for the association between sedentary time and cardiometabolic health.

4 Objectives and general outline of the thesis

The main goal of the thesis was to get a more in depth view on the associations between physical activity, sedentary behaviour, health-related fitness and cardiometabolic health. Besides this, we aimed to examine if these associations were independent of each other and if potential confounders mediated these associations (Figure 4). We hypothesized that physical activity and sedentary behaviour would be associated with health-related fitness. Furthermore, we hypothesized that physical activity and health-related fitness would be positively associated with cardiometabolic health, and that sedentary behaviour would be negatively associated with cardiometabolic health. Besides getting more insight in these associations, we wanted to examine the possible health benefits of substituting sedentary time with a more active behaviour or sleep (Figure 1). We hypothesized that substituting sedentary time with any other behaviour would be associated with improved cardiometabolic health. Up to our knowledge, we were the first to include comprehensive objective measurement of physical activity, sedentary time and sleep in these substitution analyses.

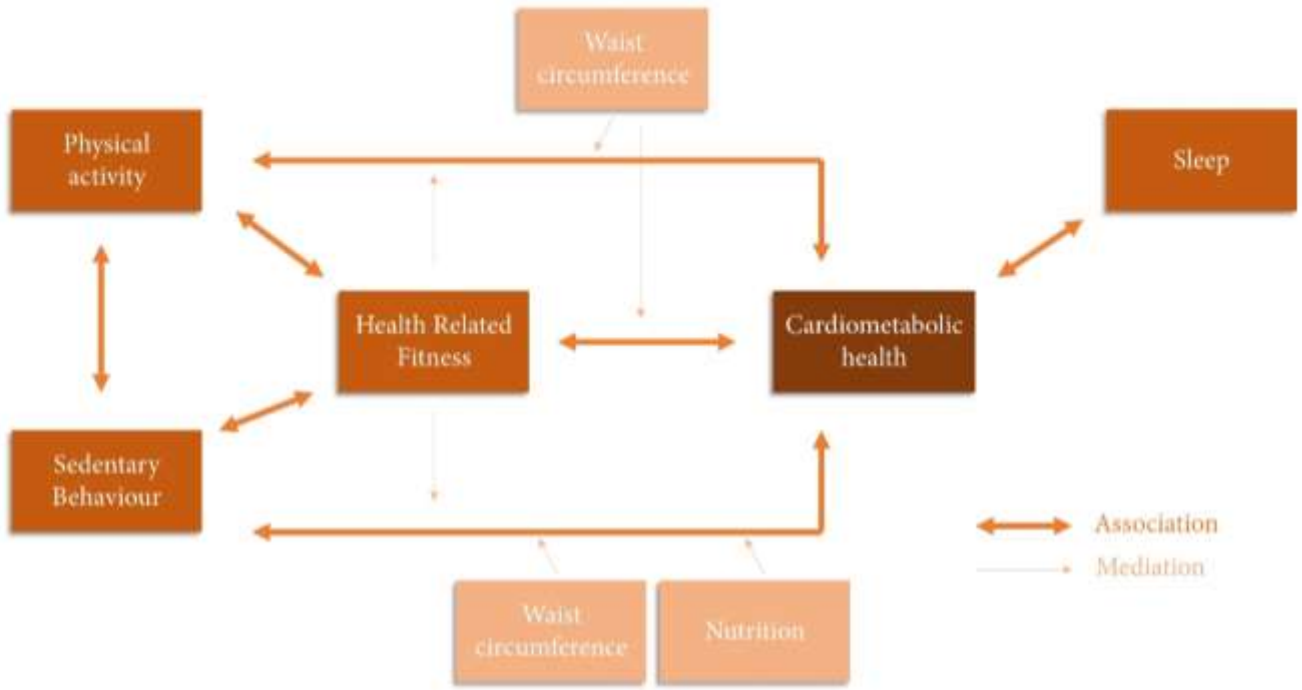


Figure 4. Representation of all hypothesized associations and mediators discussed in the present thesis.

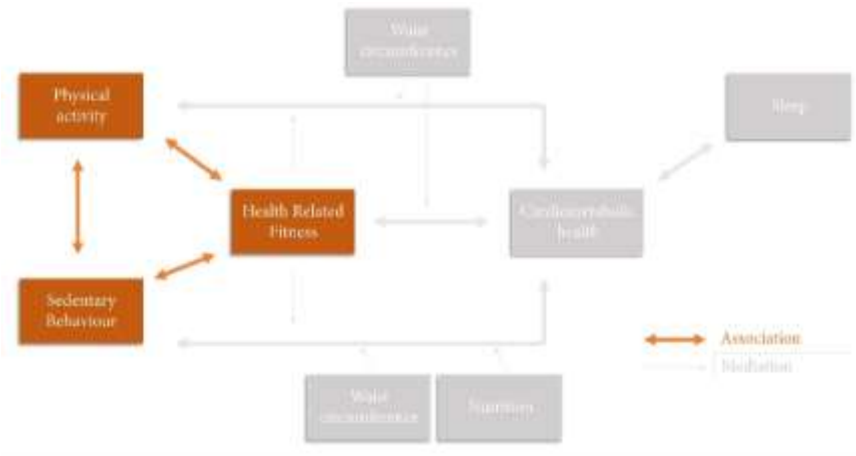
Part two of the thesis consists of five interrelated scientific papers that are published, in press or under review in an internationally peer-reviewed journal. The rationales and aims of all five papers are briefly presented below.

4.1 Paper 1: Associations between physical activity and health-related fitness – volume versus pattern

Primary research questions of Paper 1

- Is total physical (in-)activity associated with health-related fitness and its subcomponents?
- Is there a beneficial trend in health-related fitness over quartiles of physical activity volume and quartiles of the physical activity pattern?
- Which is more strongly associated with health-related fitness, the physical activity volume or the physical activity pattern?

The aim of the first paper was to get a more in depth view on the association between physical activity and health-related fitness. More physical activity and less sedentary time will likely improve someone's health-related fitness [34, 36]. However, less is known



on what the influence is of the pattern in which sedentary time and physical activity are accumulated during the day. Therefore, we addressed the question if a more stable physical activity pattern is better to improve health-related fitness than one with high and low activity intensities. That is to say, do people differ in health-related fitness if they have the same physical activity level (PAL), but a different way of reaching this (i.e., being continuously lightly active, or very sedentary with a short vigorous interval)?

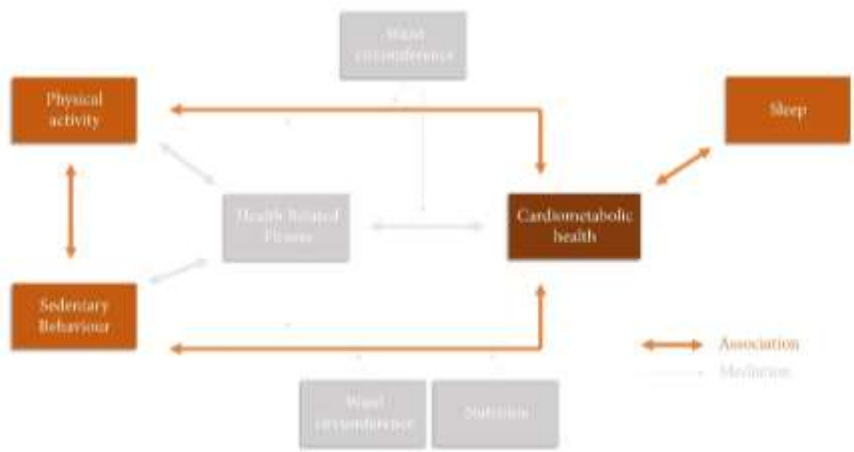
In this study the Gini index, a commonly applied statistical index in social sciences for measuring statistical dispersion [100, 101], represented the physical activity pattern. By introducing the Gini index, no cut-offs are needed to classify sedentary behaviour or physical activity and consequently physical activity patterns can be observed holistically. Therefore, the association between the pattern throughout the day of the whole range of physical activity intensities, including sleep, and health-related fitness can be assessed.

We hypothesized that both activity patterns (i.e., being continuously lightly active, or very sedentary with a short vigorous interval) result in the same level of health-related fitness, taking into account the negative effects of sedentary behaviour and the positive effects of MVPA.

4.2 Paper 2: Substituting sedentary time with light and moderate-to-vigorous physical activity is associated with better cardiometabolic health

Primary research questions of Paper 2

- Is substituting sedentary behaviour with more active behaviour (LPA or MVPA) associated with a favourable cardiometabolic risk profile?
- Is substituting sedentary behaviour with sleep associated with a favourable cardiometabolic risk profile?
- Is substituting LPA with higher-intensity activities (MVPA) associated with a favourable cardiometabolic risk profile?
- Which characteristics are significantly different in individuals with 0-1, 2, and 3 or more cardiometabolic risk factors?



The second paper aimed to apply a more novel statistical technique, namely isotemporal substitution, to systematically examine associations of clustered cardiometabolic risk and cardiometabolic risk factors, and substituting sedentary time with either sleep, LPA or MVPA. Furthermore, the extra benefit of increasing physical activity intensity from LPA to MVPA was explored. Additionally, we aimed to compare characteristics between individuals with 0-1, 2, and 3 or more cardiometabolic risk factors and means of these three groups.

Isotemporal substitution estimates the effect of replacing one form of behaviour with another form of behaviour for the same amount of time [43]. It not only estimates the effect of increasing a certain behaviour, but also integrates the effect of reducing the specific behaviour that it replaces [43]. For example, reducing sedentary time and replacing it with sleep or LPA will result in a different health benefit than replacing the same amount of sedentary time with MVPA [102-105]. Moreover, it is not clear if substituting sedentary time with LPA will already attain positive results or if it needs to be substituted by a higher-intensity activity [102-104, 106]. Furthermore, only a few papers included substituting sedentary time with sleeping time, and sleeping time with physical-active behaviours [102, 107]. It is likely that substituting sedentary time with sleep will also reduce clustered cardiometabolic risk [102], potentially only in short sleepers [107]. However, none of these studies included objectively measured sleeping time.

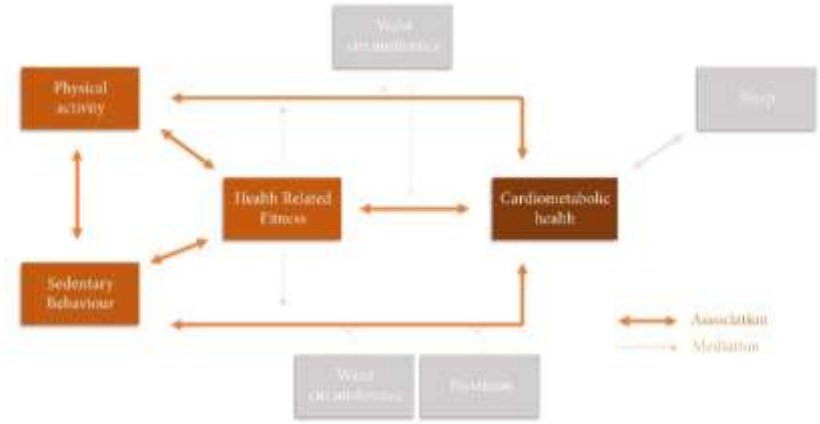
According to the literature, we hypothesize that substituting sedentary time with either sleep, LPA or MVPA, will be associated with a lower clustered cardiometabolic risk and better outcomes on five cardiometabolic risk factors. Secondly, we hypothesize that substituting LPA with MVPA will be associated with a small but significantly reduced clustered cardiometabolic risk and cardiometabolic risk factors.

4.3 Paper 3: Independent associations between sedentary time, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic health: a cross-sectional study

Primary research questions of Paper 3

- Are sedentary time, MVPA and CRF associated with clustered cardiometabolic risk and cardiometabolic risk factors?
- Are associations between sedentary time, MVPA and CRF, and clustered cardiometabolic risk and cardiometabolic risk factors independent of each other?
- What is the mediating role of CRF in the associations between sedentary time and clustered cardiometabolic risk and MVPA and clustered cardiometabolic risk?

The aim of the third paper was to examine the independent associations of objectively measured sedentary time, MVPA and CRF with clustered cardiometabolic risk and individual cardiometabolic risk factors. Furthermore, the mediating effect of CRF on the relation between MVPA or sedentary time and clustered cardiometabolic risk was analysed.



In recent years, accumulating evidence has suggested that excessive sitting and insufficient moderate-to-vigorous physical activity (MVPA) may independently contribute to unhealthier cardiometabolic risk profiles, which in turn may substantially increase the risk for incident type 2 diabetes, cardiovascular disease and premature death [13]. Another factor recognized as a strong predictive marker for cardiometabolic health, and potential mediator in the associations between MVPA, sedentary behaviour and cardiometabolic health, is cardiorespiratory fitness (CRF) [38, 92, 108]. Developing more insight into whether, and to what extent, sitting, MVPA and CRF independently shape cardiovascular health, is therefore important, in order to advance lifestyle interventions and public health guidance.

Although evidence from cross-sectional studies [24, 99, 109-111], longitudinal studies [60, 62, 63, 72, 112] and randomised controlled trials [113, 114] suggests that sedentary time, MVPA, and CRF are important predictors of various cardiometabolic risk factors [115], there are still questions regarding the specificity of these associations and the underlying relationships for predicting clustered cardiometabolic risk. More specifically, it is unclear if all three predictors independently contribute to cardiometabolic health or if they are interrelated and as such only contribute to cardiometabolic health in combination with one or more other predictors. Only few studies examined the relationship of all three parameters together for predicting clustered cardiometabolic risk and results of those studies have been equivocal [75, 94-96]. Furthermore, CRF has been proposed as a potential mediator in the association of MVPA and cardiometabolic health [38]. Moreover, to the best of our knowledge, no study has investigated the mediating effect of CRF on the relationship between sedentary time and clustered cardiometabolic risk.

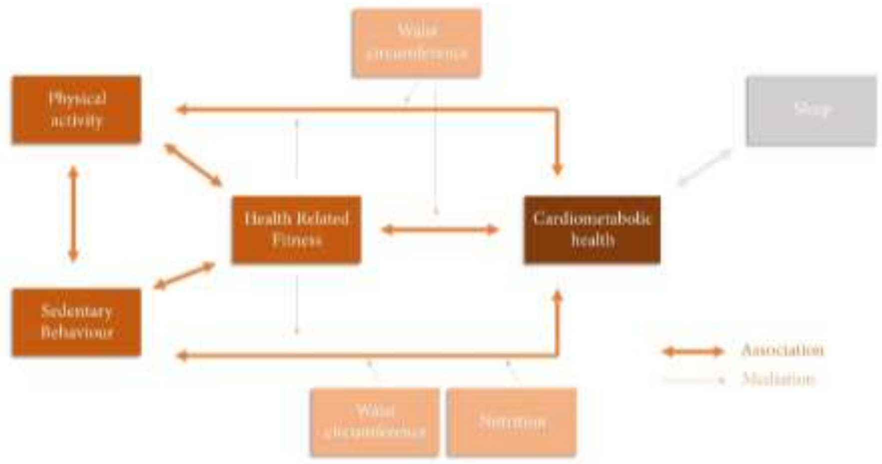
We hypothesized that objectively measured high CRF, high MVPA, and low sedentary time were independently associated with favourable cardiometabolic risk factors. Moreover, we hypothesized that CRF is a potential mediator for the association of MVPA or sedentary time and clustered cardiometabolic health.

4.4 Paper 4: Ten-year change in sedentary behaviour, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic risk: independent associations and mediation analysis

Primary research questions of Paper 4

- Are change in sedentary time, change in MVPA and change in CRF associated with change in clustered cardiometabolic risk and change in its risk factors?
- Are these associations independent of each other?
- What is the mediating role of change in CRF in the associations between change in sedentary time and change in clustered cardiometabolic risk and change in MVPA and change in clustered cardiometabolic risk?
- What is the mediating role of change in waist circumference in the associations between change in sedentary time, change in MVPA, change in CRF and change in clustered cardiometabolic risk?
- What is the mediating role of change in nutritional intake in the associations between change in sedentary time and change in clustered cardiometabolic risk?

The fourth paper follows on the third paper and examines if the cross-sectional associations found in paper 3 are also apparent over a 10-year time period. Moreover, the mediating role of CRF on associations between changes in MVPA or sedentary time and cardiometabolic health is also studied, and two other potential mediators are included, namely waist circumference and nutritional intake. The aim therefore was to examine the independent associations between change in sedentary behaviour, MVPA and objectively measured CRF with concurrent change in cardiometabolic risk over a long period of follow up. Furthermore, we aimed to examine whether any such independent associations were mediated by change in CRF (for sedentary behaviour and MVPA), change in waist circumference (for sedentary behaviour, MVPA and CRF) or nutritional intake (for sedentary behaviour).



Longitudinal studies including all three potentially important lifestyle components (sedentary behaviour, MVPA, CRF) and examining their association with cardiometabolic health are scarce [94, 95, 115, 116]. Moreover, none fully examined the potential contribution change in each of these three components could make in terms of cardiometabolic health, in relation to each other [94, 95, 116]. Furthermore, waist circumference and nutritional intake are both causally related to cardiometabolic health [117, 118]. Lower MVPA and higher sedentary behaviour may be associated with higher waist circumference [69, 119] and higher sedentary behaviour, especially TV viewing, is associated with increased snacking behaviour and changes in nutritional intake in general [27, 120, 121]. Previous longitudinal research, examining the relationship between all three exposures and cardiometabolic health, have not specifically examined the mediating role of change in CRF, waist circumference nor nutritional intake.

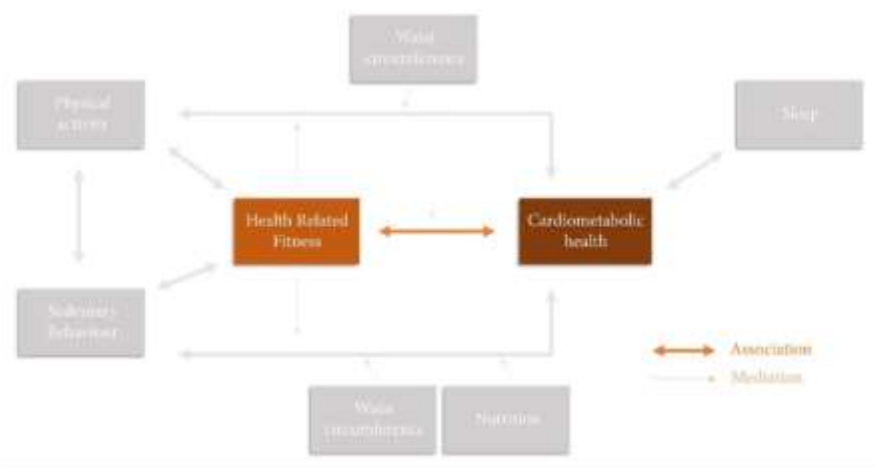
We hypothesized that both change in MVPA and change in sedentary behaviour would be independently related to change in clustered cardiometabolic risk. Similarly, we hypothesized that change in CRF would be related to change in clustered cardiometabolic risk. Furthermore, we hypothesized that change in CRF would mediate the associations between changes in MVPA or sedentary behaviour and clustered cardiometabolic risk; that change in waist circumference would mediate all associations; and that change in nutritional intake would mediate the association between change in sedentary behaviour and change in clustered cardiometabolic risk.

4.5 Paper 5: Longitudinal and cross-sectional associations between cardiorespiratory fitness, muscular fitness and cardiometabolic risk

Primary research questions of Paper 5

- Are CRF and muscular fitness associated with change in clustered cardiometabolic risk and change in its risk factors?
- Are change in CRF and change in muscular fitness associated with change in clustered cardiometabolic risk and change in its risk factors?
- Does muscular fitness add strength to the association between CRF and clustered cardiometabolic risk?
- Does change in muscular fitness add strength to the association between change in CRF and change in clustered cardiometabolic risk?

In this last paper purpose was to examine the relative importance of cross-sectional and long term changes in objectively measured CRF and muscular fitness towards cardio-metabolic health. Additionally, the primary aim was to investigate the possible additive contribution of change over a period of 10 years in objectively measured muscular fitness towards cardiometabolic health, independent of the favourable effects of change in CRF.



Numerous studies have examined the benefits of cardiorespiratory fitness on individual parameters of cardio-metabolic health and mortality [79, 92, 95, 98, 115]. Apart from CRF, muscular fitness has also been proposed as a predictor for mortality [82, 84]. However, the protective effect of muscular fitness for cardiometabolic health is less clear. Furthermore, when investigating contributions of CRF and muscular fitness towards cardiometabolic health, independent of one another, the impact of muscular fitness appears to be low or even non-existent [88-91, 122, 123]. Longitudinal studies examining the association of both CRF and muscular fitness with cardiometabolic health are scarce [87, 88, 123]. These studies concluded that muscle strength has a protective role for the metabolic syndrome [88, 123] or type 2 diabetes [87], largely independent of CRF. In conclusion, CRF appears to be the most important predictor for cardiometabolic health with some studies observing an added value of muscular fitness [89, 90, 122] and others not [88, 91, 124].

We hypothesize that both muscular fitness and CRF are associated with clustered cardiometabolic risk and several of its risk factors. Furthermore, we hypothesize that change in muscular fitness and change in CRF are also associated with change in clustered cardiometabolic risk and cardiometabolic risk factors. Finally, we hypothesize that change in muscular fitness will have a small, but significant, additive contribution towards change in cardiometabolic health, independent of the favourable effects of change in CRF.

5 Description of the sample and design of the study

In 2001 the Flemish Research Centre Sport, Physical Activity and Health was established [125]. The goal of this centre was to carry out policy relevant research concerning sport, physical activity and health in Flanders. Measurements were taken between 2002 and 2004 and funding ended in 2006. Ten years after the start of the first generation research centre, in 2011, a new Flemish Research Centre Sport was established and all participants of the first centre were invited again. Comparable to the first research centre the goal was to perform policy relevant research concerning sport, physical activity and health and measurements were taken between 2012 and 2014. The sampling procedure of the first phases of this study is previously described in detail [125].

Participants included in the present thesis visited the research centre in 2002-2004 and 2012-2014, roughly 10 years apart. Figure 5 gives an illustration of the study population. Some participants were already part of longer running cohort studies from Leuven or were the partner of someone participating in these longitudinal studies. Furthermore, additional participants who were not involved in these longitudinal studies were recruited to enlarge the study population. Consequently, participants can be divided in four main groups according to the way they were recruited.

1. The first group consists of men who participated in the Leuven Growth Study of Belgian Boys (1969-1974), during which 588 boys were followed during their six years of secondary schooling. This study was extended in the Longitudinal Study on Lifestyle, Fitness and Health,

where these men were measured again in 1986, 1991 and 1996 [126]. One hundred and ten men and 45 partners visited the research centre both in 2002-2004 and 2012-2014 and were included in the present analyses.

2. The second group consists of women who participated in the Leuven Growth Study of Flemish Girls (1979-1980). Similarly to the men, these women and their partners were invited to the research centre in 2002-2004 and 2012-2014 of which 38 women and 33 partners visited the centre at both time points.

3. In 2002, to expand the total population, the National Institute of Statistics randomly selected a community sample of 18- to 75 year old men and women. Of this subpopulation 406 participants had measurements taken at both time points.

4. The last group of participants were also added in 2002 to expand the total population and were part of a longitudinal study of the Vrije Universiteit Brussel. Here, 20 volunteers participated at both time points.

This resulted in a total sample of 652 participants. All participants were originally included in all studies incorporated in this thesis. However, when data were missing on important variables or covariates, participants were excluded.

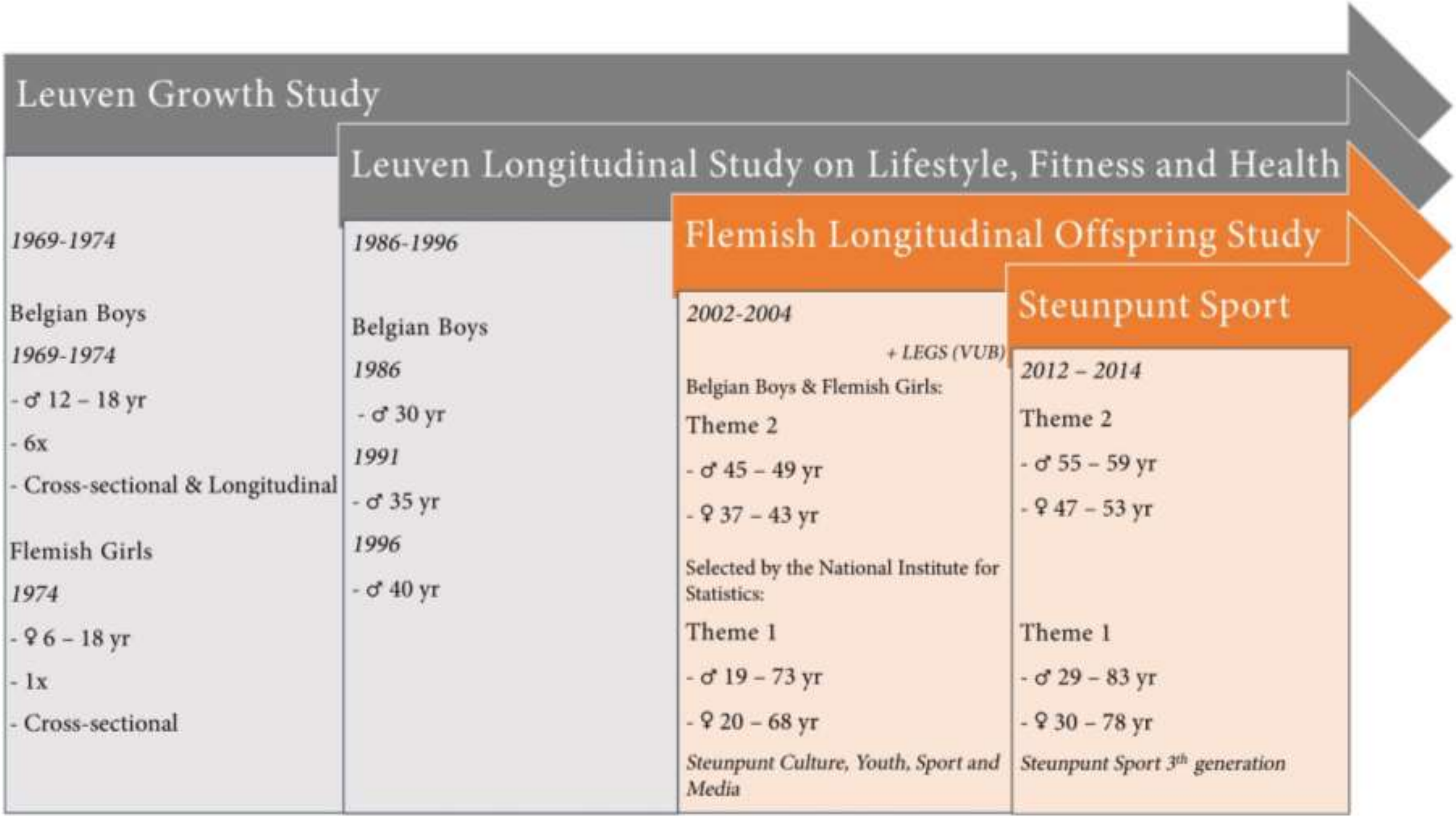


Figure 5. Illustration of the study population.

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SCIENTIFIC PAPERS

PAPER 1

Associations between physical activity and health-related fitness – volume versus pattern

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Associations between physical activity and health-related fitness – volume versus pattern

Abstract

Approximately 3.2 million people die of non-communicable diseases (NCD) each year due to insufficient physical activity. Physical activity guidelines are possibly perceived as too demanding and might thus pose a barrier. We addressed the question if a more stable physical activity pattern is associated with higher levels of health-related fitness than one with high and low intensities, regardless of the physical activity level (PAL). Physical activity was objectively measured in 296 men and women (53.7 ± 8.94 years) with the SenseWear Pro Armband®. Using this data, the PAL and a Gini index were calculated to report the physical activity pattern. Health-related fitness was expressed as a fitness index. PAL was weakly correlated to health-related fitness ($r = 0.38$, $P < .0001$). The Gini index was also weakly correlated to the fitness index ($r = 0.23$, $P < .0001$). Results of the ANCOVA showed that participants in the first quartile of PAL always scored significantly lower for health-related fitness than participants in quartile four, after adjustment for the Gini index. These results suggest that as long as the volume of physical activity is high, health-related fitness will be high as well, independent of the physical activity pattern or variability in intensities throughout the day.

Keywords

Gini-index, physical activity pattern, physical activity level, non-communicable diseases, SenseWear®

Introduction

According to the World Health Organization, approximately 3.2 million people die of non-communicable diseases (NCD), such as cardiovascular disease, cancer, respiratory disease and diabetes, each year due to insufficient physical activity (World Health Organization [WHO], 2011). Physical inactivity is one of the main modifiable and preventable risk factors that can help prevent NCD (Warburton, Nicol, & Bredin, 2006). A study by Tucker et al. shows that when physical activity is objectively measured, more than 90% of adults failed to comply with the American College of Sports Medicine (ACSM) physical activity guidelines of 150 min/week of moderate physical activity, 75 min/week of vigorous physical activity or a combination of moderate and vigorous physical activity (MVPA) (Tucker, Welk, & Beyler, 2011).

Physical activity is defined as any bodily movement produced by skeletal muscles that results in energy expenditure (Caspersen, Powell, & Christenson, 1985). Several studies have demonstrated a dose–response curve for risk for NCD showing that, although a little amount of physical activity is good, more is better, up to a certain point, with benefits of lower intensity- and shorter duration-physical activity than that recommended by the activity guidelines (Hamer & Chida, 2008; Wen, Wai, Tsai, & Chen, 2014; Woodcock, Franco, Orsini, & Roberts, 2011). Furthermore, excessive sedentary behaviour is an independent and qualitatively different risk factor for NCD in comparison to physical activity (Rezende, Rodrigues Lopes, Rey-Lopez, Matsudo, & Luiz Odo, 2014; Tremblay, Colley, Saunders, Healy, & Owen, 2010). Generally, sedentary behaviour is defined as time spent sitting or lying down, in particular, activities that require an energy expenditure lower than 1.5 metabolic equivalent units (METs) (Pate, O'Neill, & Lobelo, 2008). For both active and inactive people, sedentary behaviour demonstrates an inverse dose–response association between sitting time and mortality, independent of physical activity (Katzmarzyk, Church, Craig, & Bouchard, 2009).

Independent from physical activity and sedentary behaviour, health-related fitness is also associated with NCD. Health-related fitness is defined as a cluster of four parameters: cardiovascular fitness, muscular fitness, flexibility and body composition (American College of Sports Medicine, 2014). Because health-related fitness is a more comprehensive construct than cardiorespiratory fitness, it has not only a strong relationship with cardiovascular health and mortality, but also with the ability to perform daily activities with vigour and other hypokinetic diseases, such as lower back pain, injury risk and bone density (American College of Sports Medicine, 2014). Moreover, higher levels of health-related fitness improve the overall mortality risk profile, regardless of the individual's health status and traditional risk factors (Myers et al., 2015).

It is well understood that MVPA will improve health-related fitness (Carrick-Ranson et al., 2014; Church, 2009; Mandic, Myers, Oliveira, Abella, & Froelicher, 2010). The link between lower intensity- and shorter duration-physical activity and health-related fitness is less investigated. Therefore, our aim was to get better insights in the link between the whole range of physical activity intensities, including sleep, and the pattern throughout the day and health-related fitness. Physical activity and sedentary behaviour are continuous variables, but they are often placed on a non-continuous scale. Cut-offs are repeatedly used to classify physical activity and sedentary behaviour. In the present article, the Gini index will represent the stability of the physical activity pattern. The Gini index is a common applied statistical indices in social sciences for measuring statistical dispersion (Bonetti, Gigliarano, & Muliere, 2009; Shkolnikov, Andreev, & Begun, 2003). It is mainly used as a measure of income inequality among individuals or households. However, recently this index is applied to various medical contexts, such as inequality in health, life expectancy and health care (Bonetti et al., 2009) or as a quantification method for sedentary behaviour (Chastin & Granat, 2010). No cut-offs are needed to calculate the Gini index and therefore the physical activity pattern can be observed holistically.

In this article, we objectively measured physical activity, sedentary behaviour and health-related fitness in a sample of the Flemish adult population. We addressed the question if a more stable physical activity pattern is better to improve health-related fitness than one with high and low intensities in physical activity. That is to say, do people differ in health-related fitness if they have the same physical activity level (PAL) but a different way of reaching this (i.e., being continuously lightly active, or very sedentary with a short vigorous interval)? We hypothesised that both activity patterns result in the same level of health-related fitness, taking into account the negative effects of sedentary behaviour and the positive effects of MVPA.

METHODS

Participants and study design

In 1969, the Leuven Growth Study of Belgian Boys (LGSBB) study performed a multi-stage cluster sampling procedure resulting in 4278 selected boys (Matton et al., 2007). In the present study, 110 men are still part of that group. To expand the test group, in 2002, the National Institute of Statistics randomly selected a community sample of 18- to 75-year-old men and women in the Flemish part of Belgium. Between 2012 and 2014, all volunteers who visited the examination centre in 2002–2004 (n = 1569) were re-invited for follow-up, and 652 (42%) volunteers returned to participate. This resulted in male (n = 420) and female (n = 232) volunteers between 28.9 and 82.4 years (mean age: 56.5 ± 9.69 years). The sampling procedure of the first phases of this study is previously described in detail (Matton et al., 2007). Due to dropout during previous phases of the study, the study population is rather healthy and highly educated. For 449 participants, valid physical activity data were available. For another 499 participants, valid fitness data were available, leaving a final sample with both physical activity and fitness data of 296 (45%) participants with a mean age of 53.7 (±8.94) years. If fitness data were not available, most common reasons for exclusion were lower back pain, a higher risk of myocardial infarction, arterial hypertension and abnormalities on an electrocardiogram. An informed consent was obtained from the participants and the study was approved by the Medical Ethics Committee of the KU Leuven (s54083).

Physical activity

Objective measurement of physical activity was obtained with a SenseWear Pro 3 Armband® (BodyMedia, Inc., Pittsburgh, PA, USA), which generates reliable results for daily energy expenditure under free-living conditions (Johannsen et al., 2010). The SenseWear is a multi-sensor body monitor, worn over the triceps muscle of the right arm. Data from multiple sensors, such as two-axis accelerometer and sensors measuring heat flux, galvanic skin response, skin temperature and near body ambient temperature are combined with gender, age, body weight and height, to estimate energy expenditure, physical activity intensity and number of steps, using algorithms developed by the manufacturer (SenseWear Professional software, version 6.1). Body weight and height were measured by trained staff with participants barefoot and in underwear. Participants were asked to wear the monitor for seven consecutive days, 24 h a day, except during water-based activities. The compliance criterion was set at 1296 min (90%) a day. Furthermore, to achieve reliable estimates of physical activity patterns, only participants who met the compliance criterion of at least 3 week days and both weekend days were admitted (Scheers, Philippaerts, & Lefevre, 2012). PAL was calculated from the minute-by-minute SenseWear data as the average MET value during the measured days.

Gini index

The stability of the physical activity pattern was indicated with the Gini index, which is calculated using the accumulation of physical (in-)activity and sleep. The Gini index is a measure of statistical dispersion summarising the inequality of the physical activity pattern of each participant in a single number (Bonetti et al., 2009; Shkolnikov et al., 2003). SenseWear data were used to calculate proportionate distributions for intensity (MET.minute−1) and time (minutes). Subsequently, to create a Lorenz curve, cumulative frequencies were calculated and are plotted against each other. The difference between the Lorenz curve and the line of perfect equality (= a straight line with a slope of 1) generated the Gini index (Shkolnikov et al., 2003). This resulted in a value ranging from 0 to 1, with 0 indicating a linear distribution and a value of 1 indicating complete inequality (Shkolnikov et al., 2003). In the present context, a value of 0 indicated that someone's activity-intensity level was constant during the measurement period, meaning that this participant had not much variability in intensity and had a stable activity pattern. A value of 1 would indicate complete inequality and therefore a very unstable activity pattern. The Gini index does not take into account the intensity or volume of the total movement, therefore, in most analyses, a correction for the total volume or PAL was done.

Health-related fitness

Four parameters were measured to obtain a complete notion of the participant's health-related fitness, according to ACSM (American College of Sports Medicine, 2014). These four health-related components have a strong relation with good health, low risk of premature development of hypokinetic diseases and are characterised by the ability to perform activities of daily living with vigour (American College of Sports Medicine, 2014). As the present study is part of a longer running longitudinal design methods for all four health-related fitness, parameters are described more in detail by Duvigneaud et al. (2008). A short overview is given below.

Body composition

Percentage of body fat (fat%) was evaluated by means of bioelectrical impedance analysis (BIA) on the right side of the body with a tetrapolar BIA analyser (Bodystat 1500, Bodystat Ltd., Isle of Man, UK). Participants were measured in a supine position for 5 min with arms and legs in abduction.

Cardiorespiratory fitness

Peak VO2 was determined by means of a maximal exercise test on an electrically braked Lode Excalibur cycle Ergometer® (Lode, Groningen, The Netherlands). Oxygen consumption was measured directly with breath-by-breath respiratory gas exchange analysis, using a Cortex MetaLyzer 3B analyser (Cortex Biophysic GmbH, Leipzig, Germany) (Duvigneaud et al., 2008), which generates highly reliable results (Meyer, Georg, Becker, & Kindermann, 2001).

Muscular strength and endurance

Total muscular strength was estimated as hand grip strength, measured with a calibrated hand grip dynamometer (Jamar; Sammons Preston Rolyan, Bolingbrook, IL, USA). This is an adequate measurement for total muscle strength, because hand grip strength and total muscle strength are moderately correlated after correction for total body weight (Wind, Takken, Helders, & Engelbert, 2010). A calibrated Biodex System Pro 3® dynamometer (Biodex Medical Systems, Shirley, NY, USA) was used to measure muscular endurance. Isokinetic measurements using this device are found to be reliable and valid (Drouin, Valovich-mcLeod, Shultz, Gansneder, & Perrin, 2004). Endurance of the right upper leg was measured in a standardised manner (Duvigneaud et al., 2008). The test for muscular endurance consisted of 25 knee flexion-extension movements at $180°s^{-1}$. Total work (J) was recorded. Both muscular strength and endurance were divided by the participants' weight to correct for total body weight.

Flexibility

Total body flexibility cannot be tested with one single test. Flexibility of the hamstrings was tested, because of the importance for activities of daily living and sport performance (American College of Sports Medicine, 2014). Sit and reach is a commonly used test for hamstring flexibility as well as lower back and hip-joint flexibility (American College of Sports Medicine, 2014). Participants were asked to place the soles of their bare feet next to each other against the box and lock the knees of both legs. Then a ruler was pushed, keeping both hands parallel, as far as possible.

Fitness index

A fitness index was calculated using Z-scores of all four fitness parameters. Male and female homogenous groups were made for every 10 year age interval. Z-scores were computed for every group and are therefore age and sex corrected, because fitness is age and sex dependent.

The mean of both Z-scores of muscular endurance and muscular strength was computed to attain an average Z-score for muscular fitness. The Z-score for body composition was inversed to account for the fact that a lower fat% is better than a higher one. An average composite Z-score was created for body composition, cardiorespiratory fitness, muscular fitness and flexibility where all four parameters are equally weighed. This final score is the fitness index and is used to assess health-related fitness.

Statistical analysis

Descriptive statistics (means and standard deviations) were calculated for men and women for all variables. Correlations were calculated between the Gini index, the fitness index and PAL. Partial correlations, which is similar to a correlation where covariates are added, were performed between the Gini index and the fitness index corrected for PAL, and between PAL and the fitness index corrected for the Gini index. Additionally, a one-way analysis of variance (ANOVA) was conducted to determine statistically significant differences between the fitness index in quartiles of PAL and quartiles of the Gini index. Tukey's method, a single-step multiple comparison, was used to identify significant differences between means. Furthermore, a one-way analysis of covariance (ANCOVA) was applied to evaluate the effect of PAL with the Gini index as a covariate on health-related fitness. Similarly, a one-way ANCOVA was applied to evaluate the effect of the Gini index with PAL as a covariate on health-related fitness. Least squares means of the fitness index and all fitness parameters were computed for all quartiles of PAL and all quartiles of the Gini index and significant differences were noted.

All statistical analyses were performed using the SAS statistical program, version 9.4 (SAS institute, Cary, NC, USA). Statistical significance was set at $P < .05$ and all statistical tests were two-tailed.

RESULTS

Table 1 presents descriptive statistics for physical activity and health-related fitness of the participants categorised by sex. Table 2 presents the descriptive statistics of physical activity and the Gini index for quartiles of PAL and quartiles of the Gini index.

There was a strong correlation between PAL and the Gini index ($r = 0.76$, $P < .0001$), which means that increases in PAL were strongly related to increases in the Gini index. PAL was weakly correlated to the fitness index ($r = 0.38$, $P < .0001$). The Gini index was also weakly correlated to the fitness index (r = 0.23, P < .0001). Furthermore, there was a partial correlation between PAL and the fitness index controlled for the Gini index (r = 0.32, P = < .0001). Another partial correlation was computed to assess the relation between the Gini index and the fitness index controlled for PAL where we did not find a partial correlation (r = 0.09, P = 0.11).

Table 1. Descriptive statistics for age, physical activity & physical fitness categorized by sex

	Women (n=111)		Men (n=185)	
Variables	*M*	*SD*	*M*	*SD*
Age	52.84	8.21	54.22	9.34
Bodyweight (kg)	66.01	10.27	79.97	10.61
PAL (MET)	1.49	0.22	1.53	0.22
Gini-index	0.30	0.03	0.31	0.04
Fat%	30.34	4.93	20.34	4.61
VO_2 peak ($ml \cdot min^{-1} \cdot kg^{-1}$)	30.36	5.54	38.73	8.02
Muscular strength ($kg \cdot kg^{-1}$)	0.49	0.09	0.66	0.12
Muscular endurance ($J \cdot kg^{-1}$)	28.91	6.89	37.05	9.75
Flexibility (cm)	23.96	9.63	16.86	10.34

Note. *M* = mean, *SD* = standard deviation; VO_2peak, muscular strength & muscular endurance are relative to total bodyweight.

Table 2. Descriptives for physical activity and the Gini index over quartiles of PAL and Gini index[2]

Quartiles of PAL	Q1	*SD*	Q2	*SD*	Q3	*SD*	Q4	*SD*
PAL	1.24	0.07	1.41	0.04	1.56	0.05	1.80	0.11
Gini	0.26	0.03	0.29	0.02	0.32	0.03	0.34	0.02
Sedentary Time	1131	53	1086	62	1029	53	950	75
Light PA	210	57	247	70	253	64	229	76
MVPA	69	28	107	27	158	38	260	59
Quartiles of Gini index	**Q1**	***SD***	**Q2**	***SD***	**Q3**	***SD***	**Q4**	***SD***
PAL	1.30	0.11	1.44	0.13	1.58	0.19	1.73	0.16
Gini	0.25	0.02	0.29	0.01	0.32	0.01	0.35	0.01
Sedentary Time	1150	57	1059	75	1016	94	983	71
Light PA	217	56	266	80	247	68	212	57
MVPA	72	28	115	35	176	64	245	67

Note. PAL = Physical activity Level; Gini = Gini-index; MVPA = Moderate-and-vigorous physical activity

[2] Adapted table in supplement

Additionally, there was a significant effect of all four quartiles of PAL on the fitness index [F (3,292) = 16.99, P = < .0001] computed by means of an ANOVA. A similar, but smaller effect in the fitness index was found for all four quartiles of the Gini index [F (3,292) = 5.30, P = < .0014]. The means of the fitness index and significant differences for all quartiles of PAL and quartiles of the Gini index are reported in Figure 1. Means and significant differences of the Z-scores of all health-related fitness parameters are reported in Table 3. Furthermore, an ANCOVA was performed to control for covariates. A statistically significant difference was found between all four quartiles of PAL on the fitness index controlling for the Gini index. There was a significant effect of PAL on health-related fitness after controlling for the stability of the physical activity pattern [F (4, 291) = 13.04, P < .0001]. A similar statistical model was used to determine a difference between all four quartiles of the Gini index on the fitness index, controlling for PAL. No significant results were found with this model.

Table 3. Means of Z-scores of health-related fitness parameters for quartiles of PAL and Gini Index.

	Q1	95% CI	Q2	95% CI	Q3	95% CI	Q4	95% CI
Fat%	-0.64^{abc}	-0.86 , -0.41	-0.05^{bc}	-0.26 , 0.16	0.17^{c}	-0.05 , 0.38	0.46	0.26 , 0.67
VO_2peak (ml.min^{-1}.kg^{-1})	-0.49^{abc}	-0.71 , -0.26	-0.02^{bc}	-0.23 , 0.18	0.07^{c}	-0.14 , 0.28	0.48	0.28 , 0.69
Muscular strength (kg.kg^{-1})	-0.40^{ac}	-0.64 , -0.16	0.06	-0.16 , 0.29	-0.02	-0.25 , 0.21	0.23	0.01 , 0.45
Muscular endurance (J.kg^{-1})	-0.28^{ac}	0.52 , -0.05	0.21	0.00 , 0.43	0.06	-0.17 , 0.28	0.20	-0.02 , 0.41
Flexibility (cm)	-0.08^{c}	-0.32 , 0.16	-0.09^{c}	-0.31 , 0.14	-0.11^{c}	-0.34 , 0.12	0.20	-0.02 , 0.41
Fat%	-0.35^{bc}	-0.58 , -0.13	0.04	-0.19 , 0.26	0.09	-0.14 , 0.31	0.27	0.04 , 0.49
VO_2peak (ml.min^{-1}.kg^{-1})	-0.34^{abc}	-0.55 , -0.12	-0.15^{c}	-0.36 , 0.07	0.09^{c}	-0.12 , 0.30	0.52	0.30 , 0.73
Muscular strength (kg.kg^{-1})	-0.09^{bc}	-0.32 , 0.15	-0.03^{c}	-0.26 , 0.21	-0.11	-0.24 , 0.22	0.06	-0.18 , 0.29
Muscular endurance (J.kg^{-1})	0.04	-0.19 , 0.27	0.04	-0.19 , 0.27	0.04	-0.19 , 0.27	0.12	-0.11 , 0.35
Flexibility (cm)	-0.15	-0.38 , 0.08	0.05	-0.18 , 0.27	0.17	-0.06 , 0.40	-0.13	-0.36 , 0.10

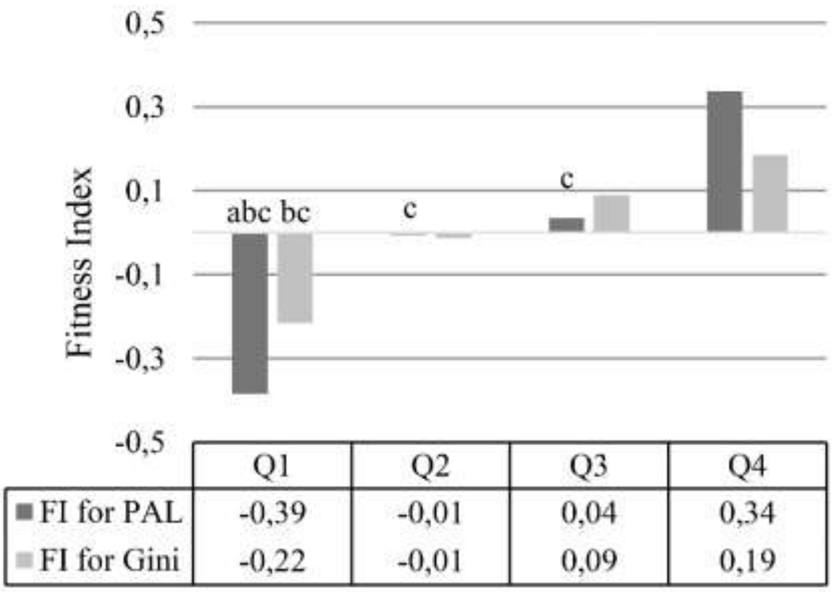

Figure 1. Means of fitness index for PAL and Gini index

Legend: Means of fitness index (FI) for quartiles of Physical Activity Level (PAL) and quartiles of the Gini index for men and women together. Significances are calculated with Tukey's method; [a] is significantly different from Q2; [b] is significantly different from Q3; [c] is significantly different from Q4; p =< .05

Figure 2 shows the least square means of the fitness index of quartiles for PAL corrected for the Gini index and for the Gini index corrected for PAL. To allow comparison of all quartiles of PAL, the least square means of the Z-scores of all health-related fitness parameters are reported in Table 3. For PAL, participants in the first quartile always scored significantly lower than participants in quartile four after adjustment for the Gini index. For all health-related fitness parameters, the lowest quartiles of PAL score poorer than the higher quartiles.

Least squares means of fitness index (FI) for quartiles of physical activity level (PAL) adjusted for the Gini index, and quartiles of the Gini index adjusted for PAL for men and women together; asignificantly different from Q2; bsignificantly different from Q3; csignificantly different from Q4; P = < .05.

The same comparison was made for all quartiles of the Gini index, after adjustment for PAL (Table 4). In this comparison, fewer differences in Z-scores were found and the differences found were smaller. There was also a less pronounced trend in the Z-scores of the four quartiles.

Table 4. Adjusted least squares means of Z-scores of health related fitness parameters for quartiles of PAL and the Gini index.

	Q1	95% CI	Q2	95% CI	Q3	95% CI	Q4	95% CI
	Physical Activity Level							
Fat%	-0.76[abc]	-1.04 , -0.48	-0.09[c]	-0.30 , 0.12	0.20[c]	-0.02 0.42	0.57	0.32 , 0.82
VO_2peak (ml.min^{-1}.kg^{-1})	-0.28[c]	-0.55 , -0.01	0.04	-0.17 , 0.25	0.01	-0.20 , 0.23	0.30	0.05 , 0.55
Muscular strength (kg.kg^{-1})	-0.61[abc]	-0.91 , -0.31	0.00[c]	-0.23 , 0.23	0.04[c]	-0.20 , 0.27	0.41	0.15 , 0.68
Muscular endurance (J.kg^{-1})	-0.39[abc]	-0.68 , -0.09	0.18	-0.04 , 0.40	0.09	-0.14 , 0.32	0.29	0.02 , 0.55
Flexibility (cm)	-0.24[c]	-0.54 , 0.06	-0.13[c]	-0.36 , 0.09	-0.06[c]	-0.30 , 0.17	0.33	0.07 , 0.60
	Gini Index							
Fat%	0.16	-0.10 , 0.42	0.02[c]	0.00 , 0.43	-0.08	-0.29 , 0.14	-0,26	-0.52 , 0.00
VO_2peak (ml.min^{-1}.kg^{-1})	-0.07	-0.32 , 0.19	-0.05	-0.27 , 0.16	0.01	-0.21 , 0.22	0.24	-0.01 , 0.50
Muscular strength (kg.kg^{-1})	0.30[bc]	0.02 , 0.57	0.11[c]	-0.13 , 0.34	0.13	-0.36 , 0.10	-0.34	-0.62 , -0.06
Muscular endurance (J.kg^{-1})	0.27	0.00 , 0.55	0.12	-0.11 , 0.35	-0.04	-0.27 , 0.20	-0.12	-0.40 , 0.16
Flexibility (cm)	-0.02	-0.31 , 0.25	0.09	-0.14 , 0.32	0.13[c]	-0.10 , 0.36	-0.25	-0.53 , 0.03

Note. [a] is significantly different from Q2; [b] is significantly different from Q3; [c] is significantly different from Q4; p =< .05

Least squares means of health-related fitness parameters for quartiles of PAL adjusted for GI, and quartiles of GI adjusted for PAL for men and women together.

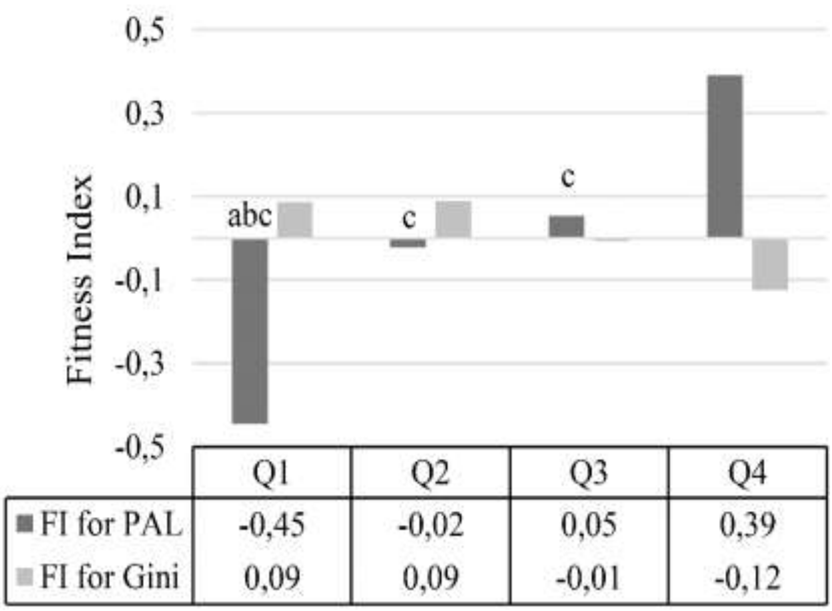

Figure 2. Adjusted least squares means of fitness index for PAL and Gini index

Legend: Least squares means of fitness index (FI) for quartiles of Physical Activity Level (PAL) adjusted for the Gini index, and quartiles of the Gini index adjusted for PAL for men and women together; [a] is significantly different from Q2; [b] is significantly different from Q3; [c] is significantly different from Q4; p =< .05

DISCUSSION

There were three major findings from this cross-sectional study. First, physical activity is related to health-related fitness, regardless of the stability of the physical activity pattern in a healthy adult population. Second, people in the lowest quartile of PAL have a lower health-related fitness than people in the highest quartile, when corrected for the stability of the physical activity pattern. Third, there was no pronounced trend in health-related fitness over quartiles of the Gini index, representing stability of the physical activity pattern. These results imply that as long the volume of physical activity is high, health-related fitness will be high as well, independent of the physical activity pattern. In other words, people's health-related fitness does not differ if they have the same volume of physical activity but a different way of reaching it (i.e., continuously lightly active, or very sedentary with a short vigorous interval). These findings are in line with our hypothesis that both activity patterns result in the same level of health-related fitness, taking into account the negative effects of sedentary behaviour and the positive effects of MVPA.

We here linked physical activity with health-related fitness. There was a moderate correlation between physical activity and health-related fitness when controlling for the Gini index. This link is important because the mortality risk reduction obtained by being fit is significantly different and stronger than obtained by merely being active (Myers et al., 2015; Williams, 2001). Put differently, the dose–response gradient for cardiorespiratory fitness is steeper than the one for physical activity (Blair, Cheng, & Holder, 2001). However, physical activity is a behaviour that can be changed, whereas health-related fitness is a physiological measure reflecting a combination of physical active behaviour, genetic potential and functional health of various organ systems, with physical activity as most important adjustable environmental component (Carnethon et al., 2010; DeFina et al., 2015). For most individuals, increases in physical activity are associated with increases in health-related fitness (Carrick-Ranson et al., 2014). The results of the present study support this. Therefore, it is said that physical activity has a double importance to risk reduction for NCD (DeFina et al., 2015). First, it has a direct effect on risk reduction for NCD. Second, being physically active will help to improve someone's health-related fitness and therefore reduce the risk for NCD (DeFina et al., 2015).

Furthermore, the findings in our study suggest that lower intensities of physical activity, in larger volumes, are also positively associated with health-related fitness. A study by Lee et al. confirms that lower intensities of physical activity, without MVPA, can have positive effects on health. A study, in which 17,000 women reported no vigorous activity, but who walked regularly, had about half the coronary heart disease (CHD) risk in comparison with women who did not walk regularly (Lee, Rexrode, Cook, Manson, & Buring, 2001). Furthermore, it was not the walking pace, but the time spent on walking that was significantly related to lower CHD rates. This implies that the volume is an important determinant for health. Additionally, a systematic review containing almost 1 million people, found that non-vigorous physical activity had a dose–response protective effect for all-cause mortality. The largest benefit was found in shifting from sedentary behaviour to low levels of activity (Woodcock et al., 2011). These results support the hypothesis that lower levels of physical activity than those advised in the current guidelines are also associated with health and a reduced all-cause mortality. In other words, risk reduction for NCD and mortality is greatest when there is a positive shift in the lowest end of the activity scale (Kodama et al., 2009; Warburton et al., 2006; Woodcock et al., 2011). Furthermore, walking, a non-vigorous physical activity, appears to be the preferred exercise among sedentary individuals taking up physical activity (Dunn et al., 1998; Hamer & Chida, 2008). Besides that, a pattern of decreasing vigorous physical activity across the life course is apparent among women, which is possibly linked to health-related attitudes and behaviours (Evenson et al., 2002). Consequently, it might seem more realistic for the majority of the adult population to sustain lower intensities of physical activity in longer duration.

Just as for physical activity, data demonstrate a dose–response association between sedentary behaviour and mortality (Katzmarzyk et al., 2009). In this article, we also assessed if people with an unstable physical activity pattern, thus those sitting for a long time but with a few peaks in physical activity, have a different health-related fitness than those with a more stable physical activity pattern. To control for total physical activity, regardless of the intensity, we controlled for PAL in all analyses. Results suggest that the stability in the physical activity pattern is not decisive for health-related fitness. In this context, we can speculate that the total volume of physical activity is more important than the variability in the pattern of the activity. Duvivier et al. confirmed that for insulin action and plasma lipids, 1 h of physical activity cannot compensate for the negative effects of sedentary behaviour. In this randomised controlled trial, reducing inactivity by increasing the time spent on walking or standing was more effective than 1 h of physical exercise, when energy expenditure was kept constant (Duvivier et al., 2013).

This study had some limitations. First, for several participants valid data was not available. In case of invalid SenseWear data, absence of data was mostly because of lack of compliance to the inclusion criteria of three weekdays and two weekend days, and 90% wearing time. We can assume that not complying with these criteria was a random event and therefore did not influence the results. An unpaired t-test confirmed this assumption as there were no significant differences between the included test group and the dropout group in age, sex, fat%, VO2peak, flexibility and muscular strength and endurance (P > .05). For another group, valid fitness data was not available. The most important reason for not having valid fitness data available was exclusion by a physician. Most common reasons for exclusion were lower back pain, a higher risk of myocardial infarction, arterial hypertension, abnormalities on an electrocardiogram or good clinical judgment. Because of these exclusion criteria, the least fit participants were probably excluded. This results in a more fit and homogenous sample. Subsequently, this could, at least in part, lead to an underestimation of the relation between health-related fitness and physical activity and its pattern. Second, according to a study of Johannsen, the SenseWear underestimates total energy expenditure, particularly at higher intensities, however our focus was not higher intensity activities, but the whole range of intensities (Johannsen et al., 2010). Furthermore, there was a rather high collinearity between PAL and the Gini index, which indicates that in general people with a higher volume of physical activity had a more unstable physical activity pattern and spent more time in MVPA. Moreover, to include the fact that the Gini index itself does not contain intensities, only variability of the pattern, we controlled for PAL in all analyses and therefore increasing the risk of over adjustment. Finally, because the sample is a highly fit Caucasian group of relatively healthy adults, the sample might not be representative for all adults.

A strength of this study was the objective measurement of physical activity in all analyses. Objective measurement provides a more accurate and complete insight into the participants' physical activity and its pattern. Another strength of objective measurement is that the whole range of energy expenditure rates can be observed (Pate et al., 2008). In the present study, SenseWear was worn 24 h a day. The entire range of activity, from completely sedentary to very vigorous, was measured in free-living conditions. The SenseWear is capable of detecting subtle changes, even in lower-intensity activities, because of the inclusion of thermal and perspiration-related sensors next to the accelerometer information (Johannsen et al., 2010). Sleep was also included in all analyses. Because on an average, almost one-third of a day is sleeping time. A meta-analysis by Ju et al. revealed a U-shaped relation between sleep duration and the risk of metabolic syndrome (Ju & Choi, 2013). It could be argued that including sleep can affect the results, because interrupting sleep will increase the Gini-index and will therefore be included as a positive aspect. However, interrupting sleep will have negative health outcomes (Rangaraj & Knutson, 2015). Therefore, all analysis were also performed excluding sleep, though this induced no significant differences in results. Furthermore, most studies addressing physical activity have used absolute cut-offs for sedentary behaviour, light, moderate and vigorous physical activity. In this study, no cut-offs are used and most analysis were done with a continuous approach because the Gini index, which is used to express the stability of the physical activity pattern, is a continuous parameter.

In summary, people do not differ in health-related fitness when having a different activity pattern. However, health-related fitness does differ by PAL suggesting that a high volume of physical activity is more important than the physical activity pattern in which this volume is attained. This might implicate that a long duration of light physical activity, can also have a positive impact on health-related fitness, similar to a shorter duration of vigorous physical activity. These observations may implicate relevant insights to health policy, because current guidelines mostly focus on minutes of MVPA and exercise and not on the total volume of physical activity. Our intention is not to devalue the well-established health benefits of higher intensities. However, results of the present study suggest that lower intensities of physical activity could also be included into the health norm as long as the physical activity volume is high. To increase generalisability, further research should extend these findings in various populations such as children or an at risk populations preferably using longitudinal approaches. In addition lifestyle interventions should focus on achieving a high PAL, on the one hand by achieving a long duration of lower intensities, on the other hand by performing a shorter duration of vigorous intensity.

ACKNOWLEDGEMENTS

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Table 2 addapted. Descriptives for physical activity and the Gini index over quartiles of PAL and Gini index

Quartiles of PAL	Q1	*SD*	Q2	*SD*	Q3	*SD*	Q4	*SD*
PAL	1.24	0.07	1.41	0.04	1.56	0.05	1.80	0.11
Gini	0.26	0.03	0.29	0.02	0.32	0.03	0.34	0.02
Sleep (minutes/day)	406	58	397	71	389	55	378	54
Sedentary Time (minutes/day)	757	81	692	95	639	72	571	78
Light PA (minutes/day)	210	57	247	70	253	64	229	76
MVPA (minutes/day)	69	28	107	27	158	38	260	59
Quartiles of Gini index	**Q1**	***SD***	**Q2**	***SD***	**Q3**	***SD***	**Q4**	***SD***
PAL	1.30	0.11	1.44	0.13	1.58	0.19	1.73	0.16
Gini	0.25	0.02	0.29	0.01	0.32	0.01	0.35	0.01
Sleep (minutes/day)	394	64	395	64	379	64	394	52
Sedentary Time (minutes/day)	757	78	665	88	636	105	589	79
Light PA (minutes/day)	217	56	266	80	247	68	212	57
MVPA (minutes/day)	72	28	115	35	176	64	245	67

Note. PAL = Physical activity Level; Gini = Gini-index; MVPA = Moderate-and-vigorous physical activity

Adapted from Knaeps et al. 2016

PAPER 2

Substituting sedentary time with light and moderate-to-vigorous physical activity is associated with better cardio-metabolic health

Knaeps, S., Bourgois, J.G., Charlier, R., Mertens, E., & Lefevre, J. (2016). Submitted to International Journal of Behavioural Nutrition and Physical Activity

Substituting sedentary time with light and moderate-to-vigorous physical activity is associated with better cardio-metabolic health

ABSTRACT

Background Apply a more novel approach to systematically examine associations of clustered cardio-metabolic risk and cardio-metabolic risk factors, and substituting sedentary time with either sleep, light physical activity (LPA) or moderate-and-vigorous physical activity (MVPA). Furthermore, the extra benefit of increasing physical activity intensity from LPA to MVPA was explored.

Methods Physical activity and sleep were objectively measured in 418 Flemish adults (55.5(±9.6) year, 64% men) with a SenseWear Pro 3 Armband. Cardio-metabolic risk factors (obesity, hyperglycemia, dyslipidemia and hypertension,), cardiorespiratory fitness and covariates were objectively measured. Isotemporal substitution analyses were performed to assess the associations between substituting time from a potentially negative behavior into another, potentially positive, behavior.

Results Substituting sedentary time with MVPA was associated with decreased clustered cardio-metabolic risk ($b = -0.02$, $p<0.05$) and substituting LPA with MVPA further improved clustered cardio-metabolic health ($b= -0.03$, $p<0.05$). Substituting sedentary time with LPA or sleep improved waist circumference, High Density Lipoprotein cholesterol, systolic and diastolic blood pressure, and waist circumference, respectively.

Conclusion Results suggest that replacing sedentary time with either sleep, LPA and MVPA was positively associated with several cardio-metabolic risk factors. Interventions for increasing cardio-metabolic health should focus on replacing sedentary time with either sleep, LPA or MVPA depending on the risk parameters that need to be targeted.

KEY WORDS

Sedentary time, light physical activity, moderate-and-vigorous physical activity, sleep, isotemporal substitution, cardio-metabolic health

BACKGROUND

The cardio-metabolic health benefits of physical activity can no longer be denied, as the lack of physical activity is one of the most important predictors of mortality and burden of disease [1]. Lately, sedentary time has also been confirmed as a cardio-metabolic health risk in itself, and meeting the guidelines for physical activity does not make up for a sedentary lifestyle [2]. Therefore, it is important to accumulate 150 minutes of moderate-intensity physical activity a week and try to limit prolonged sedentary time during waking hours [3-5]. However, as our day is limited to 24h, the question arises what changes in health will result from substituting a potentially negative behavior into another, potentially positive, behavior. Furthermore, sleep has also been suggested as an important predictor for cardio-metabolic health, where short and long sleep durations are associated with an increased risk for cardio-metabolic diseases [6].

Recently, a relatively new method of analysis, isotemporal substitution, was introduced. This technique estimates the effect of replacing one form of behavior with another form of behavior for the same amount of time [7]. An important benefit is that it not only estimates the effect of increasing a certain behavior, but also integrates the effect of reducing the specific behavior that it replaces [7]. For example, reducing sedentary time and replacing it with sleep or light-intensity physical activities (LPA) will result in a different health benefit than replacing the same amount of sedentary time with moderate-to-vigorous physical activity (MVPA) [8-11]. Results from previous research clearly state that substituting sedentary time with MVPA will lead to lower clustered cardio-metabolic health risk, than substituting with LPA or sleep. Nonetheless, some uncertainty remains if substituting sedentary time with LPA will already attain positive results [8-10, 12]. Furthermore, only a few papers included substituting sedentary time with sleeping time, and sleeping time with physical-active behaviors, such as LPA or MVPA [8, 13]. Results indicate that substituting sedentary time with sleep will also reduce clustered cardio-metabolic risk [8], potentially only in short sleepers [13]. However, none of these studies included objectively measured sleeping time.

The purpose of the current study therefore was to apply a more novel approach to systematically examine the cardio-metabolic health benefits of substitution sedentary time with either sleep, LPA or MVPA. Furthermore, the extra benefit of increasing physical activity intensity from LPA to MVPA, was also explored. Firstly, we hypothesize that substituting sedentary time with either sleep, LPA or MVPA, will be associated with a lower clustered cardio-metabolic risk and better outcomes on five cardio-metabolic health factors (waist circumference, fasting glucose, triglycerides, High Density Lipoprotein cholesterol (HDL-cholesterol) and blood pressure). Secondly, we hypothesize that substituting LPA with MVPA will be associated with a small but significantly reduced clustered cardio-metabolic risk and its factors.

METHODS

Subjects and study design

This cross-sectional study is part of a longitudinal study of which the sampling procedure is previously described in detail by Matton et al. [14]. Participants were recruited between 2002 and 2004 in Flanders, Belgium. All participants were invited again in 2012-2014, which resulted in male (n = 420) and female (n = 232) volunteers between 29 and 82 years. For 445 participants valid physical activity data were available. Some participants had missing values for cardio-metabolic markers or covariates, leaving a final sample of 410 (63%). An informed consent was obtained from the participants and the study was approved by the Medical Ethics Committee of the KU Leuven (s54083).

Physical activity, sedentary behavior and sleep

Objective measurement of physical activity was obtained with a multi-sensor SenseWear Pro 3 Armband® (BodyMedia, Inc, Pittsburgh, PA, USA), which generates valid results for daily energy expenditure under free-living conditions [15-17]. Estimates of sleep and wake parameters were extracted from multiple sensors, such as two-axis accelerometer and sensors measuring heat flux, galvanic skin response, skin temperature and near body ambient temperature and are combined with gender, age, body weight and height, using algorithms developed by the manufacturer (SenseWear Professional software, version 6.1). Participants were asked to wear the monitor for seven consecutive days, 24 hours a day, except during water-based activities. The compliance criterion was set at 1296 minutes (90 %) a day. Furthermore, to achieve reliable estimates of total physical activity, only subjects who met the compliance criterion of at least 3 week days and both weekend days were admitted, resulting in the exclusion of 107 (16%) participants [18]. The following physical activity intensity categories were assigned to wake time: Sedentary time <=1.5 MET, LPA 1.5 – 3 MET; and MVPA as >3 MET, sleep time was defined by algorithms developed by the manufacturer [17].

Cardio-metabolic risk factors and a clustered cardio-metabolic risk score

Cardio-metabolic risk factors were based on the International Diabetes Federation criteria for the Metabolic Syndrome (including waist circumference, fasting glucose, triglycerides, HDL-cholesterol and blood pressure) [19]. Because data about medicine intake or treatment was missing, risk factors were only based on the specific cut-offs for each cardio-metabolic risk factor [19]. Subsequently, a clustered cardio-metabolic risk score (CMRS) was calculated [20]. Metabolic factors were assessed by trained staff in the morning after an overnight fast. Waist circumference was measured to the nearest 0.1 cm. Systolic and diastolic blood pressure were measured by electronic monitor (Omron, The Netherlands) three times in seated position from the right arm. The means of the three measurements were used in statistical analyses. Triglycerides, plasma glucose and HDL-cholesterol were obtained from an antecubital vein and analyzed by enzymatic methods (Abbott Laboratories, Abbott Park, IL). Due to skewness, values for the latter three risk factors were first normalized (log10). Subsequently, standardized values for waist circumference, triglyceride, plasma glucose, blood pressure and the inverse of HDL-cholesterol were computed. Each cardio-metabolic variable was standardized by using the sex-specific baseline sample mean and standard deviation, derived from all men and women with baseline data for each cardio-metabolic variable. To calculate the CMRS, the sum of these standardized values was divided by the number of metabolic factors included (n=5).

Covariates

Smoking behavior was assessed using the WHO Monica Smoking Questionnaire. Participants were classified as current, former or never smokers. Education level was used as an indicator of socio-economic status, and was ranked in four categories, ranging from no degree or primary school degree; secondary school degree; professional bachelor degree; and higher.

Nutritional intake was assessed using a three-day diet record, during two weekdays and one weekend day. Participants were instructed to weigh and record all foods and drinks or alternatively estimate portions using standard household measures such as a plate, spoon or glass. The diet records were analyzed using Becel Nutrition software (Unilever Co., Rotterdam, The Netherlands) and the Healthy Eating Index, which captures the key recommendations of the 2010 Dietary Guidelines (HEI), was calculated [21].

Peak VO2 was determined by means of a maximal exercise test on an electrically braked Lode Excalibur cycle ergometer® (Lode, Groningen, The Netherlands). Oxygen consumption was measured directly with breath-by-breath respiratory gas exchange analysis, using a Cortex MetaLyzer 3B analyzer (Cortex Biophysic GmbH, Leipzig, Germany) [22]. Participants were verbally encouraged to reach a maximal level of exertion and the test was terminated when subjects were exhausted or when the physician stopped the test for medical reasons. Participants first had to pass a physical examination and people at risk for heart failure and arterial hypertension did not participate in the maximal exercise test.

Statistical analysis

Descriptive statistics (means and standard deviations) were calculated for all variables and represented for the entire sample. Comparison in characteristics between individuals with 0-1, 2, and 3 or more cardio-metabolic risk factors was performed by one-way analysis of variance and χ^2 contingency analysis, differences between means were analyzed with the Tukey method. The association between sleep and CMRS was tested for linearity to determine whether analyses needed to be corrected for sleep duration. Pearson correlations were calculated to determine associations between sleep and various physical activity intensities and cardio-metabolic health.

The isotemporal substitution model, by definition, estimates the effect of replacing one physical activity type with another physical activity type for the same amount of time [7]. An isotemporal substitution analysis (Model 1) was presented to examine the associations between replacing sedentary time with an equivalent time of sleep, LPA or MVPA and cardio-metabolic health.

Isotemporal substitution requires approximately linear associations between each exposure and the outcome, therefore together with all other relevant assumptions, linearity was tested (results not shown). All activity variables (sleep, LPA, MVPA), with the exception of sedentary time, were simultaneously included into a regression model, along with a total wear time variable and all relevant covariates (age, sex, education level, HEI and smoking status). The total wear time variable represents the time of the omitted activity and therefore total time is constant [7]. Substitution model 1 is expressed as follows:

Cardio-metabolic risk = (b1) sleep + (b2) LPA + (b3) MVPA + (b4) total wear time

+ (b5) covariates.

The remaining b1, b2, b3-coefficients represent the increase or decrease in cardio-metabolic health by substituting that activity instead of sedentary time, while holding other activity types constant.

Similarly, a model was constructed with sleep, sedentary time, MVPA, covariates and total wear time, leaving out LPA (Model 2).

Cardio-metabolic risk = (b1) sleep + (b2) sedentary time + (b3) MVPA + (b4) total wear time + (b5) covariates.

All statistical analyses were performed using the SAS statistical program, version 9.4 (SAS institute, Cary, NC, USA). Statistical significance was set at $p < .05$ and all statistical tests were two-tailed.

RESULTS

Analyses included 410 subjects, 64% men with a mean age of 55.5 (±9.6) year. The majority of the population had a higher education (58%) and had never smoked (56%) or were former smokers (36%), and have a HEI of 49 (±10). Table 1 presents the participants descriptive statistics for sleep, physical activity and cardio-metabolic markers. Total wear time was 1,424 (±12) minutes, which is 99% of a day. VO2peak of the participants (n=328) was on average 33.76 (±9.12) $ml.min^{-1}.kg^{-1}$.

Table 1. Descriptive statistics for sleep, physical activity and cardio-metabolic risk factors

	Total *(n=410)*	
Variables	**M**	**SD**
Sleep & physical activity		
Sleep (minutes/day)	389	60
Sedentary time (minutes/day)	646	110
Light physical activity (minutes/day)	238	73
MVPA (minutes/day)	151	88
Cardio-metabolic markers		
Waist Circumference (cm)	85.67	10.29
Fasting Glucose (mmol/L)	5.15	0.63
HDL-cholesterol (mmol/L)	1.53	0.39
Triglycerides (mmol/L)	1.15	0.63
Diastolic Blood Pressure (mmHg)	85.97	9.47
Systolic Blood Pressure (mmHg)	134.71	17.22

Note. M = mean, SD = standard deviation

MVPA = Moderate-to-vigorous physical activity.

Only two participants slept more than nine hours a day, but 120 (29%) participants had a short sleep duration of less than six hours, including 30 (7%) participants sleeping less than five hours a day. The association between sleep and CMRS was linear and there were no significant differences in cardio-metabolic parameters and MVPA between short and normal sleep durations (results not shown). Therefore, analyses were not stratified by sleep duration.

Forty-one % of participants had 2 or more risk factors (Table 2). These participants were older, had higher weight, and lower cardio-respiratory fitness, MVPA and education level than participants with 0-1 risk factors. Participants with 3 or more risk factors were more sedentary than participants with 0-1 risk factors.

Table 2. Characteristics of 410 participants according to the number of cardio-metabolic risk factors present

	0-1 risk factor	2 risk factors	>= 3 risk factors	P value
Prevalence	242 (59%)	107 (26%)	61 (15%)	
Sex (% male)	62	61	74	0.18
Age (years)	54 (±10)	57(±9)*	59(±9)*	<0.001
Weight (kg)	70.7(±10.1)	78(±11.4)*	88.2(±12.0)*	<0.001
VO2peak[a] ($ml.min^{-1}.kg^{-1}$)	35.9(±9.6)	30.7 (±6.9)*	29.3(±6.7)*	<0.001
Sleep (minutes/day)	384(±61)	397(±58)	390(±62)	0.17
Sedentary time (minutes/day)	632(±76)	661(±100.5)	671(±121)*	0.012
LPA (minutes/day)	237(±76)	241(±61)	242(±81)	0.82
MVPA (minutes/day)	171(±92)	126(±74)*	124(±76)*	<0.001
Healthy Eating index	50(±10)	49(±10)	47(±9)	0.21
Education level (0-4)	2.88(±0.79)	2.68(±0.85)*	2.38(±0.66)*	<0.001

[a] n=328

* mean is significantly different from 0-1 risk factor

Sedentary time was correlated with sleep ($r = -0.40$, $p < .001$), LPA ($r = -0.42$, $p < .001$) and MVPA ($r = -0.62$, $p < .001$). Sleep was correlated with LPA ($r = -0.12$, $p < .05$) and LPA with MVPA ($r = -0.21$, $p < .001$). MVPA and sleep were not significantly correlated. Pearson correlation coefficients for the association between sleep, sedentary time and physical activity and cardio-metabolic risk factors are presented in Table 3.

Table 3. Pearson correlation coefficients for the association between sleep, sedentary time and physical activity (LPA and MVPA), and cardio-metabolic health parameters

	CMRS	Waist Circumference	Fasting Glucose	HDL-cholesterol	Triglycerides	Systolic Blood Pressure	Diastolic Blood Pressure
Sleep	0.06	-0.09	0.04	0.10*	0.03	0.05	-0.03
Sedentary time	0.14**	0.29***	0.03	-0.27***	0.11**	0.12*	0.12*
LPA	0.11*	-0.04	-0.04	0.22**	-0.02	-0.15**	-0.09
MVPA	-0.30***	-0.26***	-0.03	0.09	-0.15**	-0.04	-0.05

CMRS = Clustered cardio-metabolic risk, LPA = Light physical activity, MVPA = Moderate-to-vigorous physical activity.

*p<0.05; **p<0.01;***p<0.001

Regression coefficients and 95% confidence intervals of substituting sedentary time with sleep, LPA and MVPA are reported in Table 4. Results show that substituting sedentary time with sleep was also associated with a significant decrease in waist circumference. Substituting sedentary time with sleep or LPA was not associated with clustered cardio-metabolic risk. However, substituting sedentary time with LPA was associated with an increase in HDL-cholesterol and lower waist circumference and blood pressure. Lastly, substituting sedentary time with MVPA is associated with a significantly lower clustered cardio-metabolic risk, waist circumference and triglycerides and higher HDL-cholesterol.

Table 4. Effects and 95% confidence intervals of replacing 30 minutes of sedentary time with 30 minutes of sleep, LPA or MVPA for clustered cardio-metabolic risk and cardio-metabolic health parameters

Replace 30 minutes of sedentary time with	Sleep	LPA	MVPA
Clustered cardio-metabolic risk	0.00 (-0.00; 0.00)	0.00 (-0.00; 0.00)	**-0.06 (-0.08;-0.04)**
Waist Circumference (cm)	**-0.86 (-1.33;-0.40)**	**-0.50 (-0.89;-0.11)**	**-0.95 (-1.27;-0.63)**
Fasting Glucose (mmol/L)	-0.01 (-0.04; 0.02)	-0.01 (-0.04; 0.01)	0.00 (-0.02; 0.02)
HDL-cholesterol (mmol/L)	**0.03 (-0.01; 0.05)**	**0.04 (0.03; 0.06)**	**0.02 (0.01; 0.03)**
Triglycerides (mmol/L)	0.01 (-0.04; 0.02)	-0.01 (-0.04; 0.02)	**-0.04 (-0.06;-0.01)**
Systolic Blood Pressure (mmHg)	-0.35 (-0.81; 0.11)	**-0.41 (-0.80;-0.03)**	-0.20 (-0.52; 0.12)
Diastolic Blood Pressure (mmHg)	-0.44 (-1.21; 0.33)	**-1.17 (-1.81;-0.53)**	-0.17 (-0.71; 0.36)

Data are unstandardized regression coefficients

Isotemporal substitution model including all activity variables (MVPA & LPA) and sleep; additionally adjusted for total wear time, age, sex, education level, healthy eating index and smoking status

Results in bold: p<0.05

Similarly, regression coefficients and 95% confidence intervals of substituting LPA with sleep, sedentary time and MVPA are presented in Table 5. Substituting LPA with sleep was not associated with changes in clustered cardio-metabolic risk and its factors. However, substituting LPA with sedentary time was associated with an increase in waist circumference and blood pressure and a decrease in HDL-cholesterol. Furthermore, substituting LPA with MVPA was associated with a decrease in clustered cardio-metabolic risk, a decrease in HDL-cholesterol and an increase in diastolic blood pressure.

Table 5. Effects and 95% confidence intervals of replacing 30 minutes of LPA with 30 minutes of sleep, sedentary time or MVPA for clustered cardio-metabolic risk and cardio-metabolic health parameters

Replace 30 minutes of LPA with	Sleep	Sedentary Time	MVPA
Clustered cardio-metabolic risk	-0.01 (-0.05; 0.03)	-0.01 (-0.04; 0.01)	**-0.08 (-0.11;-0.04)**
Waist Circumference (cm)	-0.36 (-0.92; 0.20)	**0.50 (0.11; 0.89)**	-0.45 (-0.90; 0.00)
Fasting Glucose (mmol/L)	0.01 (-0.03; 0.04)	0.01 (-0.01; 0.04)	-0.01 (-0.01; 0.04)
HDL-cholesterol (mmol/L)	-0.01 (-0.04; 0.01)	**-0.04 (-0.06;-0.03)**	**-0.02 (-0.04;-0.00)**
Triglycerides (mmol/L)	0.02 (-0.02; 0.06)	0.01 (-0.02; 0.04)	-0.02 (-0.05; 0.01)
Systolic Blood Pressure (mmHg)	0.07 (-0.49; 0.62)	**0.41 (0.03; 0.80)**	0.21 (-0.23; 0.66)
Diastolic Blood Pressure (mmHg)	0.74 (-0.19; 1.66)	**1.17 (0.53; 1.81)**	**1.00 (0.26; 1.74)**

Data are unstandardized regression coefficients

Isotemporal substitution model including sedentary time, MVPA and sleep; additionally adjusted for total wear time, age, sex, education level, healthy eating index and smoking status

Results in bold: $p<0.05$

DISCUSSION

The primary finding of this study is that a substitution of sedentary time with either sleep, LPA or MVPA was associated with several health improvements. Findings suggest that substituting sedentary time with LPA can improve various cardio-metabolic risk factors such as waist circumference, HDL-cholesterol, systolic and diastolic blood pressure. However, a substitution of sedentary time with MVPA, was only significantly associated with a decrease in clustered cardio-metabolic risk. Furthermore, analyses for substituting LPA with MVPA were executed to explore the extra benefit of substituting LPA with higher intensities of physical activity. Again, a significant association was present for clustered cardio-metabolic risk, but increasing exercise intensity did not lead to a further gain for other cardio-metabolic parameters. Contrary, substituting LPA with MVPA was associated with a small decrease in HDL-cholesterol and an increase in diastolic blood pressure. Biological plausible reasons for improved health with higher levels of physical activity are hypothesized to act through beneficial changes in fibrinolytic, hemodynamic and inflammatory markers, blood pressure, and the cardiovascular system [23]. However, the mechanisms that underline the associations between sleep and health are not fully understood [6]. An investigation reporting an association between sleep duration and variants of the human Circadian Locomotor Output Cycles Kaput (CLOCK) genes suggested that disruptions of the core CLOCK genes, which regulate endogenous circadian rhythmicity, are linked to perturbations in glucose metabolism, adipocyte and vascular function and obesity [24].

There was a significant trend for an increased number of cardio-metabolic risk factors with weight, VO2peak, sedentary time, MVPA and education level, similarly to the work of Sassen et al. [25]. Participants with 0-1 risk factors had a much better cardiorespiratory fitness and were more active. They spent almost one hour of their day more on MVPA than participants with more than 3 risk factors. Because there were no significant differences in sleep and LPA over the risk groups, it can be assumed that 1 hour of MVPA in the healthy participants replaced 1 hour of sedentary time in the participants with 3 or more risk factors. Finally, in accordance to previous research, participants with fewer risk factors were higher educated [26].

Results are in line with previous research applying isotemporal substitution to explore the association between sedentary time, physical activity and clustered cardio-metabolic risk [8-10]. All previous studies observed beneficial effects of substituting sedentary time with MVPA for clustered cardio-metabolic risk and several of its parameters [8, 9], starting from as little as one minute [10]. However, where some studies also found clustered cardio-metabolic risk improvements for substituting sedentary time with LPA [8, 10], our study did not observe a significant association. Nevertheless, this in line with the study by Hamer et al., who argue that the association of sedentary time and clustered cardio-metabolic risk may be dependent on the type of activity that will displace sedentary time [9]. Then again, our results suggest that for some cardio-metabolic health parameters substituting sedentary time with LPA can lead to positive effects. Therefore, not only the type of activity that displaces sedentary time is important, but also the health parameter in question, where substitution with LPA might be valuable for waist circumference, HDL-cholesterol and blood pressure.

Because a significant trend was observed in the number of risk factors with a decline in VO2peak, all analyses were further adjusted for cardiorespiratory fitness. However, results and effect sizes were of similar proportion. One other study including isotemporal substitution performed additional analyses adjusting for estimated VO2max and also found no significant change in point estimates [10]. Another study examining markers of glucose regulation explored the effects in high and low fit participants and suggested that low fit participants might benefit more from replacing sedentary time with active behavior [12].

The dose-response curve between physical activity and various health outcomes is a possible mechanism of the effect of substituting sedentary time or LPA with MVPA [27]. This implies that greater health benefits can be reached by increasing the intensity of our behavior. Therefore, replacing a less intense behavior, such as sedentary time or LPA with a more intense behavior, such as MVPA, will lead to decreased cardio-metabolic risk. Furthermore, prolonged uninterrupted periods of sedentary time are associated with lower cardio-metabolic health [5]. By reducing sedentary time and introducing more active behavior, these prolonged periods of sedentary time will likely decrease and this will in turn have positive cardio-metabolic health effects [5, 28].

To the best of our knowledge this is the first paper to include objective measurement of sleep when substituting sleep with sedentary time. Contrary to other studies, we did not observe a U-shape relation between sleep duration and cardio-metabolic health [6], possibly because of this specific population with relatively small variation and 93% of the participants sleeping time was between 5 and 9 hours a day. Additionally, no significant differences in cardio-metabolic health parameters were noted between participants with short and normal sleep duration. Therefore, unlike other studies [8, 13], analyses for sleep were not preformed separately for long and short sleep durations and sleep was not treated as a piecewise variable. However, results were similar between all three studies indicating that replacing sedentary time with sleep can have positive effects on several cardio-metabolic health parameters [8, 13].

Isotemporal substitution analyses assume a substitution of equal absolute bouts of time, however, substituting a certain bout of MVPA is a greater relative proportion of the baseline value, in comparison to the same bout of sedentary behavior or sleep. For example, substituting 30 minutes of sedentary time will only remove less than 5% of the total average sedentary time, while adding 30 minutes of MVPA appends 20% of baseline MVPA. It is likely that because of these relative differences substituting LPA by MVPA appears to have greater benefits than substituting sedentary time by MVPA (Table 4 & 5). Because, only 5% of a potentially harmful behavior (sedentary time) is changed, while 13% of LPA is changed into MVPA, possibly explaining the association with better cardiometabolic health.

Strengths of this study include the objective measurement of physical activity and sleep with a Sensewear Pro 3 and very high inclusion criteria. Average wear time was 99% of a full day (minimum 5 days) and as a consequence all activities of daily living, excluding water based activities, were measured objectively. Moreover, all participants wore the Sensewear on both weekend days, and at least three weekdays, this to generate the most complete view of their activity behavior [18]. The Sensewear is proven valid for accurate measurement of daily energy expenditure under free-living condition, with exception of higher levels of expenditure [15]. However, only a very small percentage of activities and only in few participants were performed at such a high level (5.8 (±9.1) minutes of vigorous physical activity a day). Contrary to most studies, the amount of MVPA in the current study is high, however similar to a comparable Flemish population [29], which might be due to the specific algorithms that SenseWear Pro 3 uses. Furthermore, it is possible that the recording of MVPA and ST over a 24hr period and in single minutes instead of in bouts (e.g. bouts of 10 min) may have led to a more inclusive measurement of MVPA. Alternatively, our study sample may have included healthier, more active, individuals compared to the average population.

Another strength is the use of isotemporal substitution analyses, because there are only 24h a day, adding one activity will certainly lead to reducing another activity. Consequently, the effect of increased time spent in a certain behavior, such as MVPA, might lead to different health effects when reducing different behaviors, such as sleep, LPA or sedentary behavior [7]. Finally, CRF was measured by a maximal cycle ergometer exercise test, generally considered the gold standard [30], leaving less room for measurement error.

However, this study is not without limitations. Firstly, the cross-sectional nature of the data does not allow inferences of causality. Secondly, a substantial number of participants were excluded from the analyses due to the absence of SenseWear data. A drop-out analysis confirmed our assumption that, for SenseWear data, not complying with the inclusion criteria was a random event and therefore did not influence the results (results not shown). Lastly, we could not control for medication use because data was missing, which might cause residual confounding.

In conclusion, results suggest that replacing sedentary time with either sleep, LPA and MVPA was positively associated with several cardio-metabolic health risk factors. Replacing LPA with MVPA was associated only with extra benefits for clustered cardio-metabolic risk. Interventions for increasing cardio-metabolic health should focus on replacing sedentary time with either sleep, LPA or MVPA depending on the risk parameters that need to be targeted. For example, when hypertension appears to be the principal health risk, it might be sufficient to replace bouts of sedentary time with LPA, however, when the main goal is to improve clustered cardio-metabolic risk, engaging in MVPA is probably also necessary. Increasing time of substitution might lead to additional and more pronounced cardio-metabolic health benefits [10]. Further longitudinal studies investigating substitution of different types and intensities of activities of various lengths are necessary to gain better insight in the possible cardio-metabolic health benefits.

List of abbreviations

CLOCK = Circadian Locomotor Output Cycles Kaput

CMRS = Cardio-metabolic risk score

HEI = Healthy eating index

HDL-cholesterol = High Density Lipoprotein cholesterol

LPA = Light physical activity

MVPA = Moderate-to-vigorous physical activity

Declarations

An informed consent was obtained from the participants and the study was approved by the Medical Ethics Committee of the KU Leuven (s54083).

The datasets generated during and/or analyzed during the current study are not publicly available due to the fact that the data are part of a study funded by the Flemish government and are under legal restriction, but are available from the corresponding author on reasonable request.

The authors declare that they have no competing interests

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Conceived and designed the experiments: SK JGB JL. Performed the experiments: SK EM RC. Analyzed the data: SK. Contributed reagents/materials/analysis tools: SK JL. All authors read and approved the final manuscript.

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PAPER 3

Independent associations between sedentary time, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardio-metabolic health: a cross-sectional study

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Independent associations between sedentary time, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardio-metabolic health: a cross-sectional study

Abstract

We aimed to study the independent associations of sedentary time (ST), moderate-to-vigorous physical activity (MVPA), and objectively measured cardiorespiratory fitness (CRF) with clustered cardio-metabolic risk and its individual components (waist circumference, fasting glucose, HDL-cholesterol, triglycerides and blood pressure). We also investigated whether any associations between MVPA or ST and clustered cardio-metabolic risk were mediated by CRF.

MVPA, ST, CRF and individual cardio-metabolic components were measured in a population-based sample of 341 adults (age 53.8 ± 8.9 years; 61% men) between 2012 and 2014. MVPA and ST were measured with the SenseWear pro 3 Armband and CRF was measured with a maximal exercise test. Multiple linear regression models and the product of coefficients method were used to examine independent associations and mediation effects, respectively.

Results showed that low MVPA and low CRF were associated with a higher clustered cardio-metabolic risk (β = -0.26 and β = -0.43, both $p<0.001$, respectively). CRF explained 73% of the variance in the association between MVPA and clustered cardio-metabolic risk and attenuated this association to non-significance. After mutual adjustment for MVPA and ST, CRF was the most important risk factor for a higher clustered cardio-metabolic risk (β =-0.39, $p<0.001$)

In conclusion, because of the mediating role of CRF, lifestyle-interventions need to be feasible yet challenging enough to lead to increases in CRF to improve someone's cardio-metabolic health.

Keywords

Moderate-to-vigorous physical activity, sedentary time, cardiorespiratory fitness, clustered cardio-metabolic health, SenseWear®

INTRODUCTION

According to the World Health Organization, noncommunicable diseases were responsible for 38 million (68%) of the world's deaths in 2012 [1]. A number of cardio-metabolic risk factors are closely related to these noncommunicable diseases, including visceral obesity, hypertension, hyperglycaemia, and atherogenic dislipidemia [2]. Clustering of these cardio-metabolic risk factors in the same person appears to confer a substantial additional risk for cardiovascular diseases, diabetes mellitus type 2, and all-cause mortality over and above the sum of the risk associated with each abnormality [2, 3]. Over the last few decades, the prevalence of these cardio-metabolic risk factors has increased steadily [4]. Moreover, evidence indicates that in most populations today more than one fourth of the adult population has a clustering of three or more cardio-metabolic risk factors [5].

Evidence from cross-sectional studies [6-10], longitudinal studies [11-15] and randomised controlled trials [16, 17] suggests that sedentary time (ST), moderate-to-vigorous physical activity (MVPA), and cardiorespiratory fitness (CRF) are important predictors of various cardio-metabolic risk factors [18]. However, there are still questions regarding the specificity of these associations and the underlying relationships for predicting clustered cardio-metabolic risk. For example, several studies observed an association between ST and clustered cardio-metabolic risk, independent of physical activity [19-21]. Furthermore, only few studies examined the relationship of all three parameters together for predicting clustered cardio-metabolic risk and results of those studies have been equivocal [22-25]. One study reported that people with a high ST had a 65 to 76% higher risk of developing the metabolic syndrome, although low CRF was the strongest risk factor [22]. In line with these results, another study has identified ST as a risk factor for several markers of cardio-metabolic risk, although the relationship between ST and clustered cardio-metabolic risk was remarkably less pronounced when taking CRF into account [24, 25]. In contrast to these studies, van der Velde et al. observed that not ST, but MVPA and CRF were independently associated with clustered cardio-metabolic risk [23]. Of these studies, only two included objective measurements for MVPA, ST and CRF, however CRF was not measured with a maximal exercise test [23, 25].

Based on the temporal associations of ST and MVPA with CRF [26, 27], it is possible that CRF partly mediates associations between ST, MVPA, and clustered cardio-metabolic risk [28]. In a study by Sassen et al. up to 78% of the association between average physical activity and cardiovascular disease was mediated through CRF [28]. Nevertheless, still 22% of the total variance in physical activity had a direct effect on cardiovascular disease [28], which is important because physical activity is a behaviour that can be changed whereas CRF is a physiological measure reflecting a combination of physical activity behaviors, genetic potential, and functional health of various organ systems [29]. Moreover, to the best of our knowledge, no study has investigated the mediating effect of CRF on the relationship between ST and clustered cardio-metabolic risk.

The aim of the current study was to examine the independent associations of objectively measured ST, MVPA and CRF with clustered cardio-metabolic risk and individual cardio-metabolic risk factors. Furthermore, the mediating effect of CRF on the relation between MVPA or ST and clustered cardio-metabolic risk was analysed. We hypothesized that objectively measured high CRF, high MVPA, and low ST were independently associated with a favourable cardio-metabolic risk profile. Moreover, we hypothesized that CRF is a potential mediator for the association of MVPA or ST and clustered cardio-metabolic risk.

METHODS

Participants and study design

This cross-sectional study is part of a Flemish longitudinal study of which the sampling procedure is previously described in detail by Matton et al. [30]. Participants (n = 652) were male (n = 420) and female (n = 232) volunteers between 29 and 82 years and were measured between 2012 and 2014 in Leuven, Belgium. For 203 participants (31%) valid physical activity data were not available (8% did not wear the SenseWear Pro 3 Armband and 23% did not comply with the strict inclusion criteria). Furthermore, nine participants (1.4%) did not provide a blood sample and another 89 participants (14%) did not perform a maximal cycle ergometer test. Additionally, 22 participants had missing data for covariates such as nutritional intake, education level and smoking status, leaving a final study sample of 341 participants. Written informed consent was obtained from the participants and the study was approved by the Medical Ethics Committee UZ KU Leuven (s54083).

Physical activity and sedentary time

Objective measurement of physical activity and ST was obtained with a SenseWear Pro 3 Armband (BodyMedia, Inc, Pittsburgh, PA, USA), which is proven valid for measuring daily energy expenditure under free-living conditions [31]. Participants were asked to wear the SenseWear Pro 3 Armband 24 hours a day, except during water-based activities, for seven consecutive days. The compliance criterion was set at 1296 minutes (90% of a total day). The SenseWear Pro 3 uses algorithms developed by the manufacturer (SenseWear Professional software version 6.1) to combine input from different sensors, including a two-axis accelerometer and sensors measuring heat flux, galvanic skin response, skin temperature, and near body ambient temperature, with information on gender, age, body weight, and height to estimate sleep and wake parameters. Estimated sleeping time was then excluded from all analyses. Furthermore, to achieve more reliable estimates of physical activity and ST, only participants reaching the compliance criterion for at least 3 weekdays and both weekend days were included in the study [32]. Time spent in sedentary behaviour (<= 1.5 MET), light physical activity (LPA, 1.5 - <= 3 MET) and MVPA (> 3 MET) was derived using the measured MET values.

Cardiorespiratory fitness

CRF was determined by means of a maximal exercise test on an electrically braked Lode Excalibur cycle ergometer (Lode, Groningen, the Netherlands). Peak oxygen uptake (VO_{2peak}) was measured directly with breath-by-breath respiratory gas exchange analysis, using a Cortex MetaLyzer 3B analyzer (Cortex Biophysic GmbH, Leipzig, Germany) and was defined as the highest 20-s value during the exercise test [33]. The exercise test started at a load of 20 W, which was increased with 20 W every minute until volitional exhaustion.

Clustered cardio-metabolic risk score

A cardio-metabolic risk score (CMRS), largely based on the International Diabetes Foundation criteria for the metabolic syndrome, was calculated to measure clustered cardio-metabolic risk [2, 14]. A continuous score can better capture the progressive risk increase when more risk factors are present and it has more statistical power than a dichotomous score [34]. Metabolic parameters were assessed by trained staff in the morning after an overnight fast. Waist circumference was measured to the nearest 0.1 cm. Systolic and diastolic blood pressure were measured with an electronic blood pressure monitor (Omron, the Netherlands) three times in seated position. The means of the three measurements for systolic and diastolic blood pressure were used in statistical analyses. Values for triglycerides, plasma glucose and HDL-Cholesterol (HDL-C) were obtained by enzymatic methods (Abbott Laboratories, Abbott Park, IL). Due to skewness in the latter three parameters, values were first normalized ($\log_{10}$). To create the CMRS, variables were standardised by subtracting the sex-specific sample means from the individual mean and dividing by the sex-specific standard deviation. Subsequently, these standardised values for waist circumference, blood pressure, triglyceride, plasma glucose, and the inverse of HDL-C were summarized and divided by the number of variables included (x=5), generating the CMRS. A score above zero represents higher individual cardio-metabolic markers and therefore indicates having a less preferable clustered cardio-metabolic health.

Covariates

Smoking behaviour was assessed using the WHO Monica Smoking Questionnaire [35]. Participants were classified as current, former or never smokers. Four levels of education were ranked from lowest (no degree) to highest (university degree) and were used as an indicator of socio-economic status [36]. Sugar intake, saturated fat intake and alcohol consumption were assessed using a three-day diet record, during two weekdays and one weekend day. When possible all foods and drinks were weighed and recorded. Otherwise they were estimated using standard household measures such as a plate, spoon or glass. The diet records were analysed using Becel Nutrition software (Unilever Co., Rotterdam, the Netherlands).

Statistical analysis

Descriptive statistics (median and interquartile range) were calculated for all participants. Pearson correlation coefficients were calculated between all cardio-metabolic risk factors and the three main exposures; ST, MVPA, and CRF. Additionally, Pearson correlations were calculated between the three exposure variables (Supplement Table 1).

Furthermore, multiple linear regression was used to examine the association of ST, MVPA and CRF with cardio-metabolic risk. Residuals were tested for homoscedasticity, linearity and independence. Additionally, the variance inflation factor never exceeded three, indicating that multi-collinearity was not a concern [37].

Both exposure and outcome variables were standardized in order to enable direct comparison of the effect estimates across outcome and exposure variables, and regression coefficients (95% CI) are presented. All models were initially adjusted for age, sex, waking time, smoking, alcohol consumption, sugar and saturated fat intake, and education level (Model 1). Subsequently, Model 1 was further separately adjusted for ST and MVPA (Model 1 + ST, Model 1 + MVPA).

Next, a fully adjusted model with all three exposures was composed for ST, MVPA and CRF (Model 2). Regression coefficients yielded from analyses using unstandardized exposures and outcomes are presented in Supplement 2.

Finally, mediation analyses were performed to assess whether CRF is a mediator in the associations of ST and MVPA with CMRS. Mediation analyses were performed only when ST or MVPA were independently associated with CMRS (i.e. Model 1: adjusted for covariates and each other). Mediation was established by the use of regression analyses of unstandardized exposures and outcomes including the following steps: 1) regressing the outcome (CMRS) on the exposure (ST/MVPA) (path c), 2) regressing the mediator (CRF) on the exposure (path a), 3) regressing the outcome on the mediator, adjusted for the exposure (path b). All regression analyses were adjusted for covariates and the other exposure (i.e. for ST in case of MVPA and for MVPA in case of ST). Size of the mediated effect was estimated by the product of coefficients method (a*b) by MacKinnon et al [38]. Proportion mediated was calculated by dividing the product of coefficients by the overall effect (a*b/c). The mediated effect was tested by the Sobel test [39].

Statistical analyses were performed using the SAS, version 9.4 (SAS institute, Cary, NC, USA) statistical program. Statistical significance was set at $p < .05$.

RESULTS

Table 1 presents descriptive statistics for MVPA, ST, CRF, and cardio-metabolic markers. The mean age of the participants was 53.8 ± 8.9 years. In total 207 men and 134 women (39%) were included, of whom most were former smokers (34%) or never smokers (58%). In general the study population was highly educated with more than 95% having finished secondary schooling. The mean alcohol consumption of the participants was 13.4±10.2 mg/day, which is an average of one standard drink per day. Mean sugar intake was 122±47.5 mg/day and mean saturated fat intake was 32.8±12.7 mg/day. When compared to non-participants (n= 311), participants had similar MVPA, ST, CRF and CMRS. However, participants were on average 5 years younger.

Table 1. Descriptive statistics for age, physical activity, physical fitness and cardio-metabolic markers

	Total (n=341)	
Variables	M	Q1-Q3
Age (years)	53.9	49.2 - 57.7
BMI (kg/m²)	24.3	22.8 - 26.4
Sedentary time (hours/day)	10.8	9.6 - 12.3
LPA (hours/day)	3.8	3.2 - 4.7
MVPA (hours/day)	2.1	1.4 - 3.4
VO2peak ($ml.min^{-1}.kg^{-1}$)	32	28 - 39
Cardio-metabolic markers		
Waist Circumference (cm)	81.1	77.9 - 91.2
Fasting Glucose (mmol/L)	5.06	4.78 - 5.39
HDL-cholesterol (mmol/L)	1.47	1.27 - 1.76
Triglycerides	0.99	0.76 - 1.37
Diastolic Blood Pressure (mmHg)	84.5	79.3 - 91.0
Systolic Blood Pressure (mmHg)	131.8	121.7 - 142.0

Note. M = median, Q1 = lower quartile, Q3 = upper quartile

LPA = Light physical activity, MVPA = Moderate-to-vigorous physical activity.

ST had a high negative correlation with MVPA (r = -.71, p = <.001), however not with CRF (r = -.09, p = .11). Furthermore MVPA had a moderate positive correlation with CRF (r = .43, p = <.001). Pearson correlation coefficients for the association between ST, MVPA, CRF and cardio-metabolic markers are shown in Supplement 1. In general, correlations were low to moderate, ranging from -0.42 to 0.27. As hypothesized, clustered cardio-metabolic risk was positively associated with ST and negatively with MVPA and CRF.

Results from multiple linear regression analyses using standardized and unstandardized exposure and outcome variables can be found in Table 2 and Supplement Table 2, respectively. ST was positively associated with waist circumference and negatively with HDL-C (Model 1). After adjustment for MVPA (Model 1 + MVPA) and both MVPA and CRF together (Model 2), these associations remained significant (Table 2).

Table 2. Standardized regression coefficients of sedentary behaviour, moderate-to-vigorous physical activity and cardiorespiratory fitness for clustered cardio-metabolic health and waist circumference

		ST					MVPA					CRF				
	Model			95% CI					95% CI					95% CI		
CMRS	Model 1	0.12		-0.00	,	0.23	-0.26	***	-0.37	,	-0.15	-0.43	***	-0.53	,	-0.32
	Model 1 + ST						-0.35	***	-0.50	,	-0.21	-0.43	***	-0.54	,	-0.33
	Model 1 + MVPA	-0.14		-0.30	,	0.01						-0.38	***	-0.49	,	-0.27
	Model 2	0.05		-0.11	,	0.21	-0.10		-0.26	,	0.06	-0.39	***	-0.52	,	-0.27
Waist Circumference	Model 1	0.24	***	0.14	,	0.35	-0.22	***	-0.32	,	-0.12	-0.04		-0.14	,	0.07
	Model 1 + ST						-0.12		-0.26	,	0.01	-0.04		-0.14	,	0.06
	Model 1 + MVPA	0.15	*	0.01	,	0.30						0.05		-0.06	,	0.16
	Model 2	0.15		0.04	,	0.30	-0.13		-0.29	,	0.03	-0.01		-0.11	,	0.13
Fasting Glucose	Model 1	0.03		-0.09	,	0.14	-0.01		-0.13	,	0.10	-0.03		-0.15	,	0.08
	Model 1 + ST						0.00		-0.15	,	0.16	-0.03		-0.15	,	0.08
	Model 1 + MVPA	0.03		-0.14	,	0.19				,		-0.03		-0.15	,	0.09
	Model 2	0.05		-0.13	,	0.23	0.03		-0.14	,	0.21	-0.04		-0.18	,	0.09
HDL-cholesterol	Model 1	-0.28	***	-0.39	,	-0.16	0.09		-0.03	,	0.20	-0.15	*	-0.26	,	-0.03
	Model 1 + ST				,		-0.18		-0.33	,	0.03	-0.14	*	-0.26	,	-0.03
	Model 1 + MVPA	-0.41	***	-0.57	,	-0.25				,		-0.20	**	-0.33	,	-0.08
	Model 2	-0.36	***	-0.53	,	-0.18	-0.11		-0.29	,	0.06	-0.10		-0.23	,	0.03
Triglycerides	Model 1	0.09		-0.03	,	0.21	-0.14	*	-0.25	,	-0.03	-0.20	**	-0.32	,	-0.08
	Model 1 + ST				,		-0.16	*	-0.32	,	-0.00	-0.20	***	-0.32	,	-0.08
	Model 1 + MVPA	-0.03		-0.20	,	0.14				,		-0.17	**	-0.29	,	-0.04
	Model 2	0.06		-0.12	,	0.24	-0.04		-0.22	,	0.14	-0.18	*	-0.32	,	-0.05
Diastolic Blood Pressure	Model 1	0.06		-0.06	,	0.17	-0.01		-0.12	,	0.10	-0.11	*	-0.23	,	-0.02
	Model 1 + ST				,		0.05		-0.10	,	0.21	-0.11		-0.23	,	0.00
	Model 1 + MVPA	0.10		-0.07	,	0.03				,		-0.12	*	-0.25	,	-0.00
	Model 2	0.18	**	0.01	,	0.36	0.17		0.00	,	0.34	-0.18	**	-0.31	,	-0.04
Systolic Blood Pressure	Model 1	0.06		-0.05	,	0.17	0.07		-0.03	,	0.18	0.05		-0.05	,	0.16
	Model 1 + ST				,		0.22	**	0.08	,	0.36	0.05		-0.05	,	0.16
	Model 1 + MVPA	0.22	**	0.07	,	0.37						0.03		-0.08	,	0.15
	Model 2	0.24	**	0.08	,	0.40	0.24	**	0.08	,	0.41	-0.04		-0.16	,	0.09

Data are standardized regression coefficients

ST = Sedentary Time; MVPA = moderate-to-vigorous physical activity; CRF=Cardiorespiratory fitness; CMRS = Cardio-metabolic risk score

Model 1: adjusted for age, sex, original study population, smoking, education level, alcohol intake, sugar and saturated fat intake and waking time

Model 2: adjusted for all covariates in model 1 and adjusted for ST, MVPA and CRF as applicable

*p<0.05; **p<0.01;***p<0.001

MVPA was negatively associated with CMRS, triglycerides and waist circumference (Model 1). After adjustment for ST (Model 1 + ST), this association was of similar strength for CMRS. Furthermore, a new association with systolic blood pressure became apparent. When adjusting for both ST and CRF together (Model 2), all associations attenuated to non-significance, with the exception of the association with systolic blood pressure.

CRF was negatively associated with CMRS, triglycerides and diastolic blood pressure, and positively associated with HDL-C (Model 1). Associations with CMRS, HDL-C and triglycerides remained significant after adjustment for ST or MVPA separately. Furthermore, all initial associations from Model 1 remained significant after adjustment for both ST and MVP (Model 2), with the exception of the association with HDL-C.

Finally, we investigated the mediated effect of CRF on the association between MVPA and CMRS. There was significant mediation by CRF in the association between MVPA and CMRS, (*a*b* (95% CI): -0.11 (-0.11; -0.10), $p < 0.001$). The mediated effect was calculated [4.12 * -0.03 / -0.15] * 100%; resulting in an effect of 73%, meaning that CRF explained 73% of the total variance in the association between MVPA and CMRS (Figure 1). Because there was no association between ST and CMRS (i.e. no total effect), no further mediation analysis was performed for this association.

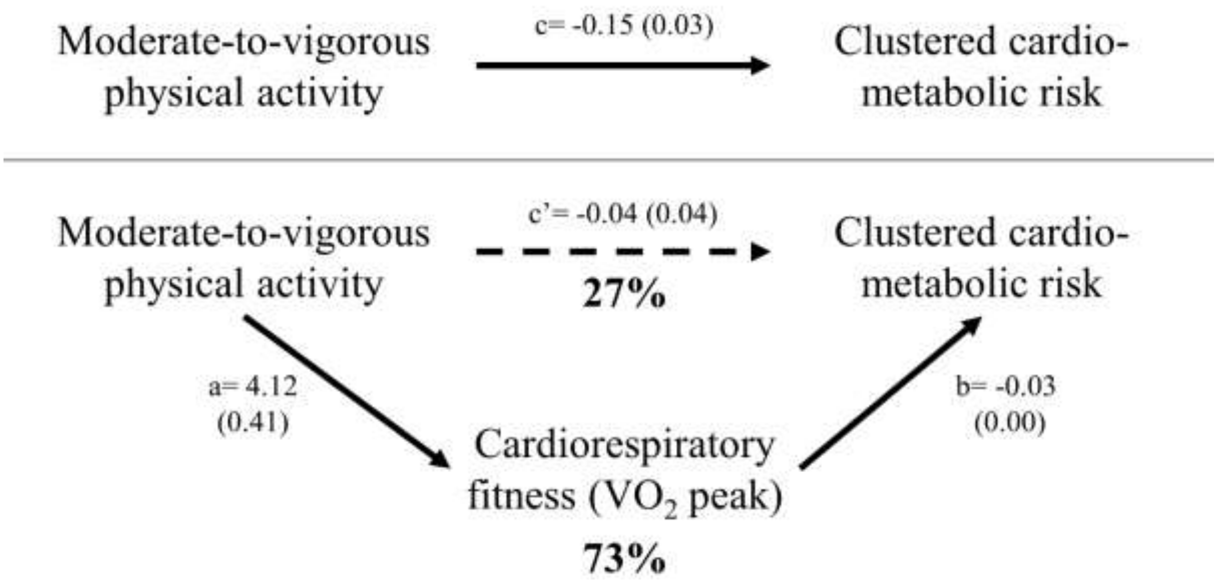

Figure 1. Mediation pathway of cardiorespiratory fitness

Legend: Pathway (regression coefficient of unstandardized exposures and outcomes (standard error)) of the association between moderate-to-vigorous physical activity (MVPA, hours/day) and clustered cardio-metabolic risk (CMRS) through cardiorespiratory fitness (CRF, ml.min-1.kg-1) corrected for all covariates: sedentary time, age, sex, waking time, smoking, alcohol consumption, suger intake, saturated fat intake and education level. CRF explains 73% of the total variance in CMRS.

DISCUSSION

The main findings of this cross-sectional study suggest that low MVPA is a significant exposure for clustered cardio-metabolic risk and that low ST is a significant exposure for a healthier waist circumference and HDL-C. Low CRF appeared to be the most important exposure for a higher clustered cardio-metabolic risk and is an important mediator in the association between MVPA and CMRS. Contrary to our research hypothesis, only CRF was associated with clustered cardio-metabolic risk independent from the other two potential exposures, MVPA and ST. However, when only examining waist circumference, ST appeared to be a more important predictor than MVPA or CRF.

Although a growing number of studies have examined the associations of ST and/or physical activity and/or CRF [7, 8, 12, 14, 18], with clustered cardio-metabolic risk, few have taken into account all three exposures simultaneously. Moreover, even fewer studies have used objective measurement for all three exposures or analysed the mediating influence of CRF [22-25, 28].

Regression coefficients from analyses using unstandardized exposure and outcome variables illustrate the clinical significance of the associations. Even in this healthy population, where 268 (79%) had ≤ 1 at risk measurements for cardio-metabolic risk factors (according to the guidelines of the International Diabetes Foundation [2]) and only 16 participants (5%) had at risk measurements for ≥ 3 cardio-metabolic risk factors, significant improvements can be achieved given that after correction for MVPA one hour less ST was associated with a 0.83 cm lower waist circumference.

Similar previous studies including all three exposures underline the importance of high CRF and support the conclusion of an independent association between CRF and clustered cardio-metabolic risk [22-24]. However, contrary to the present study, all studies found an inverse association between sedentary behaviour and cardio-metabolic risk, though in only two studies this association remained significant after adjusting for physical activity and CRF [22, 25]. Moreover, in the present study, the lack of an association between MVPA and clustered cardio-metabolic risk independent of ST and CRF appears to be in contradiction with the results of van der Velde et al. [23].

Because studies incorporating all three exposures are rather scarce, results are also compared to studies including only two exposures. For example, inconsistent findings have been reported for the association between ST and clustered cardio metabolic risk when adjusting for MVPA or CRF [9, 19, 21]. We found no evidence for an association between ST and clustered cardio-metabolic risk. . Similar results have been shown by van der Velde et al. [23] and Scheers et al. [6] who found that the inverse relationship between ST and the metabolic syndrome could not be maintained after adjusting for MVPA and/or CRF. However, some studies did observe an association between ST and clustered cardio-metabolic risk independent of MVPA [14, 19, 21]. Age might be an important moderator of the independent relationship between ST and cardio-metabolic risk. Studies with participants with an average age over 60 reported ST as an important independent predictor [19, 21], while studies in younger populations did not find an independent association between ST and clustered cardio-metabolic risk [6, 23]. Moreover, Maher et al. suggest that when adjusting for total physical activity instead of only MVPA, there is virtually no association between ST and cardio-metabolic risk factors [40].

No association between MVPA and clustered cardio-metabolic risk was found independent of both ST and CRF. This supports the hypothesis that CRF mediates the association between MVPA and clustered cardio-metabolic risk because we observed a significant association independent of ST only. In comparison, some longitudinal studies argue that a higher physical activity may lower clustered cardio-metabolic risk even in the absence of an improvement of CRF [12, 17, 41] or high ST [6]. Furthermore, a training study investigating the effect of MVPA on blood lipids and CRF observed an improved blood lipid profile in spite of a decrease in CRF [17]. However, in a study with a population of recently diagnosed type 2 diabetes patients, the observed association between MVPA and clustered cardio-metabolic risk reduced to a non-significant level after accounting for ST [21].

Our findings support the hypothesis that CRF is a significant exposure for cardio-metabolic risk and an important mediator in the association between MVPA and clustered cardio-metabolic risk. This result is in line with previous cross-sectional research [7, 28, 42, 43]. A study with more than 1500 participants concluded that both physical activity, but mainly, CRF were independently associated with lower clustered cardio-metabolic risk [44]. Moreover, Franks et al. observed a strong inverse relationship between CRF and clustered cardio-metabolic risk, and MVPA and clustered cardio-metabolic risk [8]. However, CRF modified the association between physical activity and clustered cardio-metabolic health to such an extent that in fit people the relationship did not exist [8]. Although CRF is often observed as the most important independent exposure, it is important to note that CRF can be genetically predisposed up to 47% and as such is not completely modifiable [45]. Moreover, the effect estimate of MVPA, comparable in size with the study by Sassen et al. [46], was not completely attenuated after adjustment for CRF (30% unexplained), indicating that there may be other pathways relating MVPA with cluster cardio-metabolic risk. In addition, multiple studies have shown the importance of physical activity and/or sedentary behavior for improving CRF [26, 47].

Strengths of this study include the objective measurement of ST and MVPA. Objective measurement over a substantial time period provides a more accurate and complete insight into the participants' physical activity and ST [32]. Moreover, we utilised strict inclusion criteria (90% wear time per day and 3 week days plus both weekend days) to gather the most comprehensive interpretation of ST and MVPA. It should be noted that the amount of MVPA in the current study is higher than reported in previous studies, however similar to a comparable Flemish population [6], this might be due to the specific algorithms that SenseWear Pro 3 uses. It is possible that the recording of MVPA and ST over a 24hr period and in single minutes instead of in bouts (e.g. bouts of 10 min) may have led to a more comprehensive measurement of MVPA. Alternatively, our study sample may have included healthier, more active, individuals compared to the average population Finally, CRF was measured by a maximal cycle ergometer exercise test, generally considered the gold standard [48], leaving less room for measurement error.

However, this study is not without limitations. First, the cross-sectional nature of the data does not allow inferences of causality. Second, the SenseWear Pro 3 Armband probably underestimates total energy expenditure, particularly at higher intensities [31]. However, given that the current study uses minutes per day above 3 MET to define minutes spent in MVPA, and not the energy expenditure during MVPA, it is unlikely that this has affected our results. Additionally, the SenseWear Pro 3 Armband cannot measure posture and therefore standing may have been included in ST. Third, medication use was not assessed and therefore not controlled for in analyses. Although the study population is rather healthy, this may have resulted in residual confounding. Fourth, a substantial number of participants were excluded from the analyses due to the absence of CRF data or SenseWear Pro 3 data. A drop-out analysis confirmed our assumption that, for SenseWear Pro 3 data, not complying with the inclusion criteria was a random event and therefore did not influence the results. However, the most important reason for not completing the maximal cycle ergometer test was exclusion by a physician. Most common reasons for exclusion were lower back pain, a higher risk of myocardial infarction, arterial hypertension, abnormalities on an electrocardiogram, or clinical judgment. Because of these exclusion criteria, the least fit participants were probably excluded, resulting in a more fit and therefore homogenous sample.

Finally, comparison of results from different studies is often difficult due to differences in measurements (e.g. objective measurements versus subjective measurements), variable definitions, or study populations. For example, physical activity is often defined differently (e.g. habitual physical activity [8], leisure-time physical activity [22], overall energy expenditure [11], physical activity energy expenditure [12, 41], total physical activity [40] or MVPA [6, 23]) and as a consequence, not all studies deal with the same intensities and volumes of physical activity. In a similar vein, although most studies have investigated sedentary behaviour or ST, only few look at the whole range of sedentary behaviours or ST over a 24h period of time [23, 24]. Moreover, ST is frequently based on self-report, only including television viewing [15, 49, 50], passive transportation, or both [22, 24]. With regard to the study population, it is plausible that not only age, race or gender but also health status of the population influences associations with cardio-metabolic risk. For example, the present study presents a relatively healthy and highly educated group of adults.

Conclusions

In summary, CRF appears to be the most important exposure (in comparison to ST and MVPA) for clustered cardio-metabolic risk. This study also highlights some important associations between ST, MVPA and CRF, and certain cardio-metabolic risk factors. Furthermore, our findings suggest that the risk related to low MVPA is substantially mediated by CRF. Therefore, when designing interventions for reducing cardio-metabolic risk, apart from an increase in MVPA and decrease in ST, an increase in CRF will have to be the additional goal. Consequently, exercise programs need to be feasible yet challenging enough to lead to increases in CRF.

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PAPER 4

Ten-year change in sedentary behaviour, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic risk: independent associations and mediation analysis

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Ten-year change in sedentary behaviour, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic risk: independent associations and mediation analysis

Abstract

Background We aimed to study the independent associations of 10-year change in sedentary behavior (SB), moderate-to-vigorous physical activity (MVPA) and objectively measured cardiorespiratory fitness (CRF), with concurrent change in clustered cardiometabolic risk and its individual components (waist circumference, fasting glucose, high-density lipoprotein (HDL) cholesterol, triglycerides and blood pressure). We also determined whether associations were mediated by change in CRF (for SB and MVPA), waist circumference (for SB, MVPA and CRF) and dietary intake (for SB).

Methods A population-based sample of 524 adults (age (mean±SD) 55.83±9.40; 65% men) was followed prospectively for 9.62±0.52 years. Participants self-reported SB and MVPA and performed a maximal cycle ergometer test to estimate peak oxygen uptake at baseline (2002–2004) and follow-up (2012–2014). Multiple linear regression and the product of coefficients method were used to examine independent associations and mediation effects, respectively.

Results Greater increase in SB was associated with more detrimental change in clustered cardiometabolic risk, waist circumference, HDL cholesterol and triglycerides, independently of change in MVPA. Greater decrease in MVPA was associated with greater decrease in HDL cholesterol and increase in clustered cardiometabolic risk, waist circumference and fasting glucose, independent of change in SB. Greater decrease in CRF was associated with more detrimental change in clustered cardiometabolic risk and all individual components. Change in CRF mediated the associations of change in SB and MVPA with change in clustered cardiometabolic risk, waist circumference and, only for MVPA, HDL cholesterol. Change in waist circumference mediated the associations between change in CRF and change in clustered cardiometabolic risk, fasting glucose, HDL cholesterol and triglycerides.

Conclusions A combination of decreasing SB and increasing MVPA, resulting in positive change in CRF, is likely to be most beneficial towards cardiometabolic health

Keywords

Moderate-to-vigorous physical activity, sedentary time, cardiorespiratory fitness, clustered cardio-metabolic health, SenseWear®

Introduction

In modern societies, a growing proportion of adults are inactive.[1 2] In addition, sedentary behaviour (SB; awake time sitting/reclining) is highly prevalent, even in those who are sufficiently active.[3 4] In recent years, accumulating evidence has suggested that excessive sitting and insufficient moderate-to-vigorous physical activity (MVPA) may independently contribute to unhealthier cardiometabolic risk profiles, which in turn may substantially increase the risk for incident type 2 diabetes, cardiovascular disease and premature death.[5]

Another factor recognised as a strong predictive marker for cardiometabolic health, and potential mediator in the associations between MVPA, SB and cardiometabolic health, is cardiorespiratory fitness (CRF).[6–8] Developing more insight into whether, and to what extent, changes in sitting, MVPA and CRF independently shape cardiovascular health is therefore important, in order to advance lifestyle interventions and public health guidance. However, longitudinal studies examining all three potentially important lifestyle components (SB, MVPA and CRF) in this context are scarce.[9–12] Moreover, none fully examined the potential contribution change each of these three components could make in terms of cardiometabolic health, in relation to each other.[9–11] Change in MVPA and potentially change in SB are adjustable determinants for change in CRF. [13 14] Examining whether change in CRF mediates associations between MVPA, SB and cardiometabolic health is therefore of interest, but rarely done.[7] Furthermore, all previous studies focused on a select population (men;[9 10] people with type 2 diabetes [11]) had a much shorter follow-up [10 11] and did not perform a maximal exercise test.[11]

Waist circumference and nutritional intake are both causally related to cardiometabolic health.[15 16] Lower MVPA and higher SB may be associated with higher waist circumference[17 18] and higher SB, especially TV viewing, is associated with increased snacking behaviour and changes in nutritional intake in general.[19–21] Consequently, in order to better understand the mechanisms underlying any independent associations found between change in SB, MVPA and CRF, and cardiometabolic risk, change in waist circumference and nutritional intake (the latter specifically for SB) are also important candidates to examine as potential mediators. Previous longitudinal research, examining the relationship between all three exposures and cardiometabolic health, has not specifically examined the mediating role of change in waist circumference or nutritional intake.[9–11]

The purpose of the current study therefore was to: (1) examine the independent associations between change in SB, MVPA and objectively measured CRF with concurrent change in cardiometabolic risk over a long period of follow-up, and (2) to examine whether any such independent associations were mediated by change in CRF (for SB and MVPA), change in waist circumference (for SB, MVPA and CRF) or nutritional intake (for SB).

METHODS

Participants and study design

We examined this in the longitudinal Flemish Policy Research Centre Sport study, a prospective cohort of Flemish adults aged 18–75. The sampling procedure is previously described by Duvigneaud et al.[22] In 2012–2014, all volunteers who visited the examination centre in 2002–2004 (n=1569) were reinvited for follow-up, and 652 (42%) volunteers returned to participate. Written informed consent was obtained from all participants and the study was approved by the Medical Ethics Committee of the KU Leuven (s54083).

Sedentary Behavior and Moderate-to-vigorous Physical Activity

SB and physical activity were assessed using the Flemish Physical Activity Computerized Questionnaire (FPACQ). This questionnaire generates estimates with high reliability (intraclass correlations ranging from 0.71 to 0.99) and reasonable criterion validity (correlations ranging from 0.49 to 0.57 with an objective criterion).[23 24] SB (hours/week) was estimated by asking participants to report the amount of time they spent in screen time and passive transportation. MVPA (hours/week) was estimated as a combination of sports participation and active transportation, only including activities with an intensity ≥3 METs, according to the Ainsworth compendium.[25]

Cardiorespiratory fitness

CRF was defined as peak oxygen uptake (VO_2peak) relative to total body weight and determined by means of a maximal exercise test on a cycle ergometer (Lode, Groningen, the Netherlands).[26] Oxygen consumption (VO_2) was measured directly using a Cortex MetaLyzer 3B analyzer (Cortex Biophysic GmbH, Leipzig, Germany).[22 27] Participants first had to pass a physical examination and people at risk for heart failure and arterial hypertension did not participate in the maximal exercise test.

Clustered cardio-metabolic risk score

A clustered cardiometabolic risk score (CMRS) was calculated.[28] As described in detail before,[29] metabolic parameters were assessed after an overnight fast. Waist circumference was measured to the nearest 0.1 cm. Systolic and diastolic blood pressures were measured by electronic monitor (Omron, the Netherlands). Triglycerides, plasma glucose and high-density lipoprotein (HDL) cholesterol were obtained from an antecubital vein blood sample and analysed by enzymatic methods (Abbott Laboratories, Abbott Park, Illinois, USA). Owing to skewness, values for the latter three parameters were first normalised (log10). Subsequently, standardised values for waist circumference, triglyceride, plasma glucose, blood pressure and the inverse of HDL cholesterol were computed. Each cardiometabolic variable was standardised by using the sex-specific baseline sample mean and SD, derived from all men and women with baseline data for each cardiometabolic variable. To calculate the CMRS, the sum of these standardised values was divided by the number of metabolic parameters included. In order to perform a mediation analysis considering change in abdominal adiposity as the mediator, the CMRS was also calculated without the adiposity component.[30]

Covariates

Smoking behaviour was assessed using the WHO Monica Smoking Questionnaire. Participants were classified as current, former or never smokers. Education level was used as an indicator of socioeconomic status. Sugar intake, fat intake and alcohol consumption were assessed using a 3-day diet record, during two weekdays and one weekend day. The diet records were analysed using Becel Nutrition software (Unilever Co., Rotterdam, the Netherlands). Furthermore, the Healthy Eating Index (HEI), which captures the key recommendations of the 2010 Dietary Guidelines, was calculated.[31]

Statistical analysis

A dropout analysis was performed by comparing baseline characteristics between the follow-up group and the group that participated at baseline only (unpaired Student's t-test and χ^2 test). Descriptive characteristics of the included sample were compared between baseline and follow-up (paired Student's t-test) and changes in characteristics were calculated as follow-up minus baseline.

Multiple linear regression was used to examine the association between change in the exposure variables (SB, MVPA and CRF) with change in cardiometabolic risk over 10 years. All variables were tested for normality and residuals were tested for homoscedasticity, linearity and independence. Furthermore, the variance inflation factor never exceeded two, indicating that multicollinearity was not a concern.[32]

Both exposure and outcome variables were standardised and standardised regression coefficients (95% CI) are presented, in order to enable direct comparison of the effect estimates across outcome and exposure variables (unstandardised coefficients are provided in online supplementary tables). All models were adjusted for age, sex, follow-up time, baseline value of the exposure under study, baseline value of the outcome under study; and baseline and change values in HEI, smoking and education level (model 1). Subsequently, model 1 for SB was further adjusted for change and baseline in MVPA and vice versa (model 2; see visual representation in online supplementary 1). All analyses were tested for an interaction effect with sex. Interaction effects between change in the exposures were also examined (ie, change in SB×change in MVPA, change in MVPA×change in CRF and change in SB×change in CRF). To examine whether change in CRF mediated the associations found for SB and MVPA, a mediation analysis was performed on all significant associations between change in SB or MVPA and change in cardiometabolic risk. Finally, the mediating role of change in waist circumference (for change in SB, MVPA and CRF) and in dietary intake (only for change in SB) was also examined. All mediation analyses were performed by the product of coefficients (a×b) method by MacKinnon et al.[33] The regression coefficient between the exposure and mediator (a) was adjusted for all covariates in model 2 (model 1 in case of CRF). The regression coefficient between the mediator and outcome (b) was adjusted for all covariates in model 2 (model 1 in case of CRF) and change in the relevant exposure. CIs for the mediated effect were derived with bootstrapping analysis based on 2000 repetitions.

Statistical analyses were performed using SAS, V.9.4 (SAS institute, Cary, North Carolina, USA) and Stata/SE 13.0 (Stata, College Station, Texas, USA) statistical programs.

Results

Descriptives

Of all 652 participants, full data were available at baseline and follow-up for 524 participants (65% men), with the exception of CRF data for which a subsample of 399 participants (64% men) provided all data. The average follow-up time was 9.62 (±0.52) years. Dropout analysis showed that more men than women remained in analyses. Furthermore, participants who returned for follow-up had higher CRF, MVPA and systolic blood pressure. They were more highly educated and reported higher sugar, saturated fat and fibre intake. No differences between both samples were found for SB or for any of the other outcomes and covariates.

Table 1 presents descriptive statistics of the included sample. Cardiometabolic risk deteriorated for almost all markers, and so did CRF. SB increased by, on average, 2 hours a week, whereas total MVPA stagnated. At follow-up, 43% of participants never smoked, 48% were former smokers and 9% were current smokers, where 71% did not change their smoking behaviour since baseline. Almost 60% of the participants had at least a professional bachelor's degree at follow-up, and education level improved in 16 participants. Table 2 presents a correlation matrix for baseline and change in all exposure variables.

Table 1. Descriptive statistics for age, nutrition, physical activity, cardiorespiratory fitness and cardiometabolic markers

	Baseline		Follow-up		Change	
Characteristics (n=524)	M	SD	M	SD	M	SD
Age (years)	46.13	9.54	55.83	9.40	9.70b	0.51
Nutrition						
Sugar intake (g/day)	82.28	8.59	74.74	40.70	-7.55 [b]	43.21
Saturated fat intake (g/day)	37.05	15.12	34.86	14.75	-2.19 [a]	15.63
Fibre intake (g/day)	23.58	43.46	23.87	8.17	0.30	8.34
Alcohol intake (g/day)	14.08	15.01	14.64	15.40	0.56	13.48
Total energy intake (kcal)	2405	680	2274	632	-130 [b]	662
Healthy eating Index	46.43	10.89	48.53	10.45	2.10 [b]	11.42
Cardio-metabolic markers						
Waist circumference (cm)	84.39	10.33	85.75	10.35	1.36 [b]	4.93
Triacylglycerol (mmol/l)	1.18	0.64	1.15	0.62	-0.03	0.57
HDL-cholesterol (mmol/l)	1.58	0.39	1.54	0.38	-0.04 [b]	0.27
Fasting Plasma Glucose (mmol/l)	5.10	0.49	5.13	0.61	0.03	0.50
Diastolic Blood Pressure (mmHg)	79	8.27	86	9.61	7.06 [b]	9.40
Systolic Blood Pressure (mmHg)	126	13.76	135	17.29	9.20 [b]	15.18
CMRS	0.00	0.59	0.09	0.65	0.09 [b]	0.47
CMRSno-adip	0.00	0.59	0.07	0.66	0.07 [b]	0.52
Sedentary Behaviour						
Screen Time (h/week)	13.67	7.71	15.67	8.06	2.00 [b]	7.06
Passive Transportation (h/week)	5.06	3.94	5.00	3.83	-0.06	3.99
Total (h/week)	18.73	7.93	20.67	8.70	1.94 [b]	7.76
Moderate-to-Vigorous Physical Activity						
Sport Participation (h/week)	3.96	4.29	3.00	4.01	-0.95 [b]	4.28
Active Transportation (h/week)	0.87	0.85	1.24	1.33	0.37 [b]	1.23
Total (h/week)	4.83	4.59	4.24	4.43	-0.59	4.58
Cardiorespiratory Fitness [c]						
VO2 peak ($ml.min^{-1}.kg^{-1}$)	34.60	8.34	33.65	8.76	-1.47 [b]	5.87

Note. M = mean, SD = standard deviation
[a] p<0.01; [b] p<0.001 between baseline and follow-up
[c] subgroup n=399

When comparing the total group included in main analyses (n=524) and the group with CRF data (n=399), no differences were found for MVPA, SB and most other covariates at baseline or follow-up. However, those without CRF were, on average, older, showed lower HDL cholesterol at baseline, higher triglycerides at follow-up, and higher blood pressure and waist circumference at both time points.

Table 2. Correlation matrix for baseline and change in SB, MVPA & CRF

Baseline	SB	MVPA	CRF	*Change in*	SB	MVPA	CRF
SB	1.00			SB	1.00		
MVPA	-0.03	1.00		MVPA	-0.03	1.00	
CRF	-0.05	0.31*	1.00	CRF	-0.13*	0.20*	1.00

Spearman correlation coefficients (rho) for baseline and change in SB, MVPA & CRF
SB = Sedentary Behaviour; MVPA = moderate-to-vigorous physical activity; CRF=Cardiorespiratory fitness
* p<0.05

Associations with cardiometabolic risk

Standardised and unstandardised linear regression coefficients (95% CI) are presented in table 3 and online supplementary 2, respectively. There were no interaction effects by sex for any of the associations; hence, all associations are shown for men and women combined. Greater increases in SB were associated with greater increases in CMRS, $CMRS_{no-adip}$, waist circumference, triglycerides and diastolic blood pressure, and greater decreases in HDL cholesterol after adjusting for relevant confounders (model 1). Adjustment for baseline and change in MVPA in model 2 did not attenuate these associations. Change in CRF mediated the associations with change in CMRS and change in waist circumference (CMRS: a×b (95% CI) 0.002 (0.000 to 0.004); waist circumference: 0.02 (0.00 to 0.04)), but not the associations with change in HDL cholesterol, triglycerides or diastolic blood pressure. Change in waist circumference or nutritional intake did not mediate the associations with $CMRS_{no-adip}$, HDL cholesterol, triglycerides or diastolic blood pressure.

Greater decreases in MVPA were associated with greater increases in CMRS, $CMRS_{no-adip}$, waist circumference, fasting glucose and greater decreases in HDL cholesterol (model 1). Again, adjustment for baseline and change in SB did notattenuate these associations (model 2). Similarly to SB, change in CRF mediated the associations with change in CMRS and waist circumference (CMRS: a×b (95% CI) −0.01 (−0.01 to −0.00); waist circumference: −0.09 (−0.14 to −0.04)). We also found evidence for CRF mediation for HDL cholesterol (0.08 (0.01 to 0.14)), but not for fasting glucose. Change in waist circumference did not mediate the association with $CMRS_{no-adip}$, HDL cholesterol or fasting glucose.

Table 3. Standardized regression coefficients of sedentary behavior, moderate-to-vigorous physical activity and cardiorespiratory fitness for clustered cardio-metabolic health and cardio-metabolic markers

Change in	Model	Change in SB		Change in MVPA		Change in CRF	
			95% CI		95% CI		95% CI
CMRS	1	0.20 [c]	0.11 , 0.28	-0.17 [c]	-0.27 , -0.07	-0.40 [c]	-0.49 , -0.30
	2	0.20 [c]	0.11 , 0.29	-0.17 [c]	-0.27 , -0.08		
CMRSno-adip	1	0.19 [c]	0.10 , 0.28	-0.17 [c]	-0.27 , -0.07	-0.34 [c]	-0.43 , -0.24
	2	0.19 [c]	0.10 , 0.27	-0.17 [c]	-0.26 , -0.07		
Waist Circumference	1	0.12 [b]	0.03 , 0.21	-0.11 [a]	-0.21 , -0.01	-0.40 [c]	-0.50 , -0.31
	2	0.12 [a]	0.03 , 0.21	-0.11 [a]	-0.21 , -0.01		
Fasting Glucose	1	0.01	-0.08 , 0.10	-0.10 [a]	-0.20 , 0.00	-0.13 [b]	-0.23 , -0.03
	2	0.01	-0.09 , 0.10	-0.10 [a]	-0.20 , 0.00		
HDL-cholesterol	1	-0.16 [c]	-0.25 , -0.08	0.13 [b]	0.04 , 0.23	0.23 [c]	0.14 , 0.33
	2	-0.16 [c]	-0.25 , -0.07	0.14 [b]	0.04 , 0.23		
Triglycerides	1	0.15 [c]	0.07 , 0.24	-0.09	-0.18 , 0.00	-0.21 [c]	-0.31 , -0.11
	2	0.15 [c]	0.07 , 0.24	-0.08	-0.17 , 0.01		
Diastolic Blood Pressure	1	0.12 [b]	0.03 , 0.21	-0.08	-0.18 , 0.01	-0.22 [c]	-0.31 , -0.13
	2	0.12 [b]	0.04 , 0.20	-0.09	-0.18 , 0.01		
Systolic Blood Pressure	1	0.08	-0.01 , 0.17	0.01	-0.09 , 0.10	-0.14 [b]	-0.22 , -0.05
	2	0.08	-0.01 , 0.17	0.00	-0.09 , 0.10		

Legend Table 3:

Data are standardized regression coefficients

SB = Sedentary Behaviour; MVPA = moderate-to-vigorous physical activity; CRF=Cardiorespiratory fitness

Model 1: adjusted for age, follow-up time, sex, original study population; baseline and changes in healthy eating, smoking, education level; baseline of the relevant exposure and outcome (SB & MVPA n=524; CRF n=399)

Model 2: adjusted for all covariates in model 1 and adjusted for changes and baseline MVPA for SB and vice versa

[a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$

Greater decreases in CRF were associated with greater increases in all cardiometabolic markers, except for HDL cholesterol, showing a positive association (model 1). Change in waist circumference did not mediate the associations with blood pressure. However, change in waist circumference mediated the associations with $CMRS_{no-adip}$, fasting glucose, HDL cholesterol and triglycerides ($CMRS_{no-adip}$: −0.01 (−0.01 to −0.01); fasting glucose: −0.11 (−0.18 to −0.04); HDL cholesterol: 0.15 (0.08 to 0.22); and triglycerides: −0.40 (−0.81 to −0.01)).

A sensitivity analysis was run to examine whether associations for change in SB and MVPA in models 1 and 2 were modified in the smaller subset of participants with CRF data (see online supplementary 3). In general, associations were equivalent in terms of strength and direction compared with those found in the larger sample. Associations between change in MVPA and change in blood pressure were more pronounced in the smaller sample.

Of all possible interaction effects between change in the exposures, only one interaction, more specifically between change in SB and CRF for the association with HDL cholesterol, was borderline significant (β=−0.03; −0.05; −0.00), indicating that the associations found in table 3 did not differ by different levels of change in the other exposures. Figure 1 displays the estimated marginal means (SE) for change in CMRS in six groups of participants, defined by their change in CRF (decrease (62%, white bars) and no decrease (38%, black bars) over time) and change in SB (decrease (37%), increase between 0 and 4 hours/week (24%) and increase by >4 hours/week (39%)). The direction and strength of association between change in SB and CMRS was similar among those who increased and decreased their CRF. More specifically, in those with increasing CRF, increasing SB was associated with less decrease in clustered cardiometabolic risk, and in those with decreasing CRF, increasing SB was associated with greater increase in clustered cardiometabolic risk.

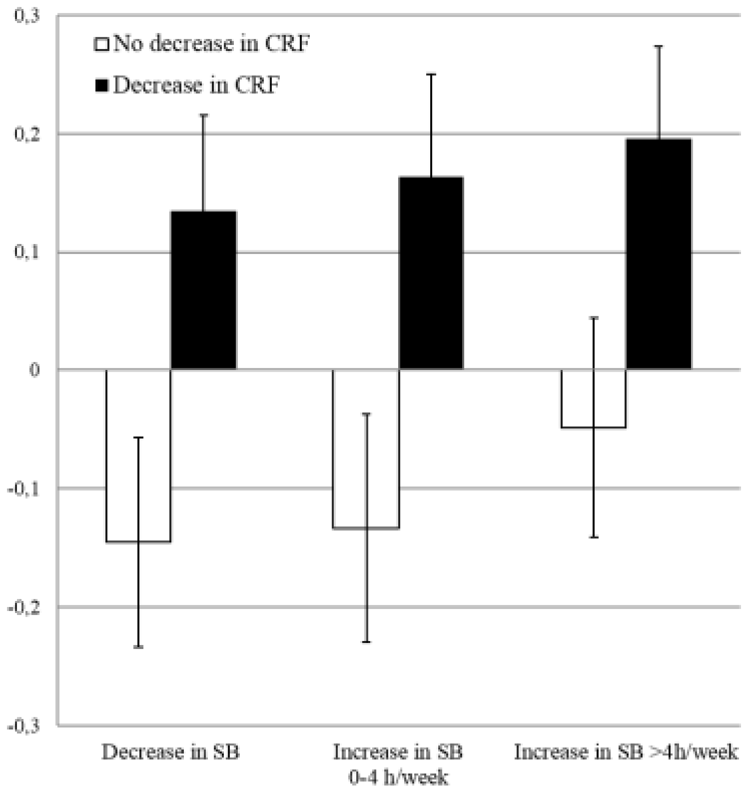

Figure 1. Estimated marginal means for change in cardiometabolic risk by change in cardiorespiratory fitness and sedentary behaviour

Legend: The estimated marginal means (SE) for change in cardiometabolic risk score in six groups of participants, defined by their change in cardiorespiratory fitness (CRF) (decrease (62%, white bars) and no decrease (38%, black bars) over time) and change in sedentary behaviour (SB) (decrease (37%), increase between 0 and 4 hours/week (24%) and increase by >4 hours/week (39%)).

DISCUSSION

Our results suggest that favourable changes in self-reported SB, MVPA and objectively measured CRF are associated with favourable changes in clustered cardiometabolic risk, independently from confounders and also from baseline and change in MVPA and SB, as applicable. Furthermore, increases in CRF were associated with favourable changes in all individual cardiometabolic risk markers; a decrease in SB, independently from change in MVPA, with a decrease in waist circumference, triglycerides, diastolic blood pressure and an increase in HDL cholesterol; and an increase in MVPA, independently from change in SB, with a decrease in waist circumference, fasting glucose and an increase in HDL cholesterol. In context, owing to the dose-dependent association between plasma triglycerides and both cardiovascular and all-cause mortality, for example, decreasing sitting time over 10 years by 2 hours/day would be associated with a 1% lower relative risk for cardiovascular and all-cause mortality, in an already relatively healthy population such as the present one.[34] Change in CRF mediated the associations between change in SB and change in CMRS and waist circumference, and the associations between change in MVPA and change in CMRS, waist circumference and HDL cholesterol. Change in waist circumference did not mediate these associations for SB or MVPA, and change in nutritional intake did not mediate the associations found for change in SB. However, change in waist circumference did mediate the association between CRF and $CMRS_{no-adip}$, fasting glucose, HDL cholesterol and triglycerides. This means that change in waist circumference might be a biologically plausible mechanism explaining the detrimental effects of low CRF on certain cardiometabolic health parameters.

Strengths and limitations

In this study, we were able to examine the relative importance of long-term changes in each of the three exposures for cardiometabolic health and their interrelationships. Previous longitudinal research examined individual associations between changes in SB, MVPA and CRF and clustered cardiometabolic risk,[11 21 35–38] but rarely included a mediation analysis for CRF. [7] Additional strengths of this study include the 10-year follow-up, which is longer than all previous studies.[11 30 36 37 39] Furthermore, to reduce measurement error, CRF was measured with a maximal cycle ergometer test and objectively measured VO_2, the gold standard for CRF testing.[26] Measuring CRF objectively in large studies is costly, not without danger and time-consuming. Therefore, CRF is generally approximated by extrapolation of a submaximal test.[11 35 38] Finally, since all analyses investigated changes within participants, interference of genetic influences is less likely.

The following limitations should, however, be considered. First, SB and MVPA were self-reported and did not cover all domains of daily living. Estimates included screen time, passive and active transportation and sports participation, and only including certain types of SB and MVPA might have introduced considerable bias. Self-report, in comparison with objective measurement, is also associated with more measurement error, increasing the risk for regression attenuation bias and potentially lowering the strength of the associations found for SB and MVPA. However, the FPACQ has been validated extensively[23 24] and shows, in comparison to other self-report measures, high test-retest reliability and criterion validity.[40] Furthermore, change in SB and MVPA is likely to be captured with smaller measurement error compared with an estimate of these behaviours at one single time-point, as participants are likely to misreport to a similar extent at both time-points.[37] Second, people at risk for heart failure and arterial hypertension did not participate in the maximal exercise test and therefore CRF data were only available for a healthier subgroup. However, a sensitivity analysis which examined whether associations for change in SB and MVPA were modified in the smaller subset of participants with CRF data compared with the total included participant group showed no substantial differences in associations. Furthermore, non-inclusion of these participants may have reduced the risk for reverse causality for the associations found. Nevertheless, associations found in this study are relative to the fairly small cohort of Caucasian adults included in analysis and need to be examined in other populations, heterogeneous in terms of ethnicity and health status. Third, although CMRS has several advantages for evaluating cardiometabolic risk over the dichotomous classification of the metabolic syndrome, [29] the score is sample specific and therefore highly dependent on the distribution of risk factors of the included participants. Finally, analyses could not be controlled for medication use. Although this was a healthy adult population, some participants would have taken relevant medication, which might cause residual confounding. However, exclusion for arterial hypertension in the subsample analyses will have minimised the impact of this for antihypertensive medication.

Comparison to previous research

To the best of our knowledge, no previous longitudinal studies examined associations between changes in SB, MVPA and CRF and cardiometabolic risk and the mediating role of CRF. However, our findings extend previous cross-sectional and longitudinal findings of associations between SB and clustered cardiometabolic risk,[9 30 41] waist circumference,[30 41] HDL cholesterol[41] and triglycerides,[30 40] independent of MVPA. Other cross-sectional[38 42] and longitudinal[10 11] studies could not confirm an association between SB and clustered cardiometabolic risk, when adjusting for MVPA.

Inconsistent findings for SB and cardiometabolic risk might be due to a number of reasons. First, in other populations with more homogeneous outcome profiles, associations with clustered cardiometabolic risk might be difficult to detect.[38 42] Second, in patient populations with higher medication use, the positive effects of lowering SB might be drowned out by the health improvements caused by the use of this medication.[11] Third, the type and measurement method of SB under study might also cause inconsistencies. Self-reported SB typically includes mainly screen time, while studies using objective measurement include all SBs.[30 43] The mean change in SB in our study was mostly driven by a change in screen time, which may be associated with a different confounding structure and different pathways explaining associations (such as differences in dietary intake), compared with studies that implemented objective measurement of total sedentary time.[11 21 38]

Change in MVPA was associated with change in clustered cardiometabolic risk, waist circumference, fasting glucose and HDL cholesterol, independent from change in SB. These associations were found to be mediated through change in CRF, except for the association with fasting glucose. When comparing to a cross-sectional study, the association between MVPA and HDL cholesterol and fasting glucose, independent of SB, was of similar strength.[38] However, for the independent association with waist circumference, this study observed a considerably smaller association.[38] Differences in study design, statistical analysis and measurement method may have caused these differences in results.

Finally, changes in CRF were associated with changes in clustered cardiometabolic risk and all individual markers. Furthermore, these associations were almost always more than twice as strong as those with changes in SB or MVPA. The difference in strength of associations is in line with previous research, where CRF is often recognised as one of the most important determinants of longevity and health.[7 9 44] However, part of this difference in strength of association might be due to the difference in measurement error between objectively measured CRF and self-reported SB and MVPA. Furthermore, CRF is a physiological measure reflecting a combination of genetic potential, behavioural and functional health of various organ systems.[45] As a result, CRF improves mostly when MVPA increases and when SB decreases.[46] Therefore, CRF was also examined as a potential mediator in all significant associations between change in SB or MVPA and change in cardiometabolic health.

Conclusions

In conclusion, favourable changes in self-reported SB and MVPA are independently associated with positive changes in clustered cardiometabolic health, and favourable changes in CRF are associated with positive changes in clustered cardiometabolic health. The associations between change in SB and MVPA and change in clustered cardiometabolic risk were mediated through changes in CRF and the association between CRF and clustered cardiometabolic risk was mediated through changes in waist circumference. On the basis of these results, a combination of decreasing SB and increasing MVPA, most likely resulting in a positive change in CRF, is most beneficial towards cardiometabolic health. Further longitudinal studies investigating changes in all three exposures measured objectively are necessary to gain better insights into their associations and inter-relationships with cardiometabolic health as well as intervention studies that try to integrate both SB and MVPA and evaluate the mediating effect of CRF.

Key Messages

- The greater increase in sedentary behaviour (SB) and decrease in moderate-to-vigorous physical activity (MVPA) was associated with greater increase in clustered cardiometabolic risk, independently from change in MVPA and SB, respectively.
- The greater decrease in cardiorespiratory fitness (CRF) was associated with greater increase in clustered cardiometabolic risk.
- The associations of change in SB and MVPA with change in clustered cardiometabolic risk were mediated through change in CRF.
- The association between change in CRF and change in clustered cardiometabolic risk was mediated through change in waist circumference.
- Clinical practice should recommend lifestyle changes resulting in a combination of decreasing SB and increasing MVPA, most likely resulting in a positive change in CRF, for the most beneficial cardiometabolic health

Acknowledgements

Contributors JL and JGB coordinated the study design. EM, RC and KW carried out the measurements. SK and KW contributed to the conception of the paper. SK performed the statistical analysis and drafted the manuscript. All the authors read and approved the final manuscript.

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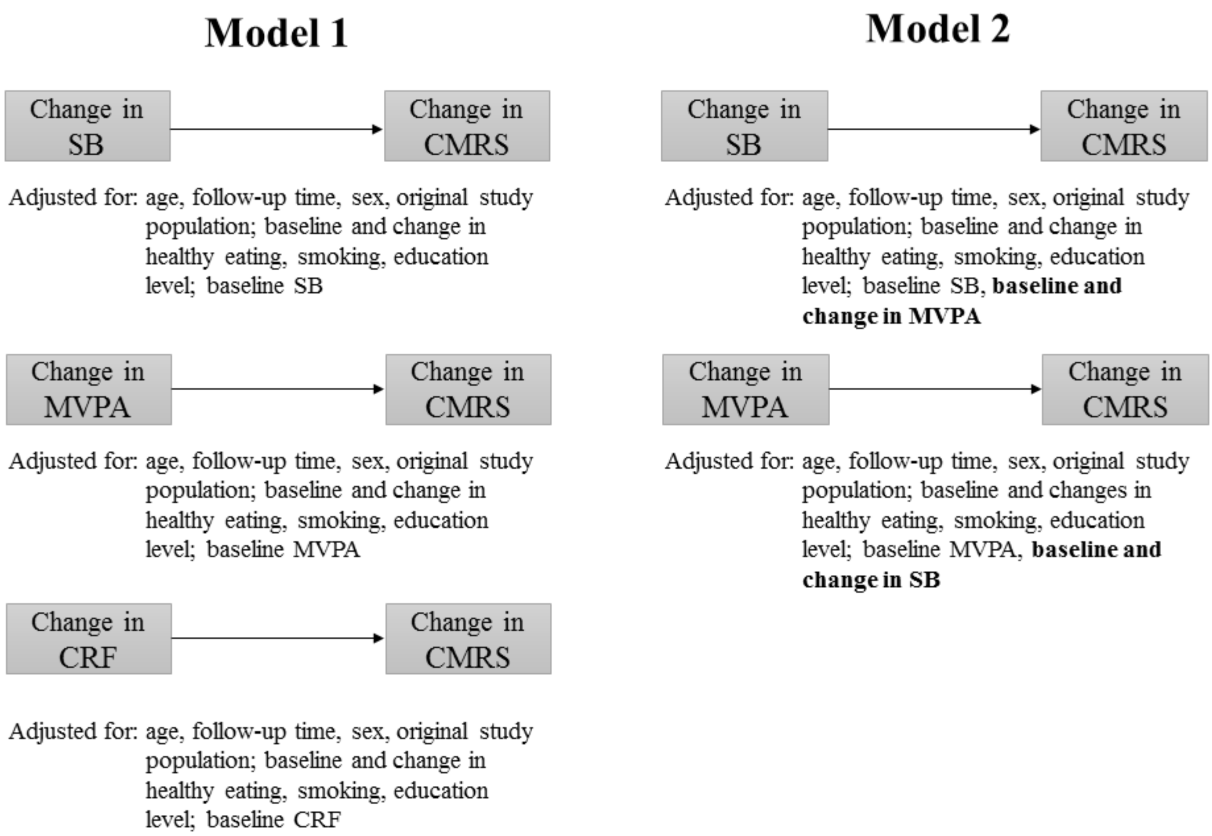
Visual representation of regression models:



Note. SB = Sedentary Behavior; MVPA = moderate-to-vigorous physical activity; CRF=Cardiorespiratory fitness, CMRS = Cardio-metabolic risk score

Supplement 1. Regression coefficients of sedentary behavior, moderate-to-vigorous physical activity and cardiorespiratory fitness for clustered cardio-metabolic health and cardio-metabolic markers

		Change in SB					Change in MVPA					Change in CRF				
Change in	Model			95% CI					95% CI					95% CI		
CMRS	1	0.01	c	0.01	,	0.02	-0.02	c	-0.03	,	-0.01	-0.03	c	-0.04	,	-0.02
	2	0.01	c	0.01	,	0.02	-0.02	c	-0.03	,	-0.01					
CMRSno-adip	1	0.01	c	0.01	,	0.02	-0.02	c	-0.03	,	-0.01	-0.03	c	-0.04	,	-0.02
	2	0.01	c	0.01	,	0.02	-0.02	c	-0.03	,	-0.01					
Waist Circumference (cm)	1	0.07	b	0.02	,	0.13	-0.12	a	-0.22	,	-0.01	-0.34	c	-0.42	,	-0.26
	2	0.08	a	0.02	,	0.13	-0.12	a	-0.23	,	-0.01					
Fasting Glucose (mmol/L)	1	0.00		-0.01	,	0.01	-0.01	a	-0.02	,	0.00	-0.01	b	-0.02	,	0.00
	2	0.00		-0.01	,	0.01	-0.01	a	-0.02	,	0.00					
HDL-cholesterol (mmol/L)	1	-0.01	c	-0.01	,	0.00	0.01	b	0.00	,	0.01	0.01	c	0.01	,	0.02
	2	-0.01	c	-0.01	,	0.00	0.01	b	0.00	,	0.01					
Triglycerides (mmol/L)	1	0.01	c	0.01	,	0.02	-0.01		-0.02	,	0.00	-0.02	c	-0.03	,	-0.01
	2	0.01	c	0.01	,	0.02	-0.01		-0.02	,	0.00					
Diastolic Blood Pressure (mmHg)	1	0.14	b	0.04	,	0.24	-0.17		-0.37	,	0.02	-0.35	c	-0.50	,	-0.21
	2	0.14	b	0.03	,	0.24	-0.18		-0.37	,	0.01					
Systolic Blood Pressure (mmHg)	1	0.16		-0.01	,	0.32	0.02		-0.29	,	0.34	-0.35	b	-0.57	,	-0.13
	2	0.15		-0.02	,	0.32	0.02		-0.30	,	0.33					

SB = Sedentary Behavior; MVPA = moderate-to-vigorous physical activity; CRF=Cardiorespiratory fitness

Model 1: adjusted for age, follow-up time, sex, original study population; baseline and changes in healthy eating, smoking, education level; baseline of the relevant exposure and outcome (SB & MVPA n=524; CRF n=399)

Model 2: adjusted for all covariates in model 1 adjusted for changes and baseline MVPA for SB and vice versa (n=524)

a p<0.05; b p<0.01 c p<0.001

Supplement 2. Standardized regression coefficients of sedentary behavior, moderate-to-vigorous physical activity and cardiorespiratory fitness for clustered cardio-metabolic health and cardio-metabolic markers in a subgroup with complete data

		Change in SB					Change in MVPA					Change in CRF				
Change in	Model			95% CI					95% CI					95% CI		
CMRS	1	0.19	c	0.08	,	0.29	-0.21	c	-0.32	,	-0.10	-0.40	c	-0.49	,	-0.30
	2	0.18	c	0.08	,	0.29	-0.21	c	-0.32	,	-0.10					
$CMRS_{no\text{-}adip}$	1	0.18	c	0.07	,	0.28	-0.20	c	-0.31	,	-0.09	-0.34	c	-0.43	,	-0.24
	2	0.17	c	0.07	,	0.28	-0.20	c	-0.31	,	-0.09					
Waist Circumference	1	0.11	a	0.01	,	0.22	-0.15	a	-0.26	,	-0.03	-0.40	c	-0.50	,	-0.31
	2	0.11	a	0.01	,	0.22	-0.15	a	-0.26	,	-0.03					
Fasting Glucose	1	0.04		-0.06	,	0.15	-0.05		-0.16	,	0.06	-0.13	b	-0.23	,	-0.03
	2	0.04		-0.06	,	0.15	-0.05		-0.16	,	0.07					
HDL-cholesterol	1	-0.12	a	-0.22	,	-0.02	0.22	c	0.11	,	0.32	0.23	c	0.14	,	0.33
	2	-0.12	a	-0.22	,	-0.02	0.21	c	0.10	,	0.32					
Triglycerides	1	0.14	b	0.04	,	0.24	-0.09		-0.20	,	0.01	-0.21	c	-0.31	,	-0.11
	2	0.14	b	0.04	,	0.24	-0.09		-0.20	,	0.01					
Diastolic Blood Pressure	1	0.10	a	0.00	,	0.20	-0.14	a	-0.25	,	-0.03	-0.22	c	-0.31	,	-0.13
	2	0.11	a	0.01	,	0.21	-0.14	a	-0.24	,	-0.03					
Systolic Blood Pressure	1	0.08		-0.02	,	0.18	-0.06		-0.17	,	0.04	-0.14	b	-0.22	,	-0.05
	2	0.09		-0.01	,	0.19	-0.06		-0.17	,	0.04					

Data are standardized regression coefficients

SB = Sedentary Behavior; MVPA = moderate-to-vigorous physical activity; CRF=Cardiorespiratory fitness

Model 1: adjusted for age, follow-up time, sex, original study population; baseline and changes in healthy eating, smoking, education level; baseline of the relevant exposure and outcome (SB, MVPA & CRF n=399)

Model 2: adjusted for all covariates in model 1 adjusted for changes and baseline MVPA for SB and vice versa;

[a] p<0.05; [b] p<0.01; [c] p<0.001

PAPER 5

Cross-sectional and longitudinal independent associations between muscular fitness, cardiorespiratory fitness and cardio-metabolic health

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Cross-sectional and longitudinal independent associations between muscular fitness, cardiorespiratory fitness and cardio-metabolic health

Abstract

Purpose The primary aim of this study was to investigate the possible additive contribution of objectively measured muscular fitness (MF) and change in MF towards cardio-metabolic health, independent of the favorable effects of change in cardiorespiratory fitness (CRF).

Methods A sample of 357 adults was followed for 9.68 (±0.50) years with a mean age of 44.51 (±0.9.26) at baseline. CRF (VO2peak, ml.min-1.kg-1) and MF (isokinetically measured peak torque, N.m.kg-1) were objectively measured at baseline (2002-2004) and follow-up (2012-2014) with a maximal cycle ergometer test and a Biodex dynamometer, respectively. A clustered cardio-metabolic risk score, largely based on the criteria for the metabolic syndrome, represented cardiometabolic health x Pearson correlation and multiple linear regression (adjusting for age, sex, education level, smoking, sedentary behavior, physical activity, diet) was used to examine the cross-sectional and longitudinal associations between fitness parameters and cardio-metabolic risk.

Results CRF and MF were cross-sectionally associated with clustered cardio-metabolic health (β = -0.46, p<0.001 and β = -0.18, p<0.01, respectively). However, only CRF was associated with clustered cardio-metabolic health independent from MF (β = -0.43, p<0.001). Examining associations with change over time in CRF or MF, only change in CRF was associated with change in clustered cardio-metabolic risk, independent from change in MF (β = -0.37, p<0.001).

Conclusion CRF is strongly associated with cardio-metabolic risk, both in the short and in the long term. However, added benefits of objectively measured MF towards better cardio-metabolic health are small, certainly looking at changes over a longer period of time.

Keywords

Muscular fitness, cardiorespiratory fitness, clustered cardio-metabolic health

INTRODUCTION

Numerous studies have examined the benefits of cardiorespiratory fitness (CRF) on individual parameters of cardio-metabolic health and mortality, whether or not independent from physical activity and sedentary behavior [1-10]. Results indicated that poor CRF is a powerful predictor for various clinical outcomes and should be incorporated in health evaluation and management [11]. Apart from CRF, muscular fitness (MF) has also been proposed as a predictor for mortality [12, 13]. However, the protective effect of MF for cardio-metabolic health is less clear. An inverse association between MF and the metabolic syndrome was observed in one study [14] and resistance training reduced the risk of coronary heart disease and the metabolic syndrome in two other studies [15, 16]. However, a small training study with eight weeks of resistance training in overweight and obese women did not observe a favorable effect [17]. Furthermore, when investigating contributions of CRF and MF towards cardio-metabolic health, independent of one another, the impact of MF appears to be low or even nonexistent [18-23]. Longitudinal observational studies examining the association of both CRF and MF with cardio-metabolic health are scarce [18, 23, 24]. These studies concluded that muscle strength has a protective role for the metabolic syndrome [18, 23] or type 2 diabetes [24], largely independent of CRF. However, mostly men were included. In conclusion, CRF appears to be the most important predictor for cardio-metabolic health with some studies observing an added value of MF [19, 20, 22] and others not [17, 18, 21].

Large epidemiological studies often approximate parameters of fitness, mostly because of time constraints, financial costs and health risks involved with maximal effort testing. However, maximal exercise tests such as through a cycle ergometer with breath-by-breath gas exchange and a calibrated isokinetic dynamometer generate more precise measurements.

The primary aim of this study was to investigate the possible additive contribution of change over a period of 10 years in objectively measured MF towards cardio-metabolic health, independent of the favorable effects of change in CRF. Contrary to other studies, in this study both CRF and MF were objectively measured with a maximal exercise test at baseline and follow-up. We hypothesize that a positive change in CRF and MF will be associated with a positive change in cardio-metabolic health. Furthermore, we hypothesize that CRF will be more strongly associated, however, we presume that MF will make a small but significant contribution to the association with cardio-metabolic health.

Methods

Participants and study design

We examined this in the longitudinal Flemish Policy Research Centre Sport study, a prospective (2002-2004 and 2012-2014) cohort of Flemish adults aged 18-75 at baseline. The sampling procedure of this study is previously described in detail by Duvigneaud et al. [25]. Between 2012 and 2014 all volunteers who visited the examination centre in 2002-2004 (n=1569) were re-invited for follow-up to repeat the complete measurement protocol. Six hundred fifty-two (42%) volunteers returned to participate. Written informed consent was obtained from all subjects and the study was approved by the Medical Ethics Committee of the KU Leuven (s54083).

Cardiorespiratory fitness (CRF)

CRF was defined as peak oxygen uptake (VO2peak) relative to body weight and determined by means of a maximal exercise test on an electrically braked Lode Excalibur cycle ergometer (Lode, Groningen, The Netherlands) [26]. A standardized step protocol started with a workload of 20 W, which was increased stepwise by 20 W.min-1. Oxygen consumption (VO2) was measured directly with breath-by-breath respiratory gas exchange analysis, using a Cortex MetaLyzer 3B analyzer (Cortex Biophysic GmbH, Leipzig, Germany) [26, 27]. Participants were verbally encouraged to reach a maximal level of exertion and the test was terminated when subjects were exhausted or when the physician stopped the test for medical reasons.

Muscular fitness (MF)

A calibrated Biodex System Pro 3® dynamometer (Biodex Medical Systems, Shirley, NY) was used to measure dynamic muscular strength of the quadriceps in a standardized manner [20]. Isokinetic measurements using this device are found to be reliable and valid [28]. Participants were asked to conduct four maximal knee extension-flexion movements at a low velocity of 60°/s. Unless medically contraindicated, peak torque (Nm) of the right upper leg was recorded, otherwise the left leg was used. Peak torque was divided by the subjects’ weight to correct for total body weight.

Clustered cardiometabolic risk

A clustered cardio-metabolic risk score (CMRS), largely based on the criteria for the metabolic syndrome, was calculated [29]. Metabolic parameters were assessed by trained staff in the morning after an overnight fast. Waist circumference was measured to the nearest 0.1 cm. Systolic and diastolic blood pressure was measured by an electronic monitor (Omron, The Netherlands) three times in seated position from the right arm. The means of the three measurements were used in statistical analyses. Triglycerides, plasma glucose and HDL-cholesterol were obtained from an antecubital vein and analysed by enzymatic methods (Abbott Laboratories, Abbott Park, IL). Due to skewness, values for the latter three parameters were first normalized (log10). Subsequently, standardized values for waist circumference, triglyceride, plasma glucose, blood pressure and the inverse of HDL-cholesterol were computed. Each cardio-metabolic variable was standardized by using the sex-specific baseline sample mean and standard deviation, derived from all men and women with baseline data for each cardio-metabolic variable. To calculate CMRS, the sum of these standardized values was divided by the number of metabolic parameters included (n=5).

Behavioural parameters

Sedentary behaviour and moderate-to-vigorous physical activity were assessed using the Flemish Physical Activity Computerized Questionnaire (FPACQ), which showed high test-retest reliability and reasonable criterion validity [30].

Smoking behaviour was assessed using the WHO Monica Smoking Questionnaire. Participants were classified as current, former or never smokers. Education level was used as an indicator of socio-economic status, and was ranked in four categories, ranging from no degree or primary school degree; secondary school degree; professional bachelor degree; and higher. Food consumption was assessed using a three-day diet record, during two weekdays and one weekend day. Participants were instructed to weigh and record all foods and drinks or alternatively estimate portions using standard household measures such as a plate, spoon or glass. The diet records were analysed using Becel Nutrition software (Unilever Co., Rotterdam, The Netherlands) and the Healthy Eating Index, which captures the key recommendations of the 2010 Dietary Guidelines (HEI), was calculated [31].

Statistical analysis

A drop-out analysis was performed by comparing baseline results between the follow-up group and the group that participated at baseline only (unpaired t-test and Chi²-test). Descriptive characteristics of the included sample were compared between baseline and follow-up (paired t-test and Chi²-test) and changes in characteristics were calculated as follow-up minus baseline.

Pearson correlations were calculated at follow-up between MF, CRF and all cardio-metabolic markers. Furthermore, a longitudinal analysis was done, calculating Pearson correlations between change in MF, change in CRF and change in cardio-metabolic markers.

Multiple linear regression was used to examine the cross-sectional association between fitness parameters and clustered cardio-metabolic risk and its separate risk factors; and longitudinal association between changes in these parameters. All variables were tested for normality and residuals were tested for homoscedasticity, linearity and independence and the variance inflation factor never exceeded two, indicating that multi-collinearity was not a concern [32]. Both exposure and outcome variables were standardized and standardized regression coefficients (95% CI) are presented, in order to enable direct comparison of the effect estimates across outcome and exposure variables. Cross-sectional Model 1 was initially adjusted for age, sex, HEI, smoking and education level. Longitudinal Model 1 was additionally adjusted for follow-up time, baseline value of the exposure under study, baseline value of CMRS, and baseline and change values of HEI, smoking and education level. Subsequently, cross-sectional Model 1 for CRF was further adjusted for MF and vice versa (Model 2). Longitudinal Model 1 for CRF was further adjusted for baseline and change in MF and vice versa (Model 2). All analyses were tested for an interaction effect with sex and age, which were found to be non-significant, so results are presented for the total included sample. Interaction effects between CRF and MF were also examined. Furthermore, logistic regression models assessed odds ratio (OR) and 95% confidence interval associated with CRF or MF and the metabolic syndrome as defined by the International Diabetes Foundation [33].

The population was divided in tertiles of CRF and tertiles of MF, stratified by sex and age. More specifically, all participants were distributed by sex in the following age groups: >40, 40-50, 50-60, 60+ year at follow-up and then each age/sex group was divided into tertiles of CRF and of MF. For the longitudinal analyses, the population was divided in tertiles of change in CRF and tertiles of change in MF, again stratified by age and sex. A one-way analysis of covariance

(ANCOVA) was applied to evaluate a trend in CMRS over tertiles of CRF and MF and tertiles of change in CRF and MF, corrected for all relevant covariates as described above. Least squares means of CMRS and all separate risk factors were computed for all tertiles of CRF and all tertiles of MF and significant differences were noted.

Results

Male participants who had dropped out had lower education and CRF, and higher triglycerides. There were no differences in MF, waist circumference, HDL-cholesterol, fasting glucose, blood pressure, smoking status, nutrition, age, moderate-to-vigorous physical activity or sedentary behaviour. Women who dropped out had lower HDL-cholesterol, CRF and moderate-to-vigorous physical activity, and higher systolic blood pressure and waist circumference. No other differences were observed between both groups.

Table 1 represents the descriptive statistics of all 357 participants for age, nutrition, cardio-metabolic markers, physical activity and fitness. In total 231 men and 126 (35%) women participated and were followed for 9.68 (±0.50) years. At follow-up 47 (9%) participants were smokers and 247 (48%) participants were former smokers. Twenty nine (8%) participants quit smoking between both time points and 4 (1%) participants started smoking. Furthermore, at follow-up 217 (61%) participants had a professional bachelor degree or higher. Change over a 10-year time period is also presented in Table 1. In general, participants' nutrition improved, but cardio-metabolic health markers, physical activity and fitness parameters decreased over time.

CRF and MF were correlated at baseline ($r = 0.38$, $p < .001$) and follow up ($r = 0.44$, $p < .001$). Change between both fitness parameters (CRF and MF) was not correlated ($r = 0.07$, $p = 0.21$). CRF at baseline was correlated with CRF at follow up ($r = 0.77$, $p < .001$) and change in CRF ($r = -0.26$, $p < .001$). Similarly, MF at baseline was correlated with MF at follow up ($r = 0.54$, $p < .001$) and change in MF ($r = -0.53$, $p < .001$.

Table 1. Descriptive statistics for age, nutrition, cardio-metabolic markers, physical activity and fitness

	Baseline		10 year follow-up		Change	
Characteristics (n=357)	M	SD	M	SD	M	SD
Age (years)	44.51	9.26	54.24	9.11	9.72***	0.49
Nutrition						
Sugar intake (g/day)	80.98	41.88	74.89	40.27	-6.09**	42.23
Saturated fat intake (g/day)	37.30	15.13	35.25	14.78	-2.06*	15.74
Fibre intake (g/day)	23.47	8.87	23.89	8.32	0.42	8.57
Alcohol intake (g/day)	14.05	14.57	14.81	15.21	0.77	12.03
Total energy intake (kcal)	2400	651	2296	643	-104**	647
Healthy eating Index	45.98	10.82	48.33	10.13	2.36***	11.15
Cardiometabolic markers						
Waist circumference (cm)	83.50	9.92	84.93	10.14	1.43***	4.64
Triacylglycerol (mmol/l)	1.15	0.58	1.15	0.61	0.00	0.58
HDL-cholesterol (mmol/l)	1.59	0.38	1.55	0.38	-0.05***	0.26
Fasting Plasma Glucose (mmol/l)	5.07	0.44	5.08	0.55	0.01	0.47
Diastolic Blood Pressute (mmHg)	78.5	7.57	85.7	9.01	7.19***	8.89
Systolic Blood Pressure (mmHg)	125	12.45	134	15.18	8.82***	13.43
CMRS	-0.07	0.54	0.02	0.62	0.10***	0.45
Physical activity						
Sedentary Behavior (h/week)	18.73	8.14	20.45	8.81	1.71***	8.04
MVPA (h/week)	4.69	4.19	4.22	4.29	-0.47*	4.04
Fitness						
VO2 peak ($ml.min^{-1}.kg^{-1}$)	35.43	8.30	34.11	8.85	-1.32***	5.89
Muscular strength ($N.m.kg^{-1}$)	2.08	0.48	1.87	0.45	-0.2***	0.45

Note. M = mean, SD = standard deviation, MVPA = Moderate-to-vigorous physical activity

*p<0.05; **p<0.01;***p<0.001 between baseline and follow-up

Table 2 presents the standardized regression coefficients of CRF and MF for clustered cardio-metabolic risk and separate risk factors, adjusted for sex, age, HEI, smoking and education level and the other exposure (MF and CRF, respectively). CRF was associated with CMRS and all separate risk factors, both in a cross-sectional and longitudinal model and after correction for MF. Contrary to these results, MF was only cross-sectionally associated with CMRS, waist circumference and HDL-cholesterol. After correcting for CRF, only the association with waist circumference remained significant. There were no significant interaction effects between CRF and MF for any of the observed associations.

Table 2. Standardized regression coefficients of cardiorespiratory fitness and muscular fitness for clustered cardio-metabolic health and separate risk factors (n=357)

		CRF					Muscular fitness				
Cross-sectional	Model			95% CI					95% CI		
CMRS	1	-0.46	***	-0.59	,	-0.32	-0.18	**	-0.29	,	-0.06
	2	-0.43	***	-0.60	,	-0.29	-0.10		-0.22	,	0.01
Waist Circumference	1	-0.47	***	-0.58	,	-0.37	-0.24	***	-0.33	,	-0.15
	2	-0.43	***	-0.53	,	-0.32	-0.17	***	-0.25	,	-0.08
Fasting Glucose	1	-0.17	*	-0.31	,	-0.03	-0.01		-0.13	,	0.10
	2	-0.17	*	-0.32	,	-0.03	0.02		-0.10	,	0.13
HDL-cholesterol	1	0.26	***	0.13	,	0.38	0.13	*	0.03	,	0.23
	2	0.24	***	0.11	,	0.36	0.09		-0.01	,	0.19
Triglycerides	1	-0.21	**	-0.36	,	-0.06	-0.09		-0.21	,	0.03
	2	-0.19	*	-0.34	,	-0.04	-0.05		-0.17	,	0.07
Systolic Blood Pressure	1	-0.13		-0.26	,	0.00	0.03		-0.07	,	0.14
	2	-0.14	*	-0.28	,	-0.01	0.07		-0.04	,	0.18
Diastolic Blood Pressure	1	-0.17	*	-0.31	,	-0.03	-0.07		-0.19	,	0.04
	2	-0.16	*	-0.31	,	-0.01	-0.04		-0.16	,	0.08

		CRF					Muscular fitness				
Change in	Model			95% CI					95% CI		
CMRS	1	-0.37	***	-0.48	,	-0.26	-0.09		-0.22	,	0.04
	2	-0.37	***	-0.48	,	-0.26	-0.04		-0.16	,	0.08
Waist Circumference	1	-0.43	***	-0.54	,	-0.32	-0.13		-0.26	,	0.00
	2	-0.42	***	-0.53	,	-0.31	-0.07		-0.19	,	0.05
Fasting Glucose	1	-0.15	*	-0.27	,	-0.03	-0.04		-0.16	,	0.09
	2	-0.17	**	-0.29	,	-0.05	-0.01		-0.14	,	0.12
HDL-cholesterol	1	0.20	***	0.10	,	0.31	0.10		-0.02	,	0.22
	2	0.19	***	0.09	,	0.30	0.08		-0.03	,	0.20
Triglycerides	1	-0.17	**	-0.27	,	-0.06	-0.07		-0.19	,	0.05
	2	-0.16	**	-0.27	,	-0.05	-0.05		-0.17	,	0.07
Systolic Blood Pressure	1	-0.13	**	-0.24	,	-0.02	0.04		-0.08	,	0.16
	2	-0.14	**	-0.25	,	-0.03	0.05		-0.07	,	0.17
Diastolic Blood Pressure	1	-0.17	**	-0.28	,	-0.06	-0.04		-0.16	,	0.08
	2	-0.16	**	-0.27	,	-0.05	-0.03		-0.15	,	0.10

Data are standardized regression coefficients; CRF=Cardiorespiratory fitness

Model 1: cross-sectional model adjusted for: age, sex, original study population, healthy eating index (HEI), smoking, education level, relevant exposure; longitudinal model adjusted for: age, sex, original study population, follow-up time, baseline and changes in HEI, smoking & education level; baseline and change of the relevant exposure

Model 2: adjusted for all covariates in model 1 with additional adjustment for: cross-sectional model: muscular fitness for CRF and vice versa; longitudinal model: baseline and change in muscular fitness for CRF and vice versa

*p<0.05;
p<0.01;*p<0.001

After controlling for age, sex, education, smoking, HEI, physical activity and sedentary behavior CRF and change in CRF were associated with the likelihood of having the metabolic syndrome as defined by the International Diabetes Foundation [33] (OR = 0.87, 95% CI = 0.80-0.94 and OR = 0.85, 95% CI = 0.78-0.94, respectively). Odds-ratios for MF and change in MF were not significant. Additionally controlling for MF did not alter the OR for CRF or change in CRF and the likelihood of having the metabolic syndrome (OR = 0.87, 95% CI = 0.80-0.95 and OR = 0.86, 95% CI = 0.78-0.95, respectively).

Least squares means at follow-up and least squares means of change over time of clustered cardio-metabolic risk and cardio-metabolic risk markers by tertiles of CRF and tertiles of change in CRF are presented in Table 3. Furthermore, the trend over the tertiles and significant differences between means are presented. Similarly, Table 4 represents these results for muscular fitness.

Table 3. Comparison between least squares means of clustered cardio-metabolic risk and cardio-metabolic risk markers by tertiles of cardiorespiratory fitness at follow-up, and by tertiles of changes in cardiorespiratory fitness over a 10-year time period

	Lowest tertile (n=131)		Middle tertile (n=114)			Highest tertile (n=112)			p
CMRS	0.06	(-0.03;0.15)	-0.07	(0.17;0.2)	a	-0.22	(0.32;0.12)	ab	***
Waist Circumference (cm)	85.85	(84.62;87.08)	83.36	(82.07;84.66)	a	80.89	(79.54;82.24)	ab	***
Fasting Glucose (mmol)	5.11	(5.04;5.18)	5.03	(4.95;5.10)		5.07	(4.99;5.15)		***
HDL-cholesterol (mmol)	1.53	(1.47;1.59)	1.59	(1.53;1.65)		1.68	(1.62;1.74)	ab	***
Triglycerides (mmol)	1.19	(1.10;1.30)	1.18	(1.07;1.28)		1.07	(0.96;1.18)		***
Systolic Blood Pressure (mmHg)	125	(123;127)	125	(123;127)		125	(123;127)		***
Diastolic Blood Pressure (mmHg)	79	(78;80)	78	(77;80)		78	(77;79)		***
change in	Lowest tertile (n=118)		Middle tertile (n=116)			Highest tertile (n=123)			
CMRS	0.20	(0.13;0.28)	0.14	(0.06;0.22)		-0.05	(-0.13;0.02)	ab	***
Waist Circumference (cm)	2.93	(2.14;3.72)	1.34	(0.55;2.13)	a	0.07	(-0.71;0.85)	ab	***
Fasting Glucose (mmol)	0.2	(-0.06;0.11)	0.08	(-0.01;0.16)	a	-0.07	(-0.16;0.01)	b	
HDL-cholesterol (mmol)	-0.06	(-0.11;-0.01)	-0.06	(-0.11;-0.01)		-0.03	(-0.07;0.02)		*
Triglycerides (mmol)	0.10	(-0.01;0.20)	0.00	(-0.10;0.11)		-0.09	(-0.19;0.01)	a	*
Systolic Blood Pressure (mmHg)	10.28	(7.92;12.65)	8.64	(6.28;11.00)		7.59	(5.27;9.92)		***
Diastolic Blood Pressure (mmHg)	9.35	(7.78;10.92)	6.93	(5.36;8.50)	a	5.35	(3.81;6.89)	a	***

Note. CMRS = Clustered Cardio-Metabolic Risk Score

[a] Significantly different from lowest tertile, [b] significantly different from middle tertile
*p<0.05; **p<0.01;***p<0.001

Table 4. Comparison between least squares means of clustered cardio-metabolic risk and cardio-metabolic risk markers by tertiles of muscular fitness at follow-up, and by tertiles in changes of muscular fitness over a 10-year time period

	Lowest tertile (n=116)		Middle tertile (n=118)		Highest tertile (n=123)		p
CMRS	0.03	(-0.07.0.13)	-0.09	(-0.19;0.00)	-0.15	(-0.24;-0.05) [a]	***
Waist Circumference (cm)	85.4	(84.1;86.7)	83.3	(82.0;84.6) [a]	81.9	(80.6;83.2) [a]	***
Fasting Glucose (mmol)	5.08	(5.00;5.15)	5.07	(5.00;5.15)	5.06	(4.99;5.14)	***
HDL-cholesterol (mmol)	1.53	(1.48;1.59)	1.61	(1.56;1.67) [a]	1.63	(1.58;1.69)	***
Triglycerides (mmol)	1.16	(1.06;1.26)	1.18	(1.07;1.28)	1.11	(1.01;1.22)	**
Systolic Blood Pressure (mmHg)	125	(123;128)	124	(122;126)	125	(122;127)	***
Diastolic Blood Pressure (mmHg)	79	(78;80)	78	(77;79)	78	(77;80)	***
change in	Lowest tertile (n=119)		Middle tertile (n=115)		Highest tertile (n=123)		
CMRS	0.12	(0.04;0.21)	0.09	(0.001;0.17)	0.08	(-0.01;0.16)	***
Waist Circumference (cm)	1.27	(0.37;2.17)	1.7	(0.87;2.53)	1.33	(0.43;2.23)	***
Fasting Glucose (mmol)	0.06	(-0.03;0.16)	0.01	(-0.08;0.09)	-0.04	(-0.14;0.05)	
HDL-cholesterol (mmol)	-0.09	(-0.14;-0.04)	-0.02	(-0.07;0.02)	-0.03	(-0.08;0.02)	**
Triglycerides (mmol)	-0.02	(-0.13;0.10)	0.05	(-0.06;0.16)	-0.03	(-0.14;0.09)	
Systolic Blood Pressure (mmHg)	8.38	(5.80;10.97)	8.19	(5.80;10.59)	9.84	(7.24;12.44)	**
Diastolic Blood Pressure (mmHg)	7.32	(5.58;9.07)	7.02	(5.41;8.64)	7.20	(5.45;8.95)	*

Note. CMRS = Clustered Cardio-Metabolic Risk Score

[a] Significantly different from lowest tertile

*p<0.05; **p<0.01;***p<0.001

Cross-sectionally, Figure 1 shows that regardless of MF, each higher CRF level was gradually correlated with significantly lower clustered cardio-metabolic risk. Although a similar, but smaller, trend is apparent for each higher MF level regardless of CRF, this was not significant. Similarly, Figure 2 illustrates the change in clustered cardio-metabolic risk for tertiles of change in CRF and MF. Again, the trend over tertiles of change in CRF was significant, but this was not the case for MF.

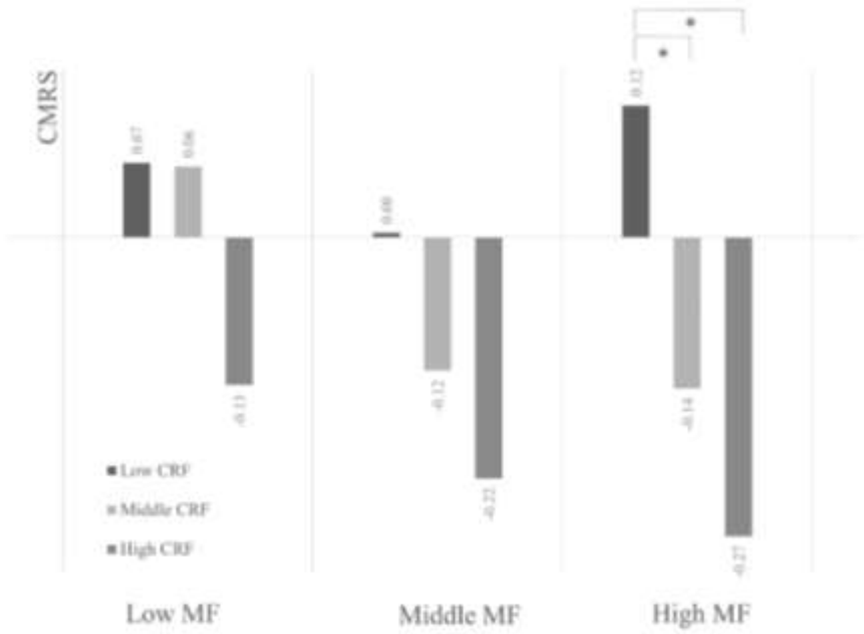

Figure 1. Least squares means of clustered cardiometabolic health by groups of combined tertiles of MF and CRF, '*' indicating significant difference between groups.

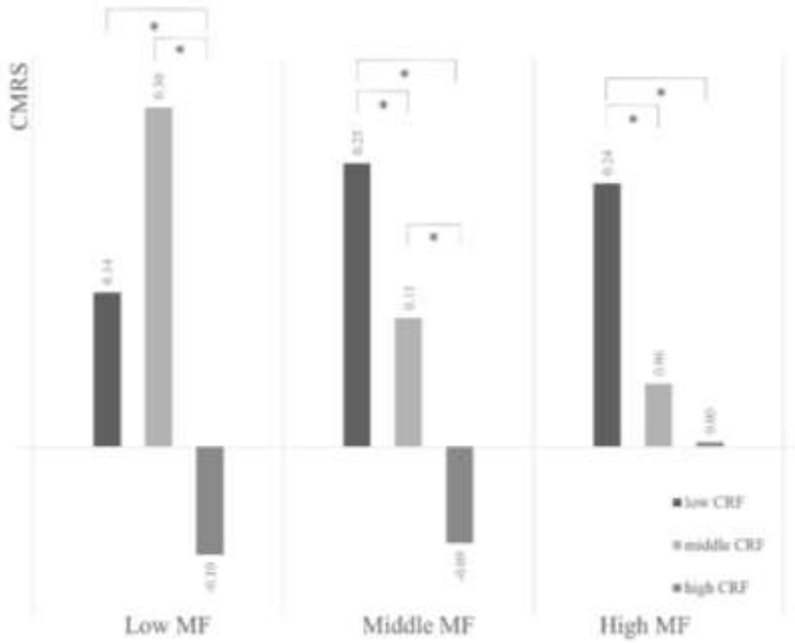

Figure 2. Least squares means of change in clustered cardiometabolic health by groups of combined tertiles of change in MF and change in CRF, '*' indicating significant difference between groups.

DISCUSSION

In the present study, we were able to examine the relative importance of objectively measured CRF and MF towards cardio-metabolic health, using cross-sectional and 10-year change data. Results indicate that CRF is more strongly associated with cardio-metabolic health than MF, both in the cross-sectional and longitudinal analyses. Furthermore, in cross-sectional analysis the association between MF and clustered cardio-metabolic risk, independent of CRF, was not significant. When observing changes over a 10-year time period changes in CRF were significantly associated with changes in clustered cardio-metabolic health and its markers. Changes in MF were not associated with changes in clustered cardio-metabolic health and its markers. Therefore, we conclude that both CRF and MF can contribute to cardio-metabolic health. However, over a longer period of time, high CRF is much more important than MF for a favorable cardio-metabolic health profile.

The importance of CRF has been demonstrated by multiple cross-sectional, longitudinal and interventional studies [4, 11, 22, 34-39]. Furthermore, CRF has often been proposed as a stronger predictor for an increased risk of death than clinical variables or established risk factors such as hypertension, smoking or ECG abnormalities [11, 40]. Furthermore, poor CRF is a modifiable risk factor and both CRF at baseline and increasing fitness over time have been demonstrated to improve prognosis [1, 40]. Biological plausible reasons for improved health with improved fitness are hypothesized to act through beneficial fibrinolytic, hemodynamic, inflammatory markers, blood pressure, and the cardiovascular system [41, 42].

Results for associations between MF and cardio-metabolic health have been equivocal. Cross-sectional analyses only including MF often find protective associations between MF and clustered cardio-metabolic health [14, 19, 43]. This is similar to results of the present study, where after correction for confounders, but not CRF, MF was negatively associated with a CMRS. However, when examining the associations between MF and clustered cardio-metabolic risk independent from CRF, association were much lower and often lost significance [18-20]. Earnest et al. observed no improvements in clustered cardio-metabolic health after nine months of resistance training in type 2 diabetes patients [44]. In contrast, nine months of aerobic training was associated with improved clustered cardio-metabolic health. However, a combination of aerobic and resistance training achieved a slightly better cardio-metabolic profile than aerobic training alone [44]. Nonetheless, a nine month training program by Bateman et al. in healthy adults did not observe any benefits for a combined training program for improving clustered cardio-metabolic health. A meta-analysis observed that resistance training can reduce blood pressure, body fat and plasma triglycerides, but not other blood lipids or fasting plasma [45]. The diversity in the response of separate cardio-metabolic markers may influence the associations with clustered cardio-metabolic health.

Longitudinal studies examining associations between MF and CRF and clustered cardio-metabolic risk are scarce and mostly include men [18, 23, 24]. It is possible that a publication bias arose and non-significant results never made it to publication, because contrary to our results, all these studies found significant longitudinal associations between MF and clustered cardio-metabolic risk. A study by Jurca et al. found a strong inverse gradient of metabolic syndrome incidence rates across quartiles of MF. However, after adjustment for CRF this association attenuated to being marginally not significant [18]. Another longitudinal study examined fitness at 18 years and the relation to risk for type 2 diabetes in adulthood [24]. Results indicate that both low CRF and low MF at age 18 were associated with long-term risk for type 2 diabetes; a combination of both was associated with the highest risk. Furthermore, this study observed an interaction between low CRF and low MF, unlike results from the present paper [24]. A similar study confirmed that childhood MF predicts adult clustered cardio-metabolic health, independent of CRF [23]. Nonetheless, none of these studies examined the associations between change over time and only included baseline measurement of MF.

Strengths of this study include the combined cross-sectional and longitudinal analyses over a ten-year follow-up with measurement of CRF and MF at both time points. Participants were healthy adults, and unlike most other studies examining MF, also included women. Furthermore, to reduce measurement error, both CRF and MF were objectively measured with a maximal exercise test. CRF was measured with a maximal cycle ergometer test and objectively measured oxygen consumption, which is the gold standard for CRF-testing [26]. MF was measured with maximal repetitions on a calibrated isokinetic dynamometer [28]. Finally, because all longitudinal analyses investigated changes within-participants, interference of genetic influences is less likely.

The following limitations should, however, be considered. First, muscular fitness only comprised of one component, namely muscular strength of the quadriceps. However, quadriceps muscle strength has previously been related to overall limb strength [46] and several health outcomes [47, 48]. Second, no causal inference can be made from change-by-change analyses. Third, only 42% of participants returned for follow-up measurements. A drop-out analyses showed that for men there were hardly any differences between participants who dropped out or returned for follow-up measurements. For women, there were more differences and in general a healthier subgroup returned for follow-up measurements. Fourth, people at risk for heart failure, abnormalities in an electrocardiogram, lower back pain or arterial hypertension did not participate in the maximal exercise test and therefore CRF data was only available for a healthier subgroup, limiting external validity of these findings to healthy Caucasian populations. However, non-inclusion of these participants may have reduced the risk for reverse causality for the associations found. Finally, analyses could not be controlled for medication use. Although this was a healthy adult population, some participants would have taken medication against hypertension, dyslipidemia or dysglycemia. Though, exclusion for arterial hypertension in the subsample analyses will have minimized the impact of this for antihypertensive medication. Other possible confounders that were not included are occupation, and family history of cardio-metabolic diseases, which might have caused residual confounding.

In conclusion, both CRF and MF were cross-sectionally associated with clustered cardio-metabolic health. However, when examining the independent association of the other fitness component, only CRF was associated with clustered cardio-metabolic health independent from MF. When examining associations with change over time in CRF or MF, only change in CRF was associated with change in clustered cardio-metabolic risk, independent from change in MF. Therefore, although individuals with high muscular fitness might have lower mortality [12, 48], higher functionality at older age [50] and lower incidence of type 2 diabetes [24], this study observed that for healthy adults additional benefits of higher objectively measured muscular fitness towards better cardio-metabolic health may be limited especially in the long-term. Further longitudinal studies with larger sample sizes and intervention studies should further investigate independent associations between MF, CRF and clustered cardio-metabolic health in a variety of populations, preferably with highly accurate measurements such as objective measurement with a maximal exercise test.

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SUMMARY AND GENERAL DISCUSSION

Summary and general discussion

Even in 400 BC Hippocrates understood the importance of physical activity and the deleterious effects of inactivity when he said that "All parts of the body that have a function, if used in moderation and exercised in labours in which each is accustomed, become thereby healthy, well-developed and age more slowly, but if unused and left idle they become liable to disease, defective in growth, and age quickly". If he knew what we know now, he would probably have added that sitting too much does not produce health either. In addition, he might also have wanted to add that physical activity and lack of sedentary behaviour will probably lead to improved cardiorespiratory fitness (CRF), which is even more strongly associated with cardiometabolic health. Similarly, Edward Stanley raised a valuable point in 1873 when stating that "Those who think they have not time for bodily exercise will sooner or later have to find time for illness". Consequently, we can conclude without doubt that "Exercise is Medicine" and physicians should prescribe an active lifestyle for health [1].

1 Summary of main results and conclusions

This chapter will briefly discuss the main results and conclusions of the five included papers. In the next chapter, practical implications for theory and practice, an overarching discussion including the results of the five papers and the present literature will be made.

1.1 Paper 1: Associations between physical activity and health-related fitness – volume versus pattern

Key findings of Paper 1

- In a healthy adult population, physical activity is associated with health-related fitness, regardless of the variability of the physical activity pattern.
- Participants in the lowest quartile of physical activity level (PAL) have a lower health-related fitness than participants in the highest quartile, when corrected for the variability of the physical activity pattern.
- Participants in the lowest quartile of variability in physical activity did not have lower health-related fitness than participants in the highest quartile, when corrected for PAL.

The first paper addressed the question if stability in a physical activity pattern was associated with health-related fitness. The Gini index, a coefficient which measures statistical dispersion, was applied to assess the variability in the physical activity pattern. We hypothesized that being continuously lightly active, or very sedentary with short vigorous intervals, has the same potential for improving health-related fitness, taking into account the positive outcomes of MVPA and negative outcomes of sedentary behaviour.

Results were based on measurements of 296 healthy adults (63% men). PAL was calculated from the minute-by-minute SenseWear data as the average MET value during all measured days. The Gini-index, representing the variability of the activity pattern, was calculated using the accumulation of physical (in-)activity and sleep. Health-related fitness consisted of four objectively measured components (body composition, cardiorespiratory fitness (CRF), muscular fitness and flexibility). Health-related fitness was associated with PAL, independent of the Gini index. Health-related fitness was however not associated with the Gini index, independent of PAL. Furthermore, there was a significant trend of improved health-related fitness over quartiles of PAL. This trend was not apparent over quartiles of the Gini-index.

The three main findings were in line with the previously stipulated hypothesis. First, physical activity is associated with health-related fitness, regardless of the variability of the physical activity pattern in a healthy adult population. Second, participants in the lowest quartile of PAL have a lower health-related fitness than participants in the highest quartile, when corrected for the variability of the physical activity pattern. Third, there was no pronounced trend in health-related fitness over quartiles of the Gini index, representing variability of the physical activity pattern. These results imply that as long as the volume of physical activity is high, health-related fitness will be high as well, independent of the physical activity pattern. In other words, people's health-related fitness does not differ if they have the same volume of physical activity but a different way of reaching it.

Results of the first paper support previous research stating that sedentary behaviour and physical activity, even in low intensities, are associated with health-related fitness [2, 3]. Furthermore, results highlighted the importance of physical activity volume, measured as PAL. Similarly, it has previously been suggested that not the walking pace, but the time spent walking (volume) is an important determinant for health-related fitness and health [4]. These results might implicate that a long duration of light physical activity (LPA), can also have a positive impact on health-related fitness, similar to a shorter duration of vigorous physical activity.

These observations may contribute to health policy, because current guidelines mostly focus on minutes of MVPA and exercise and not on the total volume of physical activity.

1.2 Paper 2: Substituting sedentary time for physical activity is related to cardiometabolic risk

Key findings of Paper 2

- Substituting sedentary time with MVPA is associated with a lower clustered cardiometabolic risk, waist circumference and triglycerides and higher HDL-cholesterol.
- Substituting sedentary time with LPA is associated with an increase in HDL-cholesterol and lower waist circumference and blood pressure.
- Substituting sedentary time with sleep is associated with a decrease in waist circumference.
- Substituting LPA with MVPA is associated with a decrease in clustered cardiometabolic risk, a decrease in HDL-cholesterol and an increase in diastolic blood pressure.

The purpose of the second paper was to apply a more novel approach, namely isotemporal substitution, to systematically examine the cardiometabolic health benefits of substitution sedentary time with either sleep, LPA or MVPA. Furthermore, the extra benefit of increasing physical activity intensity from LPA to MVPA, was explored. Additionally, an evaluation in characteristics between individuals with 0-1, 2, and 3 or more cardiometabolic risk factors was made and means of these three groups of individuals were compared.

Results show that substituting sedentary time with MVPA is associated with a significantly lower clustered cardiometabolic risk, waist circumference and triglycerides and higher HDL-cholesterol. Substituting sedentary time with sleep or LPA was not associated with lower clustered cardiometabolic risk. However, substituting sedentary time with LPA was associated with an increase in HDL-cholesterol and lower waist circumference and blood pressure. Substituting sedentary time with sleep was also associated with a significant decrease in waist circumference. Similarly, substituting LPA with sedentary time was associated with an increase in waist circumference and diastolic blood pressure and a decrease in HDL-cholesterol. Furthermore, substituting LPA with MVPA was associated with a decrease in clustered cardiometabolic risk, a decrease in HDL-cholesterol and an increase in diastolic blood pressure.

Finally, substituting LPA with sleep was not associated with changes in clustered cardiometabolic risk and its factors.

When evaluating the characteristics of groups with different numbers of cardiometabolic risk factors, 41% of participants had 2 or more risk factors. These participants were older, had higher weight, and lower cardiorespiratory fitness, MVPA and education level than participants with 0-1 risk factors. Participants with 3 or more risk factors were more sedentary than participants with 0-1 risk factors.

All previous studies including isotemporal substitution observed beneficial effects of substituting sedentary time with MVPA for clustered cardiometabolic risk and several of its parameters [5-7]. However, only some studies also observed clustered cardiometabolic risk improvements for substituting sedentary time with LPA [5, 6], whereas our study did not observe a significant association. However, our results suggest that for some cardiometabolic health parameters, substituting sedentary time with LPA can lead to positive effects. Substituting sedentary time with sleep was associated with a decrease in waist circumference which is in line with the few previous studies including substitution with sleep [5, 8]. In conclusion, results suggest that not only the type of activity that displaces sedentary time is important, but also the health parameter in question, where substitution with LPA might be valuable for waist circumference, HDL-cholesterol and blood pressure.

1.3 Paper 3: Independent associations between sedentary time, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic health: a cross-sectional study

Key findings of Paper 3

- Sedentary time had a positive association with waist circumference and a negative association with HDL-cholesterol, both independent from MVPA.
- MVPA had a negative association with clustered cardiometabolic risk, triglycerides and systolic blood pressure, all independent from sedentary time.
- CRF was positively associated with clustered cardiometabolic health, triglycerides and diastolic blood pressure and negatively with HDL-cholesterol, independent of sedentary time and MVPA.
- A mediation analysis showed that CMRS explained 73% of the association between MVPA and CRF, independent from sedentary time.

The aim of the third paper was to examine the independent associations of objectively measured sedentary time, moderate-to-vigorous physical activity (MVPA) and CRF with clustered cardiometabolic risk and individual cardiometabolic risk factors. Furthermore, the mediating effect of CRF on the relation between MVPA or sedentary time and clustered cardiometabolic risk was analysed.

Included in analyses were 341 healthy Flemish adults (61% men) who participated in a maximal cycle ergometer exercise test and wore the SenseWear Pro 3 Armband for at least 5 consecutive days, including both weekend days. Results indicate that MVPA, but not sedentary time, was independently of the other behaviour associated with clustered cardiometabolic risk. CRF however, was even more strongly associated with clustered cardiometabolic health, independent of sedentary time and MVPA. Sedentary time was independently of MVPA associated with waist circumference and HDL-cholesterol and MVPA was independently of sedentary time associated with triglycerides and systolic blood pressure. Furthermore, CRF was, independently from sedentary time and MVPA, associated with HDL-cholesterol, triglycerides and diastolic blood pressure. Results from the mediation analysis showed that CRF explained 73% of the association between MVPA and CRF, independent from sedentary time.

Similar previous studies including all three exposures underline the importance of high CRF and support the conclusion of an independent association between CRF and clustered cardiometabolic risk [9-12]. However, contrary to the present study, all studies found a significant inverse association between sedentary behaviour and cardiometabolic risk, though in only two studies this association remained significant after adjusting for physical activity and CRF [10, 12]. Moreover, in the present study, the lack of an association between MVPA and clustered cardiometabolic risk independent of sedentary time and CRF appears to be in contradiction with the results of van der Velde et al. [9]. However, this supports the hypothesis that CRF mediates the association between MVPA and clustered cardiometabolic risk [13].

1.4 Paper 4: Ten-year change in sedentary behaviour, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic risk: independent associations and mediation analysis

Key findings of Paper 4

- The greater increase in screen time and passive transportation and decrease in MVPA was associated with greater increase in clustered cardiometabolic risk, independently from change in MVPA and screen time and passive transportation, respectively.
- The greater decrease in CRF was associated with greater increase in clustered cardiometabolic risk.
- The associations of change in screen time and passive transportation and MVPA with change in clustered cardiometabolic risk were mediated through change in CRF.
- The association between change in CRF and change in clustered cardiometabolic risk was mediated through change in waist circumference.

The objective of the fourth paper was to (1) examine the independent associations between change in sedentary behaviour (only including screen time and passive transportation), MVPA and objectively measured CRF with concurrent change in cardiometabolic risk over a long period of follow-up, and (2) to examine whether any such independent associations were mediated by change in CRF (for sedentary behaviour and MVPA), change in waist circumference (for sedentary behaviour, MVPA and CRF) or nutritional intake (for sedentary behaviour).

Full data were available at baseline and follow-up for 524 participants (65% men), with the exception of CRF data for which a subsample of 399 participants (64% men) provided all data.

The average follow-up time was 9.62 (±0.52) years. Over this time cardiometabolic risk deteriorated for almost all markers, and so did CRF. Sedentary behaviour (screen time and passive transportation) increased by, on average, 2 hours a week, whereas total MVPA stagnated. Results suggest that favourable changes in self-reported sedentary behaviour, MVPA and objectively measured CRF are associated with favourable changes in clustered cardiometabolic risk. A decrease in screen time and passive transportation was associated, independently from change in MVPA, with a decrease in waist circumference, triglycerides, diastolic blood pressure and an increase in HDL cholesterol; and an increase in MVPA, independently from change in sedentary behaviour, with a decrease in waist circumference, fasting glucose and an increase in HDL cholesterol. Change in CRF mediated the associations between change in sedentary behaviour and change in CMRS and waist circumference, and the associations between change in MVPA and change in CMRS, waist circumference and HDL cholesterol. Change in waist circumference did not mediate these associations for sedentary behaviour or MVPA, and change in nutritional intake did not mediate the associations found for change in sedentary behaviour. Furthermore, increases in CRF were associated with favourable changes in all individual cardiometabolic risk markers. However, change in waist circumference did mediate the association between CRF and $CMRS_{no-adip}$, fasting glucose, HDL cholesterol and triglycerides. On the basis of these results, a combination of decreasing sedentary behaviour and increasing MVPA, most likely resulting in a positive change in CRF, is most beneficial towards cardiometabolic health.

Our findings extend previous cross-sectional and longitudinal findings of associations between sedentary behaviour and clustered cardiometabolic risk, waist circumference, HDL cholesterol and triglycerides, independent of MVPA [10, 14, 15]. However, other cross-sectional [9, 16] and longitudinal [11, 17] studies could not confirm an association between sedentary behaviour and clustered cardiometabolic risk, when adjusting for MVPA. It is possible that our results found this significant association because only screen time and passive transportation were included. Likewise, results for the associations of MVPA independent of sedentary behaviour was of similar strength compared to a cross-sectional study, the association between MVPA and HDL cholesterol and fasting glucose, independent of sedentary behaviour [9]. However, for the independent association with waist circumference, this study observed a considerably smaller association [9]. Inconsistent findings might be due to a number of reasons including, but not limited to, type of included MVPA and sedentary behaviour with self-report, diverse study populations with various outcome profiles, statistical analysis or medication use in patient populations.

The results that changes in CRF were associated with changes in clustered cardiometabolic risk and all individual markers and that these associations were almost always more than twice as strong as those with changes in sedentary behaviour or MVPA is also in line with previous research. Therefore, CRF is often recognised as one of the most important determinants of longevity and health [10, 13, 18]. CRF is, however, a physiological measure reflecting a combination of genetic potential, behavioural and functional health of various organ systems [19]. As a result, CRF improves mostly when MVPA increases and when sedentary behaviour decreases [2], although all are also determined by multiple factors such as heredity, the environment and personal attributes [20].

1.5 Paper 5: Longitudinal and cross-sectional associations between cardiorespiratory fitness, muscular fitness and cardiometabolic risk

Key findings of Paper 5

- The greater increase in screen time and passive transportation and decrease in MVPA was associated with greater increase in clustered cardiometabolic risk, independently from change in MVPA and screen time and passive transportation, respectively.
- The greater decrease in CRF was associated with greater increase in clustered cardiometabolic risk.
- The associations of change in screen time and passive transportation and MVPA with change in clustered cardiometabolic risk were mediated through change in CRF.
- The association between change in CRF and change in clustered cardiometabolic risk was mediated through change in waist circumference.

The aim of the final paper was to investigate the possible additive contribution of change over a period of 10 years in objectively measured muscular fitness towards cardiometabolic health, independent of the favourable effects of change in CRF. Furthermore, cross-sectional and longitudinal associations with cardiometabolic health were also examined independent from relevant covariates.

In total, 357 participants (65% men) provided data for fitness, cardiometabolic risk factors and covariates, such as nutrition, smoking and education. Results showed that CRF was negatively associated with clustered cardiometabolic risk and triglycerides. Moreover, muscular fitness was negatively associated with clustered cardiometabolic risk and HDL-cholesterol. In a longitudinal analysis, change in CRF was associated with change in all cardiometabolic markers. Conversely, change in muscular fitness was not associated with change in any of the cardiometabolic markers. Additionally, a trend in means at follow-up and means of change over time of clustered cardiometabolic risk and cardiometabolic risk markers by tertiles of CRF and muscular fitness was assessed. At follow-up, there was a significant trend for clustered cardiometabolic risk and all cardiometabolic markers with the most favourable mean in the tertile with the highest CRF. For muscular fitness this trend is less apparent and only significant for clustered cardiometabolic risk, waist circumference and HDL-cholesterol. In the longitudinal analyses, the trend over tertiles of change in CRF for change in means over a 10-year time period is significant for clustered cardiometabolic risk, waist circumference, fasting glucose, HDL-cholesterol and diastolic blood pressure, where participants in the highest tertile had the largest improvement in cardiometabolic markers over time. For change in muscular fitness, there was only a significant trend for fasting glucose.

Out of these results we concluded that CRF is more strongly associated with cardiometabolic health than muscular fitness, both in cross-sectional and longitudinal analyses. The beneficial contribution towards clustered cardiometabolic health of muscular fitness, independent of CRF, was not significant. In conclusion, both CRF and muscular fitness can contribute to cardiometabolic health, however, associations with muscular fitness are not independent of CRF. Furthermore, over a longer period of time, high CRF is much more important than muscular fitness for a favourable cardiometabolic health profile.

Results in the literature are comparable to the results of this study. Cross-sectional analyses only including muscular fitness often find protective associations for clustered cardiometabolic health [21-23]. After correction for confounders, but not CRF, muscular fitness was negatively associated with a CMRS. However, when examining the associations between muscular fitness and clustered cardiometabolic risk independent from CRF, associations are much lower and often loose significance [23-25] , similarly to the results of this paper. Longitudinal studies examining associations between muscular fitness and CRF and clustered cardiometabolic risk are scarce, and mostly report significant associations between muscular fitness and cardiometabolic risk, unlike results from this paper [16, 21, 22]. Nonetheless, none of these studies examined the associations between change over time and only included baseline measurement of muscular fitness.

2 General discussion

2.1 Practical implications for theory and practice

Physical activity, sedentary behaviour and cardiometabolic health

The three papers that discussed physical activity and clustered cardiometabolic health, all had similar conclusions and confirmed existing literature. Physical activity, starting from low volumes and intensities, has major effects on non-communicable diseases and mortality, and decreasing the prevalence of physical inactivity could improve health substantially [26, 27]. Conversely, the results regarding sedentary behaviour were somewhat more equivocal. Cross-sectionally, sedentary behaviour was associated with some cardiometabolic risk factors (waist circumference, HDL-cholesterol and blood pressure) but not clustered cardiometabolic risk. However, replacing sedentary behaviour with physical active behaviour, mostly MVPA, did have an association with clustered cardiometabolic health and multiple risk factors [28]. Comparing these results with larger studies, we can presume that sedentary behaviour is positively associated with cardiometabolic disease, its risk factors and mortality [15, 29]. There are many plausible pathways through which physical activity results in better cardiometabolic health. First of all there are many cardiovascular adjustments, the liver increases the release of glucose, the adipocyte increases the hydrolysis of its triglyceride stores, and on a whole-body level the endothelial function improves [30].

The discrepancy in results for sedentary behaviour between the cross-sectional and longitudinal analyses is in line with a study by Stamatakis et al. who found that associations between sedentary behaviour and cardiometabolic risk factors were more consistent when sedentary behaviour is measured by self-report that includes TV viewing (as in Paper 4) in comparison to objective measurement including all sedentary behaviours (Paper 3) [31]. And indeed, a sub analysis in the data of Paper 4 showed that screen time was much stronger associated with cardiometabolic health in comparison to passive transportation (results not shown). Furthermore, literature shows that the length of the bouts of sedentary behaviour, and not only total sedentary behaviour, appears to be important when evaluating cardiometabolic health [16, 32, 33], which was unfortunately not included in the analyses in this thesis.

Results of this thesis confirm current knowledge that state that the associations between MVPA and cardiometabolic health, and between sedentary behaviour and cardiometabolic health are statistically independent of one another [34-36]. It is possible to have high MVPA and high sedentary behaviour or low MVPA and low sedentary behaviour or any other possible combination (high MVPA and low sedentary behaviour; low MVPA and high sedentary behaviour). Still, the question arises if one behaviour can make up for the other. Research shows that performing MVPA is associated with better health, even in those with concomitant high sedentary behaviour [37]. Moreover, high levels of MVPA seem to eliminate increased mortality risk associated with high sedentary behaviour, although not the increased risk associated with TV-viewing time [38]. In conclusion, both MVPA and low sedentary behaviour are important for favourable cardiometabolic health independent of each other [35, 36], nevertheless, high MVPA can, to some extent, compensate for the detrimental effects of sedentary behaviour.

Physical fitness and cardiometabolic health

From the results of the first paper (Paper 1), it can be concluded that the total volume of physical activity is more strongly associated with health-related fitness than the variability in the pattern [39]. Total volume is defined as: the time spent in an activity   the intensity of this activity. Thus, to have a high volume either time, intensity or both need to be high. This is in line with studies showing health benefits of long walks [40-42] or high-intensity interval training [43]. The final three papers in this thesis (Paper 3, 4 & 5) focused on the association of two components of health-related fitness (CRF and muscular fitness) and cardiometabolic health. The motivation for dropping both other components was that (1) body composition can be seen as part of cardiometabolic health and was therefore an outcome instead of an exposure and (2) flexibility has a less plausible underlying biological pathway with cardiometabolic health.

The last three papers (Paper 3, 4 & 5) have consistent findings regarding the association of CFR with cardiometabolic risk and cardiometabolic risk factors. Associations between CRF and cardiometabolic risk were always stronger than those with sedentary behaviour, MVPA or muscular fitness [36]. This is in line with previous research where CRF is often recognised as one of the most important contributors of health and longevity [10, 13, 18]. In the cross-sectional regression model, higher CRF was significantly associated with lower clustered cardiometabolic risk, lower HDL cholesterol and higher triglycerides and diastolic blood pressure [36]. Over a period of 10 years, change in CRF was significantly associated with change in clustered cardiometabolic health and change in all cardiometabolic risk markers [35]. Although both analyses do not provide prove of causality, we can assume that a higher CRF will lead to a better cardiometabolic health profile. Nevertheless, reverse or reciprocal causality cannot be excluded. Furthermore, CRF seems to be an important mediator in the associations between physical activity of sedentary behaviour and cardiometabolic health [35, 36], although adjustment for CRF did not significantly change the results of the associations of substitution of sedentary time by physical active behaviour [28].

Results regarding muscular fitness were less consistent [44]. Cross-sectional analyses showed small but significant associations with clustered cardiometabolic risk. These associations were however not apparent when assessing the association between change in muscular fitness and change in clustered cardiometabolic risk [44]. Moreover, associations between CRF and clustered cardiometabolic risk where independent from muscular fitness, both cross-sectionally and longitudinally. Contrary, the significant association between muscular fitness and clustered cardiometabolic health was not independent of CRF. Therefore, although individuals with high muscular fitness might have lower mortality [45, 46], higher functionality at older age [47] and lower incidence of type 2 diabetes [48], results of our research indicate that for healthy adults benefits of higher objectively measured muscular fitness towards better cardiometabolic health are small, definitely looking at changes over a long period of time [44]. Moreover, high CRF is a much more important exposure for clustered cardiometabolic health [44]. However, CRF can be considered as the combined capacities of the central nervous system to recruit motor units, the pulmonary and cardiovascular systems to deliver O2 to contracting skeletal muscles, and the ability of those muscles to consume O2 in the oxidative, metabolic pathways [30]. Consequently, muscular fitness can be regarded as an important component of CRF, and there its importance should not be underestimated.

2.2 Practical implications

Use of physical activity, sedentary behaviour and CRF

Out of the results included in the present thesis and the literature we can conclude that physical fitness, high physical activity as well as low sedentary behaviour are important predictors for cardiometabolic health and mortality [28, 35, 36, 39, 44]. Regression coefficients from analyses using unstandardized exposure and outcome variables illustrate the clinical significance of the associations. Even in this healthy population, where most participants had ≤ 1 at risk measurements for cardio-metabolic risk factors (according to the guidelines of the International Diabetes Foundation (IDF) [49]) and only a small percentage was diagnosed with the Metabolic Syndrome, significant health improvements could be achieved. For example, substituting 30 minutes of sedentary behaviour with physical activity was associated with a lowered waist circumference of one cm or a lowered diastolic blood pressure of one mmHg [28]. Although on an individual level this appears to be a rather small change, on a population level this can have significant consequences. Furthermore, this was a fairly healthy population, and as stated before, people in the lowest levels of fitness and physical activity and highest level of sedentary behaviour can make the most progress. Therefore, in the actual Flemish population a substitution of 30 minutes of sedentary behaviour by a more active behaviour will probably be accompanied with a more substantial health improvement.

A main difference, however, between an active lifestyle and physical fitness, is that both sedentary behaviour and MVPA are behaviours, whereas CRF is mostly a physiological measure reflecting a combination of genetic potential, behavioural and functional health of various organ systems [19]. Figure 1 show the complex relation among physical activity, physical fitness and health, where other factors, each to a different extend, influence these relationships [20]. To improve CRF the most important behavioural changes that need to be made are reducing sedentary behaviour and increasing LPA and MVPA [2]. However, because CRF and an active lifestyle are substantially different in nature, they both have various benefits for different settings and outcomes.

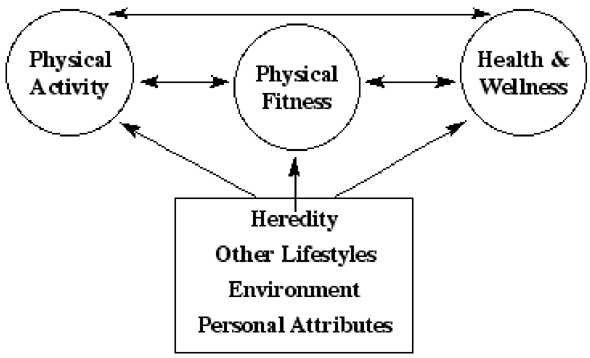

Figure 1. Complex relationships among physical activity, physical fitness, health, wellness and other factors (Adapted from Bouchard, et al. 2012)

CRF is probably most valuable in a clinical setting, because it is a physiological measure. It is a valuable clinical tool for assessing and predicting someone's cardiometabolic health profile and mortality risk and is a reliable metric to assess the ability of the cardiovascular system to sustain prolonged physical work [50, 51]. Furthermore, CRF has been shown to be the most powerful predictor of morbidity and mortality, way beyond classical cardiovascular disease risk factors such as smoking, cholesterol, hypertension and diabetes [50, 52-54]. And even though physical activity is the most important behaviour preceding changes in CRF, the literature and results of this thesis show that CRF is a stronger predictor of cardiometabolic health and mortality [35, 36, 55]. Another benefit of CRF as a clinical tool is that movement scientists or physicians can either estimate CRF with a few simple field tests or accurately measure it with a maximal exercise test [56]. This results in the advantage that clinicians can quickly measure a reliable outcome rather than depend on a reported behaviour [50]. Therefore, for clinical practice, CRF can be regarded as a surrogate measurement for measuring physical (in-) activity and sedentary behaviour and help predicting cardiometabolic disease risk and mortality [55].

On the other hand however, CRF is less useful for public health promotion, as it is the behaviour proceeding changes in CRF that needs to be targeted to improve CRF [2, 57]. Therefore, physical activity and sedentary behaviour are most likely to find their merit in public health promotion, because they represent changeable behaviours. Consequently, public health campaigns should focus on promoting an active lifestyle with more LPA, MVPA and a reduced sedentary behaviour. Furthermore, literature shows that not only sedentary behaviour should be reduced, but that continuous sedentary behaviour should also be broken down into short bouts [16, 32]. Important to note is that sedentary behaviour, LPA and MVPA are behaviours that are intertwined, because it is impossible to change one behaviour without changing to another behaviour (presuming sleep as a constant) [6]. Results of this thesis show that substituting sedentary behaviour with MVPA will probably lead to the largest health improvements, even though, substituting sedentary behaviour with LPA will also attain health benefits and might be more feasible and easy to integrate in someone's lifestyle [28]. Consequently, it is most beneficial to substitute sedentary behaviour by LPA or MVPA and not an active behaviour by LPA or MPVA (eg. substituting LPA with MVPA [6], although substituting LPA with MVPA will lead to small extra health benefits [35]). However, the ultimate goal of the overall physical activity level (reducing sedentary behaviour and performing regular physical activity) needs to be improvements in CRF as this is more strongly associated with clustered cardiometabolic risk and its risk factors [35, 36, 55].

"Moving a little"

It is understandable that increasing CRF needs to be one of the main goals of increasing physical activity and reducing sedentary behaviour. The question arises what the optimal dose, intensity and volume are for health-enhancing physical active behaviour. The first physical activity guidelines were rooted in an 'athletic' paradigm and stressed vigorous exercise. In the 1990s this approach was challenged by a more inclusive population-based attitude stating that "every adult should accumulate at least 30 minutes of MVPA, and most preferably, all days of the week" [58]. The current European physical activity guidelines are still in line with those of the 90s (30 minutes of moderate physical activity on at least five days a week, 20 minutes of vigorous physical activity on three days a week or an equivalent combination of MVPA) [59]. Nevertheless, experts continue to debate about the optimal (both minimal and maximal) physical activity level and upper threshold for sedentary behaviour, and they are quick to recognize that “moving a little is better than doing nothing at all” [50].

For motivational purposes it might be very important to include in the guidelines the idea of “moving a little is better than doing nothing at all” and that LPA will also lead to substantial health benefits [4, 28, 40]. Some countries are starting to add these directives to their national guidelines. Australian guidelines literally state: “Doing any physical activity is better than doing none. If you currently do no physical activity, start by doing some, and gradually build up to the recommended amount”. Moreover, the Flemish guidelines already include a low-threshold guideline with shorter durations and lower-intensity activities such as walking slowly. They could however be improved by adding the statement that some activity is better than none. Similarly, all health-professionals should emphasize the importance of any physical activity, even if the volume or intensity of the activity or exercise does not meet the guidelines [4, 60, 61]. Hopefully, this way, no one will be discouraged when they want to adopt a more active lifestyle, but perceive the guidelines as too demanding. In addition, when an individual has become active, introducing some periods of more vigorous physical activity or exercise is necessary, as it appears to confer additional cardiovascular protection. Notwithstanding that it remains debated whether additional cardiovascular protection can be obtained by exercise way beyond the guidelines [62, 63].

Just as with physical activity, CRF does not need to be exceptionally high to provide significant protection against cardiometabolic diseases [18, 39]. In several different generally healthy populations, a VO2peak of ≥28 ml.kg-1.min-1 is associated with substantial morbidity and mortality protection, with greater benefits associated with higher CRF [52, 54]. This fitness-health curve is not linear but curvilinear, with lower levels of fitness being associated with considerably higher levels of risk compared to higher fitness levels (Figure 2) [55, 64-66]. Furthermore, associations between cardiometabolic health and physical activity are altered by level of CRF [67, 68]. The fitter someone is, the less ‘influence’ physical activity has on cardiometabolic health and mortality [67]. Additionally, muscular fitness also moderates the association between physical activity and mortality [67]. This leads to the conclusion that the least fit sub-groups could benefit most from physical activity interventions and that public health campaigns should largely target this unfit and inactive population [65, 67].

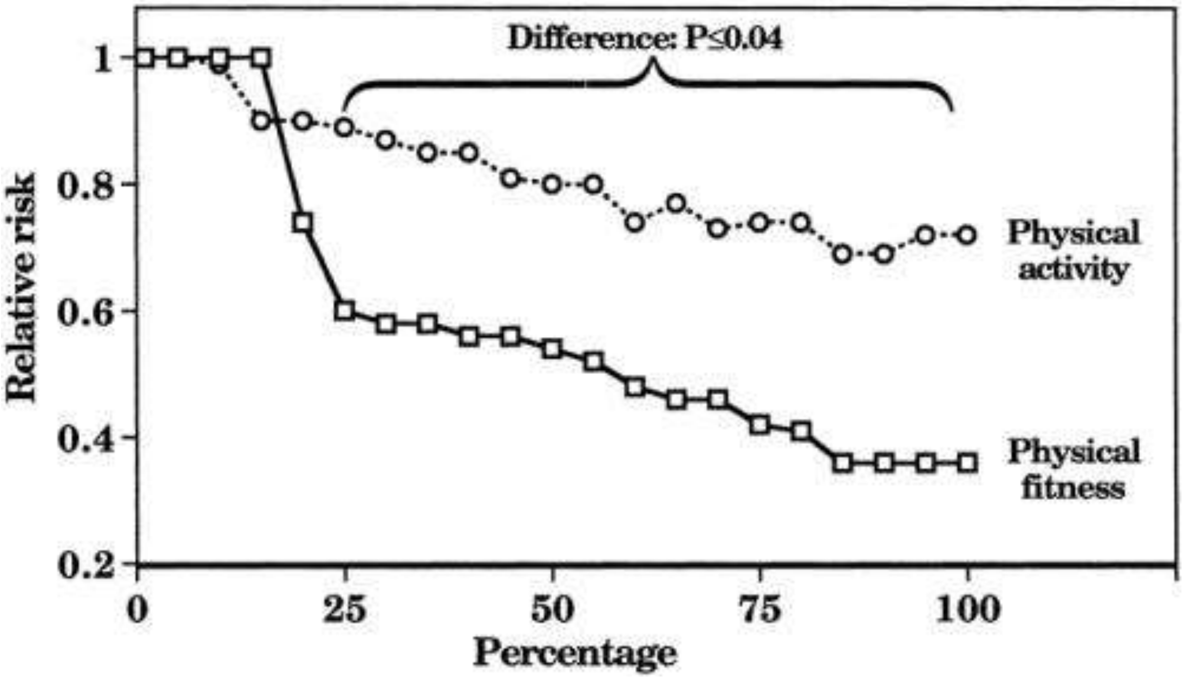

Figure 2. Estimated dose-response curve for the relative risk of both coronary heart disease and cardiovascular disease by sample percentages of fitness and physical activity (Williams, 2001).

Conclusion

To the best of our knowledge, we were the first to include baseline and follow-up measurement of physical activity, sedentary behaviour and cardiorespiratory fitness into one analysis, where fitness was measured with a maximal exercise test. We conclude that a physically active lifestyle with more MVPA and low sedentary behaviour will partially improve health-related fitness [2, 39]. Substituting sedentary time with a more active behaviour or sleep was also associated with better cardiometabolic health [28]. Rigorous and comprehensive measurement methods showed that high MVPA, low sedentary behaviour and high physical fitness are positively associated with cardiometabolic health and affect several cardiometabolic risk factors both in the short and in the long term [28, 35, 36, 44, 69]. Furthermore, results from the cross-sectional and longitudinal analyses included in this thesis indicate that these associations are mostly independent of each other and that CRF is an important mediator in the relationship between MVPA or sedentary behaviour (screen time and passive transportation) and cardiometabolic health [35, 36]. Change in nutritional intake was not a mediator in the association between change in sedentary behaviour and change in cardiometabolic health, conversely waist circumference was a mediator in the association between change in CRF and change in some cardiometabolic health factors.

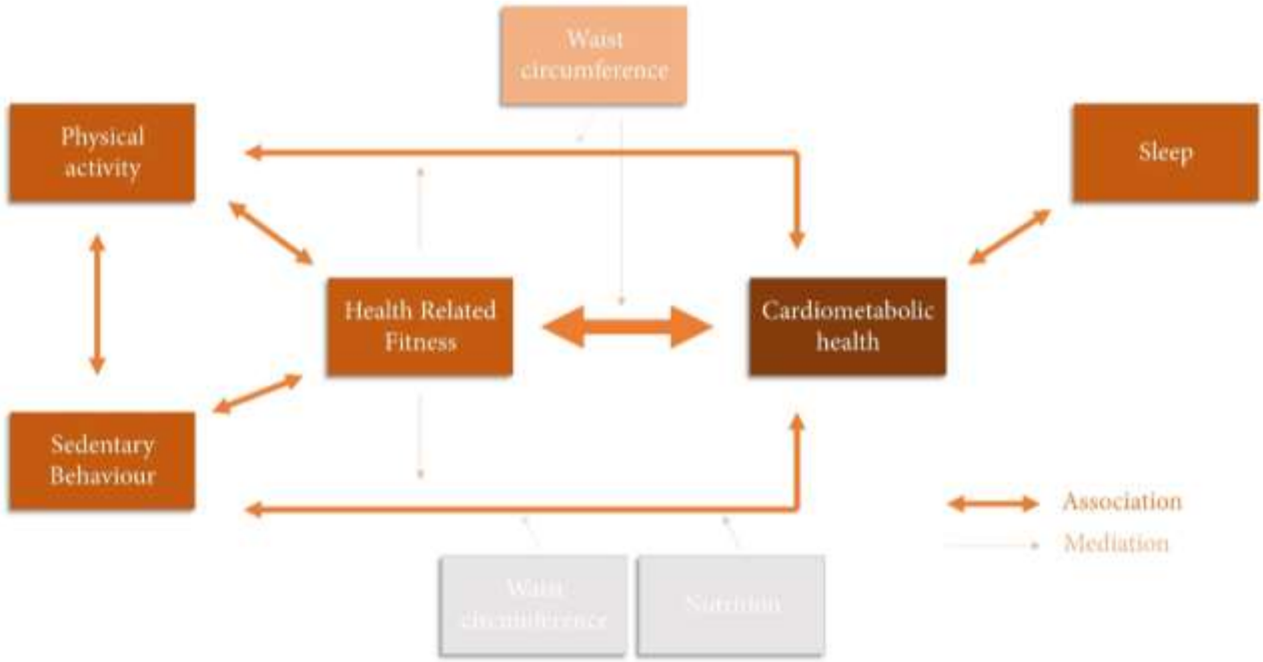

Figure 3. Visual representation of all associations confirmed in the present thesis.

Literature shows that all three exposures have a curvilinear relation with health, indicating that unfit and inactive people with high sedentary behaviour have considerably high cardiometabolic risk with more cardiometabolic risk factors (Figure 2) [54, 55, 70]. Therefore, it is important to target these groups first. The ultimate goal for these groups needs to be reaching the physical activity guidelines and raising their CRF, however, doing some activity will already lead to significant health improvements and is preferable to no physical activity [55].

2.3 Strengths, limitations and future research

Strengths and limitations

The measurement methods used in this thesis are associated with specific advantages and disadvantages and are important to consider when evaluating all results. A major strength of the present thesis is the combination of both cross-sectional and longitudinal analyses, as the combination of both techniques can lead to more in-depth conclusions. Furthermore, the rigorous and comprehensive measurement methods of physical activity, health-related fitness and cardio-metabolic health are also an important strength to consider. Finally, internal and external validity are important factors to take into account when generalising results. All strengths and limitations are discussed below.

Measurement techniques

The rigorous and comprehensive measurement methods are a very important strength of this thesis. Physical fitness was as much as possible measured with an objective approach and a maximal test. CRF was measured with the gold standard, a maximal exercise test on an electrically braked Lode Excalibur cycle ergometer (Lode, Groningen, The Netherlands) with directly measured breath-by-breath respiratory gas exchange analysis, using a Cortex MetaLyzer 3B analyser [56, 71]. Muscular fitness was also maximally assessed with a calibrated Biodex System Pro 3® dynamometer (Biodex Medical Systems, Shirley, NY) [72]. Major strengths of these measurements are the reliability, validity and sensitivity to within-participant changes over time [71, 72]. This is an advantage over other large epidemiological studies that often use approximated parameters of physical fitness, mostly because of time constraints, financial costs and risks involved with maximal exercise tests. The risk of maximal exercise testing generated also in this thesis some limitations. All participants had to undertake a physical examination before participating in the maximal tests and participants with lower back pain, a higher risk of myocardial infarction, arterial hypertension, abnormalities on an electrocardiogram were excluded. This resulted in the exclusion of more than 150 participants and reduced the sample size considerably. Furthermore, due to the exclusion of more unfit and unhealthy participants, the included population was rather homogeneous, which can lead to an underestimation of the actual associations observed in this thesis. Another factor potentially increasing the risk of over adjustment, is that both CRF and muscular fitness are relative to total body weight, which is correlated with waist circumference (which is part of the outcome).

Another strong measurement method, although only available at follow-up, was objective physical activity assessment with the SenseWear Pro 3 Armband. The SenseWear Armband has been validated at length in multiple contexts and is a reliable and valid measurement tool [73-75]. The synchronous measurement of acceleration and other physiological responses allows for accurate assessment of energy expenditure during all types of physical (in-)activity and sedentary behaviour. In comparison to most other studies we applied strong inclusion criteria to reach the most comprehensive assessment of total physical activity, sedentary behaviour and sleep. Inclusion criteria were set at 1296 minutes a day (90%), for at least three weekdays and both weekend days according to the recommendations to reliably assess habitual physical activity by Scheers et al. [76]. Limitations of the SenseWear Armband are the underestimation of total energy expenditure, mostly at high intensities, compared to double labelled water [73, 74] and the SenseWear Armband cannot be worn during water-based activities. Consequently, these activities are not measured and can decrease the actual physical activity level. Because swimming is the preferred recreational sport for almost 12% of the Flemish adults, this may decrease total MVPA significantly [77]. Another limitation is that the SenseWear Armband cannot differentiate between sitting and standing, and as a result, some standing will be included in sedentary time.

For longitudinal analyses including sedentary behaviour and physical activity the Flemish Physical Activity Computerised Questionnaire (FPACQ) was used. This is a questionnaire which collects information about sedentary behaviour and physical activity at work, for transportation and during leisure time throughout a usual week. This questionnaire is extensively validated and generates estimates with high reliability (intraclass correlations ranging from .67 to .99) and criterion validity ranging from low to high (correlations ranging from .21 to .88 with an objective criterion) [78, 79]. Although it remains a subjective measurement, the FPACQ has better reliability and validity in comparison to similar questionnaires and single-item questionnaires [78, 80]. A limitation of the FPACQ is that it only assesses a part of physical activity and sedentary behaviour. Included in this thesis for MVPA were a combination of sports participation and active transportation, only including activities with an intensity ≥3 METs, according to the Ainsworth compendium [81]. For sedentary behaviour the reported amount of time they spent in screen time and passive transportation was included. Because not all parts of physical activity and sedentary behaviour were included, it is most likely that MVPA and sedentary behaviour were substantially underestimated, which potentially diminished the strength of association with cardiometabolic health. Furthermore, the mean change in sedentary behaviour in the longitudinal study (Paper 4) was mostly driven by a change in screen time, which may be associated with a different confounding structure and different pathways explaining associations (such as differences in dietary intake), compared with studies that implemented objective measurement of total sedentary time.

Because several parameters may affect the association between physical activity, sedentary behaviour, CRF and cardiometabolic health, multiple questionnaires and objective measurements were performed to gather as much relevant, and possibly confounding information about participants. For example, nutrition and smoking are possible confounders because total energy intake, sugar intake, and smoking can be associated with cardiometabolic health [82, 83]. Nutrition was assessed with a three-day food record and key recommendations of the 2010 Dietary Guidelines were included with the Healthy Eating Index [84]. Past and present smoking behaviour was assessed with the WHO Monica Smoking Questionnaire. Another important covariate is the social economic status of the participant as it has significant influence on physical activity and sedentary behaviour [85-87]. However, some data about the participants remains unknown, of which medication use is the most important one. Although the population assessed in this thesis was relatively healthy, some would have taken medication against hypertension, dyslipidemia or dysglycemia. There is a chance that in participants with higher medication use, the positive effects of sedentary behaviour and physical activity might be drowned out by the health-improvements caused by this medication-use. Other possible confounders that were not incorporated were occupation, income and family history of cardiometabolic risk. This could have caused residual confounding.

Statistical analyses

Besides these strong measurement techniques, novel applications of statistical methods were applied in the first two papers (Paper 1 & 2). In the first paper the Gini index was used to explore the influence of the variability of the physical activity pattern. The Gini index measures statistical dispersion and is a common applied statistical coefficient in social sciences. It is mainly used as a measure of income inequality among individuals or households. However, recently this index is applied to various medical contexts, such as inequality in health, life expectancy and health care [88] or as a quantification method for sedentary behaviour [89]. A major strength of this technique is that no cut-offs are needed to calculate the Gini index and therefore the physical activity pattern can be observed holistically. Furthermore, this technique had never been applied to the full physical activity pattern (Figure 3). Limitations were that there was collinearity between the Gini index and total volume of physical activity and that the Gini index itself does not contain intensities.

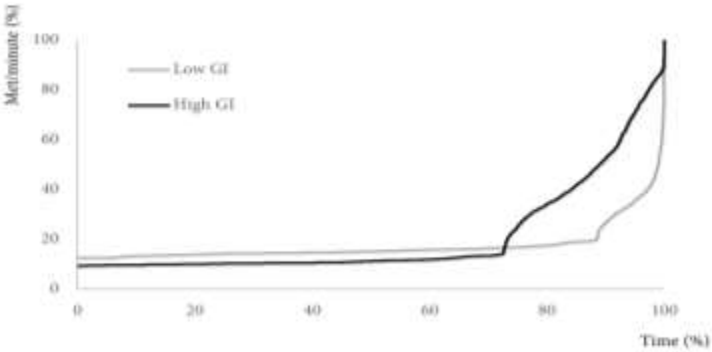

Figure 3 is a representation of a participant with a high and one with a low Gini Index (GI). The figure shows that a high GI has more variation in pattern than a low GI. Participants with a high GI perform more time at more diverse intensities (Met/minute) in comparison to participants with a low GI.

Another novel application of a statistical technique used in this thesis is the isotemporal substitution method (Paper 2). This technique estimates the effect of replacing one form of behaviour with another form of behaviour for the same amount of time [90]. The most important strength is that it not only estimates the effect of increasing a certain behaviour, but also integrates the effect of reducing the specific behaviour that it replaces [90]. Isotemporal substitution can take into account that reducing sedentary time and replacing it with sleep or LPA will result in a different health benefit than replacing the same amount of sedentary time with MVPA [5-7, 91]. To the best of our knowledge paper 2 is the first paper to include substitution with objectively measured physical activity, sedentary behaviour and sleep.

Cardiometabolic health was represented by a continuous cardiometabolic risk score (CMRS), largely based on the IDF criteria for the metabolic syndrome [49, 92]. To calculate CMRS metabolic parameters, blood pressure and waist circumference were assessed by trained staff in the morning after an overnight fast. A sample of fasting blood plasma was taken from an antecubital vein of subjects. Standardized values for waist circumference, triglyceride, plasma glucose, blood pressure and the inverse of HDL-cholesterol were computed. Each cardiometabolic risk factor was standardized by using the sex-specific sample mean and standard deviation, derived from all men and women with data for each cardiometabolic risk factor. To calculate the CMRS, the sum of these standardized values was divided by the number of metabolic parameters included (n=5). A score above zero represents higher individual cardiometabolic factors and therefore indicates having a less preferable clustered cardiometabolic health.

A continuous cardiometabolic risk score that includes the same five risk factors as the metabolic syndrome was applied instead of the metabolic syndrome itself, which is a dichotomous approach. A continuous score is more appropriate for epidemiological studies [92], firstly because cardiovascular and diabetes risk increase progressively with an increasing number of risk factors. Strengths of a continuous score are that it can better capture the progressive risk increase when more risk factors are present and it has more statistical power than a dichotomous score [92]. Furthermore, it takes into account that each risk factor is continuous and can vary substantially even in two participants who score too high according to the IDF criteria. A limitation to the risk score applied in this thesis is that all five risk factor equally contribute to the score, it is however likely that not all five risk factors are equally strong related to cardiometabolic health. Therefore, some continuous risk scores perform a principal component analysis to weigh each factor [92]. However, the IDF criteria does not weigh factors either, but includes central obesity as a prerequisite risk factor [49]. Another limitation of the continuous score is that it is sample specific as it is calculated with means and standard deviations of this sample. However, to have the most representative estimation CMRS was calculated on data of all participants with data for cardiometabolic risk factors.

Cross-sectional and longitudinal designs

Firstly, cross-sectional studies have the benefit that they can include the newest and most precise methods and do not have to rely on the methods chosen at baseline, which can be outdated. Particularly in this thesis, the main advantage of the cross-sectional analyses was the inclusion of objective measurement for sedentary behaviour and MVPA. Objective measurement was not available at baseline (first measurement 2002-2004), because at that time it was still very expensive and not feasible at large cohorts. Nevertheless, cross-sectional analyses have some major disadvantages. Mainly the fact that causality cannot be established, because all exposures and outcomes were measured at the same time [93]. Causality can only be deduced from biological plausible mechanisms. Consequently, we can assume that an active lifestyle with low sedentary behaviour and high CRF, through beneficial changes in fibrinolytic, hemodynamic, inflammatory markers, and the cardiovascular system, will have a positive impact on cardiometabolic health [94, 95]. It is however not completely unlikely that participants with better cardiometabolic health (eg. lower waist circumference) are less restricted and more encouraged in their physical-active behaviour and, therefore, enjoy physical activity more and participate in more MVPA and less sedentary behaviour. For example, a study by Ekelund et al. showed that weight gain predicts sedentary behaviour, but sedentary behaviour did not predict future obesity [96].

Longitudinal studies have more statistical advantages, but are more expensive and time consuming to conduct [93]. Furthermore, mediation effects can be studied more meaningfully in a longitudinal dataset, because a cause-and-effect relationship can be established, which is not possible with cross-sectional analyses. However, most longitudinal analyses included in this thesis are change-by-change analyses, and although they have many advantages, they do not allow for interference of causality either. In the medium-sized sample included in all analyses, this type of longitudinal approach was preferred because there were only two data points, which were far apart in time. The major strength of change-by-change analyses is the examination of within-participant effects. These are less prone to unmeasured time-independent confounders such as genetic factors and nurture, because each individual acts as his own control [15]. However, interindividual differences in change-measures can still be influenced by genes, nature and environment.

Internal and external validity

Internal validity is the potential for confounding factors to interfere with the relationship between the independent and dependent variables [97]. All cross-sectional and longitudinal research designs are vulnerable to threats to internal validity, such as selection bias, the Hawthorne effect, attrition, instrumentation, and regression to the mean. First, selection bias means that there is a selective drop-out, which in this case might mean participants who do not enjoy taking physical activity tests. However, to evaluate the selection bias, a drop-out analysis was performed comparing people who did return for follow-up and to those who did not return.

Notwithstanding that this was not possible in the cross-sectional analyses. Secondly, the tendency to perform better, merely because someone is being observed is another threat. This is also known as the Hawthorne effect or observer effect. However, we assume this influence is minimal, definitely in the long run, because the SenseWear Pro 3 is relatively convenient and discreet to wear and participants wore the monitor for at least five days. A third potential threat to the internal validity is attrition and refers to the gradual reduction in sample size, which in the cohort used in this thesis was rather large, as only 42% of all participants returned for follow-up. Again, a drop-out analysis was performed to compare the baseline values of participants who returned for follow-up and those who did not. Fourth, instrumentation involves the fact that measurement techniques evolve over time and are not always the best option at follow-up (eg. questionnaire vs. SenseWear). Fifth, regression to the mean indicates that repeated measures on the same variable have the tendency to be closer to the mean, which is mostly a problem in a sample that is only measured twice. Furthermore, another potential threat to the validity is testing, where performance by participants is enhanced due to practice or familiarity with the measurement tools and/or procedures. Though, this is probably not the case in the present thesis as measurement were almost 10 years apart.

Besides internal validity, there are also important points of external validity to take into account. External validity refers to the extent which results of a study can be generalized outside the experimental situation [97]. Treats to external validity are interaction of treatment and selection, the specific setting and the time when the study was performed. Firstly, the population included in this thesis was a healthy, fairly well educated, Caucasian adult population including both men and women of a wide age range. Contrary to most studies, the amount of MVPA in the current study is high, however similar to a comparable Flemish population where physical activity was measured similarly [16]. In comparison to a sample representative for the whole Belgian population participants of this study spend on average 30 to 50 minutes more in MVPA [98]. The difference in MVPA might, however, be due to the specific algorithms the SenseWear Pro 3 uses. Furthermore, it is possible that the recording of MVPA and sedentary time over a 24hr period and in single minutes instead of in bouts (e.g. bouts of 10 min) may have led to a more inclusive measurement of MVPA. Alternatively, our study sample may have included healthier, more active, individuals compared to the average population. Nonetheless, we assume that these results are applicable on a rather large population of Flemish adults, however not everyone. Furthermore, the total population was only medium-sized and physical activity, sedentary behaviour and mostly physical fitness are dependent of age and sex [99]. This can hamper conclusions and power for specific subpopulations, as subgroups were very small. Furthermore, results cannot be extrapolated to patient populations, or children and adolescents.

Secondly, physical activity and sedentary behaviour were measured in daily life, and therefore are less influenced by the experimental setting. Moreover, we had measurement randomly throughout the year, so the seasoning effect was also limited [100]. All other measurements were taken in a more experimental setting at the lab, and may therefore be less generable, however very reliable and valid. Bringing together both field tests and lab tests will probably result in a more complete assessment of the participants. Finally, as with all results, these results are time specific and are representative for a population in modern times with low physical activity at work and considerable screen time.

Future research

Longitudinal studies with objective measurement of physical activity and sedentary behaviour at multiple time points are necessary to gain better insight in the temporal sequence and cause-and-effect relationship of physical activity, sedentary behaviour, CRF and cardiometabolic health. Very long-term and genetically controlled studies investigating the effects of physical activity, sedentary behaviour and CRF on cardiometabolic health are lacking [101]. Furthermore, novel and more precise measurements are being developed and need to be integrated in the latest epidemiological studies. For example, in this thesis fasting plasma glucose was assessed as a measure for glycaemia, however, it has been shown that sedentary behaviour has an association with 2-h plasma glucose but not with fasting plasma glucose [102]. Apart from 2-h plasma glucose, HbA1C is a more sensitive measurement for early detection of insulin impairment [103].

Besides more epidemiological studies, more interventional studies and fundamental research are needed. Interventional studies need to thoroughly explore the effects of short and long term behavioural changes. Furthermore, they need to explore how these changes can be stimulated and maintained since many physical activity interventions have tremendous potential for the prevention of non-communicable diseases [104]. However, public health campaigns have struggled to implement these interventions on a large scale as interventions are often effective in highly controlled research settings, but fail to achieve successful scaling-up to a non-experimental context [105]. Besides these interventional studies, fundamental research is necessary to get a better insight in the underlying pathways in which physical activity, sedentary behaviour and CRF affect cardiometabolic health. To date many metabolic and regulatory pathways that underlie physical activity-induced adaptations in cardiometabolic health can be clarified, although many stay unresolved. Furthermore, genes play an important role in these pathways and more genetic factors need to be identified [106, 107].

3 Closing statement

It is clear that physical activity, sedentary behaviour and CRF are associated with cardiometabolic health and have the potential to improve someone's health. But an active lifestyle has much more important beneficial outcomes than cardiometabolic health. It is positively associated with other health outcomes, such as cancer, depression and quality of life [26, 108, 109]. Furthermore, it can make you feel better, help social and cultural integration or help women emancipate [110]. Physical activity as a tool to stimulate all these positive consequences has not been applied as much as it should. Promotion of physical activity can and should be a more important matter for the regulation of sport, public health, education, public environment and transportation. It is important to start enjoying and integrating physical activity at a young age as it tracks through adulthood. Furthermore, with a population that is getting older, it is important to keep everyone physically and mentally healthy as long as possible. Physical activity will probably help. Therefore, an active lifestyle should be more stimulated in all ages, social classes, environments and ethnicities among the Flemish population.

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APPENDICES

Appendices

1 Brief summary

In recent years, accumulating evidence suggested that **excessive sitting** and **insufficient physical activity** may independently contribute to **unhealthier cardiometabolic risk profiles**, which in turn may substantially increase the risk for incident type 2 diabetes, cardiovascular diseases and premature death. **Physical fitness** is another factor recognized as a strong predictive marker for cardiometabolic health, and potential mediator in the associations between physical activity, sedentary behaviour and cardiometabolic health. Developing more insight into whether, and to what extent, sitting, physical activity and physical fitness independently shape cardiovascular health, is therefore important in order to advance lifestyle interventions and public health guidance. Five papers were written in order to reach a better insight into the associations between physical activity, sedentary behaviour, physical fitness, clustered cardiometabolic health.

The first paper took a deeper look into the association between sedentary behaviour, physical activity and health-related fitness. The second paper examined the association between substituting two different behaviours and cardiometabolic health. In the third and fourth paper cross-sectional and longitudinal analyses assessed the associations between sedentary behaviour, MVPA and cardiorespiratory fitness (CRF). Furthermore, the mediating role of CRF on potential associations between sedentary time, MVPA and clustered cardio-metabolic risk was examined. The fifth and final paper aimed to investigate the possible additive contribution of change over a period of 10 years in objectively measured muscular fitness towards cardiometabolic health, independent of the favourable effects of change in CRF.

The conclusion of this thesis is that a physical active lifestyle with high physical activity and low sedentary behaviour will lead to better health-related fitness. All three exposures (high physical activity, low sedentary behaviour and high fitness) are associated with cardiometabolic health and affect several cardiometabolic risk factors such as diabetes type 2, hypercholesterolemia, obesity and hypertension.

2 Korte samenvatting

De afgelopen jaren is er meermaals aangetoond dat **overmatig zitten** en **weinig bewegen** onafhankelijk van elkaar bijdragen aan een **ongezond cardio-metabool profiel**. Deze stijging verhoogt op zijn beurt het risico op type 2 diabetes, hart- en vaatziektes en vroegtijdige dood. Een andere factor, erkend als een sterke voorspeller voor de cardio-metabole gezondheid, is **fysieke fitheid**. Fysieke fitheid is ook een mogelijke mediator in de associaties tussen fysieke activiteit, sedentair gedrag en cardio-metabool risico. Het is daarom belangrijk om meer inzicht te ontwikkelen in welke mate zitten, bewegen en fysieke fitheid onafhankelijk van elkaar bijdragen aan de cardio-metabole gezondheid.

De eerste paper geeft onderzocht de samenhang tussen zitten, bewegen en fitheid. De tweede paper analyseerde het verband tussen het verwisselen van twee verschillende gedragingen (bijvoorbeeld zitten en intens bewegen) en cardio-metabole gezondheid. In de derde en vierde paper beoordeelden cross-sectionele en lang termijn analyses de verbanden tussen zitten, intens bewegen en cardiorespiratoire fitheid. Bovendien werd de mediërende rol van fitheid op mogelijke verbanden tussen zitten, intens bewegen en cardio-metabole risicofactoren onderzocht. De vijfde en laatste paper onderzocht de mogelijke additieve bijdrage van verandering in musculaire fitheid naar cardio-metabole gezondheid toe over een periode van 10 jaar.

We concluderen uit deze papers dat een actieve levensstijl met veel beweging en weinig overmatig zitten zal leiden tot een betere fitheid. Deze drie variabelen (veel bewegen, weinig zitten en hoge fitheid) zijn geassocieerd met cardio-metabole gezondheid en hebben invloed op enkele onderliggende cardio-metabole risicofactoren zoals obesitas, hypercholesterolemie, diabetes type 2 en hypertensie.

3 Appositions/Bijstellingen

Bijstelling 1:

Hoewel genetica een zeer belangrijke factor is voor gezondheid, mag daarbij de invloed van gedrag, opvoeding en omgeving niet uit het oog verloren worden.

Bijstelling 2:

Het uitvoeren van longitudinaal onderzoek wordt vaak ondergefinancierd, omdat de onderzoeksresultaten pas na lange termijn beschikbaar zijn.

Bijstelling 3:

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5 Professional career of Sara Knaeps

5.1 Curriculum Vitae

2014-2016	PhD Student KU Leuven Faculty of Kinesiology & Rehabilitation Sciences Deparment of Kinesiology – Physical Activity, Sports & Health Research Group
2015	Internship PhD student University of Cambridge School of Clinical Medicine MRC Epidemiology Unit

5.2 Publications

Articles in peer-reviewed journals

Knaeps, S., Bourgois, J. G., Charlier, R., Mertens, E., Lefevre, J., & Wijndaele, K. (2016). Ten-year change in sedentary behaviour, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic risk: independent associations and mediation analysis. Br J Sports Med.

Knaeps, S., Bourgois, J. G., Charlier, R., Mertens, E., & Lefevre, J. (2016). Associations between physical activity and health-related fitness - volume versus pattern. J Sports Sci, 1-8.

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Mertens, E., Charlier, R., **Knaeps, S**., Clarys, P., Deforche, B., Thomis, M., . . . Lefevre, J. (2016). Leeftijdsgerelateerde veranderingen en stabiliteit van gezondheidsgerelateerd gedrag en fysieke fitheid bij Vlaamse volwassenen. Retrieved from www.steunpuntsport.be

Conference abstracts

Knaeps, S., Boen, F., Scheerder, J., de Carvalho, M. (2013). Relationship between sportscape perception, place attachment, and soccer attendance in Belgium - A case study. European Association for Sport Management Conference. Istanbul, 11-15 September 2013.

Knaeps, S., Bourgois, J., Charlier, R., Mertens, E., Lefevre, J. (2015). Physical activity patterns are associated with health-related physical fitness in Flemish adults. Advancing Behavior Change Science - International Society of Behavioral Nutrition and Physical Activity. Edinburgh, 3-6 June 2015.

Knaeps, S., Bourgois, J., Charlier, R., Mertens, E., Lefevre, J., Wijndaele K. (2015). Ten-year change in sedentary behavior and cardiorespiratory fitness are independently associated with clustered cardio-metabolic risk. Annual Meeting – American College of Sports Medicine. Boston (MA), 1-5 June 2016.

Knaeps, S., Bourgois, J. G., Charlier, R., Mertens, E., Lefevre, J., Wijndaele, K (2015) Poster: Ten-year change in sedentary behavior and cardiorespiratory fitness are independently associated with clustered cardio-metabolic risk Methods in Epidemiology Symposium, Centraal Auditorium at UZ Leuven, Leuven, 17 September 2015.

Knaeps, S., Bourgois, J. G., Charlier, R., Mertens, E., Lefevre, J., Wijndaele, K. (2015) Ten-year change in sedentary behavior and cardiorespiratory fitness are independently associated with clustered cardio-metabolic risk 20ste Symposium Vereniging Kinesiologie, December 2015, KU Leuven

Charlier R, Caspers M, **Knaeps S**, Mertens E, Lambrechts D, Lefevre J, Thomis M (2015) Use of different genetic predisposition score techniques to predict muscle mass and muscle function over the adult lifespan in Flemish Caucasians. Methods in Epidemiology Symposium, Centraal Auditorium at UZ Leuven, Leuven, 17 September 2015.

Caspers M, Charlier R, **Knaeps S**, Mertens E, Lambrechts D, Lefevre J, De Bock K, Thomis M (2015) (Epi)genetic variation in ageing of metabolic fitness. Methods in Epidemiology Symposium, Centraal Auditorium at UZ Leuven, Leuven, 17 September 2015.

Other

Sport in Vlaanderen onderzocht, Resultaten en aanbevelingen voor beleid en praktijk (2016), Acco Leuven / Den Haag, Marc Theeboom (red.). Chapter: Veranderingen in fysieke activiteit, sedentair gedrag en cardiorespiratoire fitheid zijn gerelateerd aan cardio-metabole gezondheid; **Sara Knaeps**, Katrien Wijndaele, Evelien Mertens, Ruben Charlier, Jan G. Bourgois en Johan Lefevre

Leven we gezonder dan tien jaar geleden? Evoluties in fysieke fitheid, fysieke activiteit, sedentair gedrag en voeding over tien jaar tijd. **Sara Knaeps**, Ruben Charlier, Evelien Mertens, Jan Bourgois, Johan Lefevre; Tijdschrift voor Lichamelijke Opvoeding

Sitting – The new smoking or the new sensationalism, **Sara Knaeps**, KU Leuven Blogt

5.3 Awards & Grants

Dag Van de Nieuwsgierigheid (Radio één) Science Slam - Winner of the Jury Price – Zitten het nieuwe roken? (2015)

"Gaston Beunen" VK Prijs voor jonge onderzoekers – 1st place oral presentation – Ten-year change in sedentary behavior and cardiorespiratory fitness are independently associated with clustered cardio-metabolic risk (2015)

Faculty Mobility Fund, UGent (2015)

First Contact Initiatives, KU Leuven (2015)

Erasmus+, European Commission (2015)

Faculty Mobility Fund, UGent (2016)

Broaden Your Horizon, KU Leuven (2016)

Congrestoelage, Academische Stichting Leuven (2016)