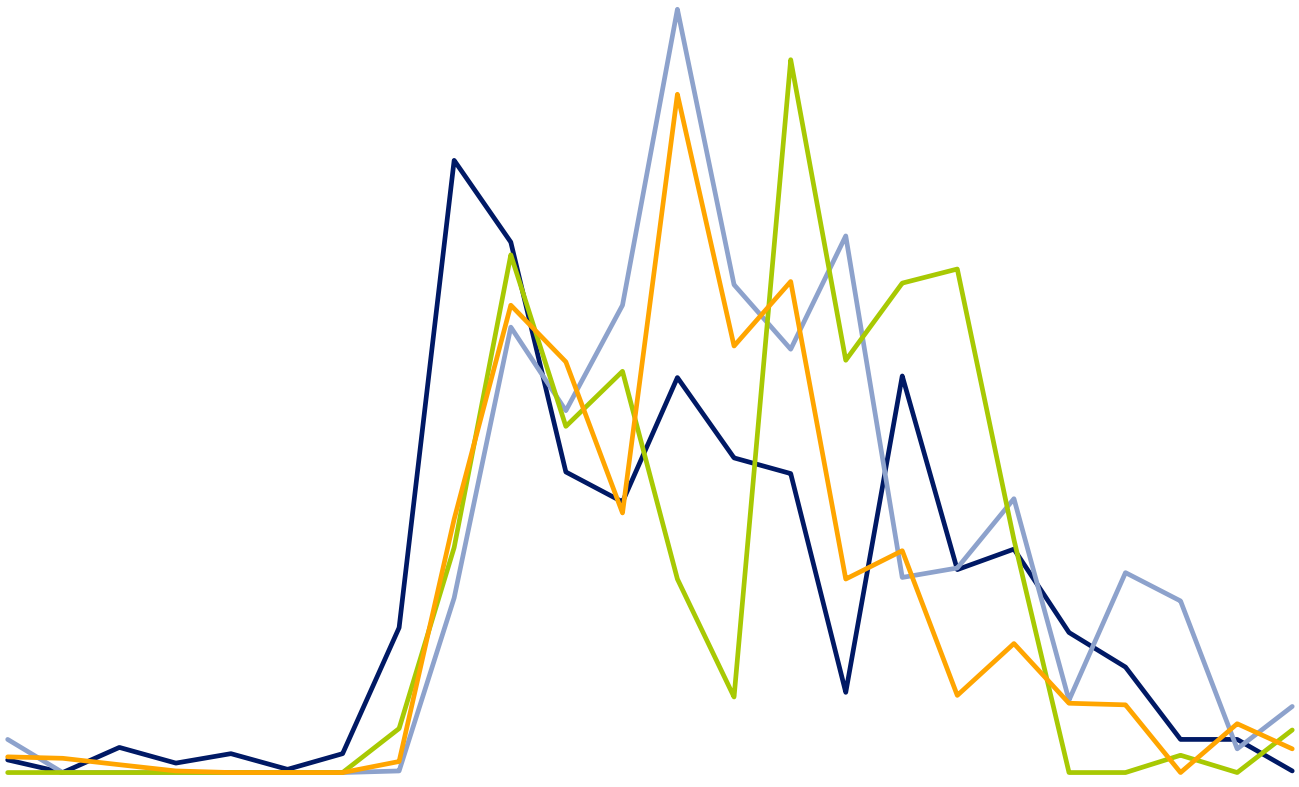
FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

The role of different dimensions of physical activity and sedentary behavior in glucose regulation

PhD Thesis

Anne-Louise Smidt Hansen

2012



Steno

Title: The role of different dimensions of physical activity and sedentary behavior in glucose regulation

Author: Anne-Louise Smidt Hansen, B.M.L.T., MSc.
asih@steno.dk *or* al.smidthansen@gmail.com

Institution: Epidemiology Research Group, Steno Diabetes Center A/S, Gentofte, Denmark
Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Supervisors: Daniel R Witte, MD, PhD
Epidemiology Research Group, Steno Diabetes Center A/S, Gentofte, Denmark
Centre de Recherche Public de la Santé, Strassen, Luxembourg

Jørn W Helge, MSc., PhD
Centre for Healthy Ageing, Institute of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Mette Aadahl, PT, MPH, PhD
Research Centre for Prevention and Health, the Capital Region of Denmark, Glostrup, Denmark

Opponents: Professor Allan Vaag (Chair), MD, PhD, DMSc.
Department of Orthopaedics and Internal Medicine, University of Copenhagen, Copenhagen, Denmark

Professor Ulf Ekelund, MSc., PhD
The Norwegian School of Sport Sciences, Oslo, Norway

Dr. Andreas Holtermann, MSc., PhD
National Research Centre for the Working Environment, Copenhagen, Denmark

Submitted: The thesis was submitted 21st December 2012 to the Graduate School of The Faculty of Health and Medical Sciences, University of Copenhagen

Cover page: Physical activity patterns from four participants of the ADDITION-PRO study (median daily physical activity energy expenditure by hour of day)

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PREFACE

During the last seven years, I have been involved in several smaller and larger clinical- and research projects. Looking back, a common factor in all of them has been focusing on dimensions of physical activity, biochemistry, and methodology. As a medical laboratory technologist, I was 'brought up' with clinical biochemistry; with a natural skepticism towards any method applied; and, with a systematical approach. At the university, my focus moved from 'cell' to 'human body' and I learned about health science in a broader perspective. Time had come, when I was to choose a topic for my first empirical university project. At this writing, it is exactly five years since I arranged to go to Greenland to participate as a research assistant in the 'Inuit Health in Transition' study, to help out with blood sampling and other tasks. They used the ActiHeart activity monitor and the International Physical Activity Questionnaire (IPAQ) in that study, and before I knew it I was writing my project about physical activity in Greenland. I have been drawn ever since. . . I was fascinated by the area of physical activity – with all the challenges of measuring this complex behavior. This led me to my Master's thesis (comparison of IPAQ and ActiHeart in a Kenyan population), and to Steno Diabetes Center.

During my time employed at the Epidemiology Research group at Steno Diabetes Center (2009-2012), I have been one of four study coordinators of the 'ADDITION-PRO' study, a follow-up study of persons identified by a diabetes screening program. Through that study, I have gained extensive experience with the practical challenges and implications of running a multicenter study: communication with collaborators, training of staff, and with collection and management of data. This data management also involved deriving the physical activity data from the 'Health2008' and the ADDITION-PRO study as measured by the ActiHeart activity monitor. When the data collection of ADDITION-PRO was nearly finished, I got the chance to visit the 'Baker IDI Heart and Diabetes Institute' in Melbourne, Australia, for a four months research stay, to work on the data regarding sedentary behavior in the 'AusDiab' and the 'Victorian Health Monitor' studies. I learned a lot.

On the side, during the last four years, I have been employed as a teaching assistant at the University of Copenhagen (integrated course in biomedicine at the Master of Health Science program) and have been an appointed examiner for the medical laboratory technology program, trying to keep up to date with current knowledge.

Altogether, I feel privileged, that by writing my thesis I can round of three busy years with a comfortable feeling that I got the most out of it!

Anne-Louise Smidt Hansen, December 2012

LIST OF PAPERS

This thesis constitutes a summary based on the following three papers, which has been conducted in collaboration with Steno Diabetes Center, the Research Centre for Prevention and Health in Glostrup, and with the Baker IDI Heart and Diabetes Institute in Melbourne. The papers are referred to by their roman numerals:

I) Physical activity patterns and glucose metabolism in an adult Danish population: the Health2008 study
(Submitted to: *Journal of Epidemiology and Community Health*)

II) Combined heart rate- and accelerometer- assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes. The ADDITION-PRO study
(Submitted to: *Diabetes Care*)

III) Adverse associations of increases in television viewing time with 5-year changes in glucose homeostasis: the AusDiab study (*Diabetic Medicine* 2012; 29 (7):918-925)

ABBREVIATIONS

MVPA, moderate-to-vigorous physical activity; **PAEE**, physical activity energy expenditure; **OGTT**, oral glucose tolerance test; **i-IFG**, isolated impaired fasting glycaemia; **i-IGT**, isolated impaired glucose tolerance; **T2DM**, type 2 diabetes mellitus; **PAI**, physical activity intensity; **PAI_{HR}** , physical activity intensity as measured by heart rate; **PAI_{ACC}** , physical activity intensity as measured by accelerometry; **RPAQ**, recent physical activity questionnaire; **PAS2**, physical activity scale II, **METs**, metabolic equivalents, **RMR**, resting metabolic rate; **BMR**, basal metabolic rate; **TV**, television; **HOMA**, homeostasis model assessment; **HOMA-IR**, HOMA-derived insulin resistance; **HOMA-B**, HOMA-derived beta cell function; **$ISI_{0,120}$**, insulin sensitivity index; **SB**, sedentary behavior; **NEAT**, non-exercise induced activity thermogenesis; **HIT**, high intensity interval training; **HbA_{1c}**, glycated hemoglobin A_{1c}, **$AGEs_{skin}$**, advanced glycation endproducts in the skin; **GLUT4**, glucose transport protein 4; **DI**, disposition index; **IGI**, Insulinogenic index.

INTRODUCTION

Physical activity levels in daily life are highly personal and influenced for each individual by different stimulants and barriers (1), such as individual, societal, and physical environmental barriers. To overcome these barriers, being physically active must to a high degree be intentional, and ideally, incorporated into daily living. The challenge in health promotion and clinical care is for the individual and for practitioners working with him/her to understand these stimulants and barriers sufficiently to achieve incorporation of physical activity into daily life.

During the health examinations for one of the studies on which this thesis is based, I met Paul, a 64-year old man working as an accountant. He lives in the city and every-day he travels to and from work by car (a 20 minute ride). At work, he sits for most of the day interrupted only by some activity when going for meetings, to pick up a cup of coffee, and when going for lunch. Paul's doctor has told him to be more physically active as he is at risk of developing diabetes due to being overweight, having an inactive lifestyle, and since his father had diabetes. However, Paul does not feel sick. He unwinds after a stressful day at work by watching television and reading the newspaper, only interrupted by dinner and a short walk with his old dog. His wife, who is now retired, does all the house work. In general, Paul's leisure time is characterized by sitting activities during the weekdays. On the weekends, however, Paul feels more energized than during the week, and he often visits the golf course with an old friend or enjoys a walk in the park with his wife. Even though Paul spends an average of 45 minutes with activities of moderate intensity per day during the week, fulfilling the current Danish physical activity recommendations (2), he also spends more than 75% of the day with sedentary activities (including sleeping). Consequently, he has relatively low total physical activity energy expenditure (2084 kJ/day), even for a man of his age. Pauls' physical activity pattern reflects a typical physical activity level and pattern in an elderly population from a western-European country, were 18-63% of the population does not fulfill the global physical activity recommendations (3). It furthermore illustrates the challenges we face if we want individuals like Paul to increase their physical activity levels.

In cross-sectional and prospective studies, physical activity has been found to be related to a better glucose metabolic profile (4-7) and increasing physical activity can reduce the risk of developing type 2 diabetes by 15-60% (8-10). Accordingly, time spent in sedentary activities is positively associated with detrimental health effects (11). In order to successfully tailor strategies and interventions to promote physical activity, it is important to know the status of a population's physical activity level and to understand how different groups of individuals accumulate physical activity throughout the day. Secondly, it is of great interest to know which activities individuals prefer. Thirdly, it is of high

importance to be able to understand and explain to which degree physical activity affects glucose metabolism, and thus, to be able to highlight any beneficial health effects.

AIMS

Based on the hypothesis that different dimensions of physical activity and sedentary behavior play a parallel role as determinants of dysglycaemia and type 2 diabetes, this thesis aims to:

- Identify patterns of physical activity by using available information on physical activity derived from both objective and self-report methods (Part I: Patterns of physical activity in a Danish population – Paper I and II)
- To quantify the associations of different physical activity dimensions and sedentary behavior with indicators of glucose homeostasis (Part II: Physical activity and sedentary behavior in relation to glucose homeostasis, Paper I, II and III).

BACKGROUND

Physical activity and the movement continuum

Definition of physical activity

In human energy metabolism, three components make up the total energy expenditure: diet induced thermogenesis; resting metabolic rate (including sleeping metabolism, basal metabolism, and arousal metabolism); and, energy expended during physical activity (12).

Physical activity has been defined as *'any bodily movement produced by skeletal muscles that results in energy expenditure'* (13), and refers to the activities performed during daily life while, for example being at work, and during leisure time. It is a complex and multidimensional behavior that includes the type and total volume of physical activity (including the frequency, intensity, and duration of physical activity bouts) and the context in which physical activity is performed (14). Physical activity is not to be confused with 'exercise' which is just a part of physical activity. Exercise refers to planned and structured bodily movement, often with the aim of improving or maintaining physical fitness components (13;15). In contrast to physical activity, the term 'physical inactivity' is used when there is a lack of physical activity, i.e. when being insufficiently physically active as according to official recommendations (15). During the last decade, physical activity recommendations have been published by global and national health authorities (2;15-17), based on research into the literature of health enhancing physical activity. Briefly, the current global recommendations on physical activity are divided into different age-groups: children and adolescents, adults, and elderly. For adults, a minimum of 150 minutes of moderate-to-vigorous physical activity (MVPA) per week, or 75 minutes

of vigorous physical activity, or equivalent combinations of MVPA are recommended. In addition, two weekly bouts of muscle-strengthening activities, and, for additional health beneficial effects, it is recommended that adults should increase their MVPA to 300 minutes per week (15).

The 'movement continuum'

The total volume of physical activity performed, the 'physical activity energy expenditure' (PAEE), is often measured in kilojoules or kilo-calories per time unit (18). Another way to quantify physical activity is by describing the intensity with which the physical activity is performed. This can be expressed as: absolute intensity, which covers the absolute PAEE estimates of specific activities; relative intensity, which is the individual effort of the specific activity; and perceived intensity, which is how hard a person perceives an activity to be (19). The intensity is often combined with the duration of the task, constituting the time spent in certain physical activity intensities. The intensity of an activity can be expressed in metabolic equivalents (METs), defined as multiples of the resting metabolic rate (20;21). Traditionally, epidemiological studies have classified physical activity intensity into different intensity categories using cut-points based on METs for specific activities, derived from different laboratory studies (22). The activity intensity is illustrated in the 'movement continuum' (Figure 1), ranging from sleep to vigorous physical activity intensity.

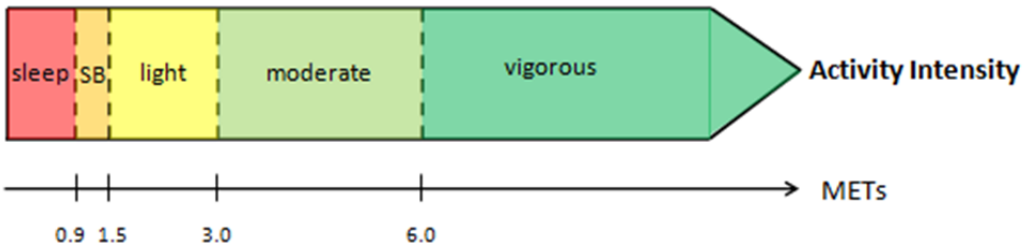

Figure 1 The movement continuum: illustrating different physical activity intensities. Modified from Tremblay et al.(23). METs= metabolic equivalent tasks. SB= sedentary behavior.

Sedentary behavior

At the very left end of the movement continuum (Figure 1) is 'sedentary behavior'. Sedentary behavior can be defined as *'any waking behavior characterized by an energy expenditure ≤1.5 METS while in a sitting or reclining posture'* (24). Sedentary behavior is mainly characterized by sitting activities and should be regarded as a separate entity from being inactive (25;26). While 'being inactive' refers to a person not meeting physical activity recommendations (15), a person can be highly sedentary despite meeting physical activity recommendations (27). However, persons spending a large amount of time being sedentary decreases their time with activities of light-,

moderate-, and vigorous- intensities (28). In epidemiological studies, sedentary behavior has traditionally included activities such as watching television, reading and other sitting activities (29).

Measuring physical activity and sedentary behavior in epidemiological settings

Physical activity

Measuring the multiple dimensions of physical activity requires different measurement methods, and in epidemiological settings, the choice of method is often a trade-off between precision and feasibility (30). Until recently, physical activity measures in population based studies have been obtained by subjective methods such as physical activity questionnaires (31;32). In general, self-report methods such as physical activity diaries, questionnaires, and logs are of low costs and easy to administer. Although they are prone to bias and error when estimating the intensity and duration of physical activity (19), they do provide information regarding the context of the physical activity. In contrast, objective methods to assess physical activity levels are often more precise in determining the absolute physical activity level (33), albeit without providing information about the type or context of the physical activity. Figure 2 illustrates how different physical activity dimensions can be obtained using self-report and objective methods. In laboratory- and clinical- studies, measures of maximal oxygen consumption (VO_2max) have been widely used, since this is a measure of a person's cardio-respiratory fitness level. However, this requires an extensive test set-up (34) and the testing is time consuming in epidemiological settings. When estimating total energy expenditure in everyday life, the 'doubly labeled water' (DLW) method is considered the gold standard (35). However, the DLW method cannot distinguish between the intensity, duration and frequency of the performed physical activity. Additionally, the low feasibility and high costs of this method has compelled the use of more feasible measurement methods, such as wearable accelerometers and heart rate monitors (32;36). Although the advantages of accelerometers and heart rate monitors include more precise monitoring of PAEE, these monitors also have some limitations. For example, the accelerometer is known to have limited capacity to register upper-extremity physical activity (37). Likewise, heart rate monitors have some limitations, since they are known to overestimate PAEE due to bias in heart rate when performing activities of lower activity intensities (38).

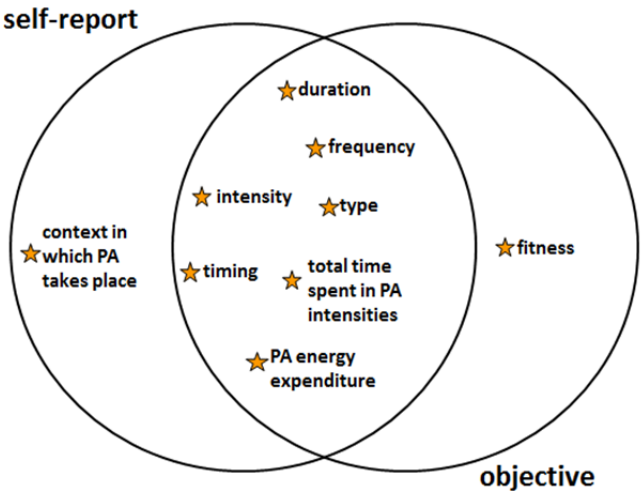

Figure 2 Different dimensions of physical activity that can be obtained with self-report or objective measurement methods. PA= physical activity

Accordingly, activity monitors combining heart rate monitoring and accelerometry have the potential to give more precise estimates of PAEE than the subjective methods and the individual measured movement and heart rate (39-41).

Sedentary behavior

Sedentary behavior has gained increasing focus in epidemiological physical activity research during the last decade (42). Traditionally, health assessment questionnaires, as well as activity monitors, have not focused on measuring sedentary behavior but on measuring physical activity or inactivity. In spite of this, questionnaires asking about time spent sitting or time spent watching TV have been used as surrogate measures of sedentary behavior, since TV-viewing time has been shown to be a rough marker of sedentary time (43) and to be one of the greatest contributors to self-reported daily sitting time on the weekends (44). However, there are some limitations when measuring sedentary behavior by self-report methods (29): It is difficult to remember sitting activities, and measures of sitting time from existing physical activity questionnaires have shown a tendency towards underreporting (29;45). To overcome this, new questionnaires asking about time spent sitting in various domains have been developed (44;46;47). In spite of acceptable reliability of the questionnaires, the studies have found modest validity of total sitting time when comparing with objectively measured sedentary time. More recently, activity monitors have been used to objectively measure time spent with sedentary activities (48-50). The objective monitors provide reliable and valid measures of the time spent with sedentary activities (51). However, there is still a need for self-report measures of sitting time, in order to obtain information on the context of sitting time, and to understand the environmental determinants that might influence sedentary behavior (49;52). Since the different measurement methods obtain different dimensions of physical activity, the choice of method is extremely dependent on the questions that are to be answered.

Physical activity patterns

The multidimensional concept of physical activity indicates that it is possible to accumulate physical activity in many ways. In some studies, groups of individuals accumulating physical activity in the same way are found (53-55). For example, persons who accumulate a large quantity of physical activity during a short period of time – mostly during weekends – have been called 'Weekend Warriors' (56). Additionally a subgroup of persons who are highly active during the week but less active in the weekends has been identified (53). These results were found in a large, heterogeneous US sample, and evidence that similar subgroups of persons with different PA patterns are present in other populations are scarce. Furthermore, it has been suggested that different patterns of physical activity have different effects on health outcomes (57;58).

These studies are a first step toward an approach where individual physical activity patterns, rather than only amounts of time or intensities, are the target for change. Furthermore, changing an activity pattern can perhaps lead to a longer lasting effect, as it increases long-term activity by a change to habitual patterns rather than depending on repeated conscious decisions to exercise.

Physical activity and fitness in a public health perspective

Physical activity is known to have several beneficial effects on cardiometabolic health (59;60), and increased fitness level, expressed as maximal oxygen consumption, has shown to decrease the risk of cardiovascular mortality (61). In Figure 3 (modified from (62)), the relative risk of mortality by maximal oxygen consumption (fitness level) (from Blair et al (63)), is shown together with the distribution of the maximal oxygen consumption from a Swedish population based study. The dark solid line reflects the risk of cardiovascular death associated with lower fitness levels. However, the overlay of this curve to the distribution shows clearly that these very high risk levels only apply to a minority of the distribution. The majority of people are at levels of fitness associated only with a mild risk elevation. This means that if efforts to increase fitness levels in the population focus solely on those with the highest risk, the impact amongst these individuals will be large, but the impact on cardiovascular death in the population will be small. In order to achieve large effects on cardiovascular deaths in the population, risk also needs to be lowered for the large group at intermediate fitness levels. In other words the entire fitness distribution needs to be shifted. The dotted line illustrates how the relative risk of mortality can be lowered if the population's distribution of maximal oxygen distribution is shifted to the right (higher fitness level). As such, from a public health perspective, even small increases in maximal oxygen consumption would potentially have a significant effect on the risk of mortality.

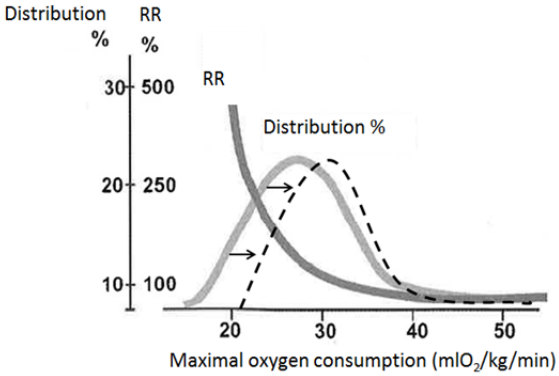

Figure 3. Shifting the maximal oxygen consumption distribution towards the right results in decreased risk of cardiovascular mortality (modified from (62)).
RR= relative risk of cardiovascular death by maximal oxygen consumption

In theory, this phenomenon is described 'the prevention paradox' (64). Examples of preventing cardiovascular disease by lowering, for example, serum cholesterol levels in an entire population (a 'left-shift' of the population distribution of the exposure) have been given in the literature (65). In

contrast to the left-shift of the population distributions in these examples, when fitness levels or physical activity energy expenditure are the exposure variables, the distribution of the exposure should be shifted towards the right in order to prevent disease development. This way of thinking might be applicable when elaborating on 'metabolic' fitness as well (e.g. the effect of physical activity level on glucose metabolism).

Glucose homeostasis

To maintain normal bodily functions, the human body requires intake of carbohydrates, lipids and protein. After intake of food, carbohydrates are processed in the liver and released as, for example, glucose in the blood stream, ready to be distributed as fuel to the organs or stored as glycogen in deposits in skeletal muscles or the liver. The glucose concentration, however, has to be regulated, as the body has to cope with periods with glucose excess and deficits. This regulation is maintained by feedback mechanisms involving insulin and glucagon as the key hormones (66). In type 2 diabetes patients, the glucose level is elevated in the fasting or post-prandial states, or in both (67). As a consequence of the elevated levels of glucose in the blood, the proportion of glycated hemoglobin A_{1c} (HbA_{1c}) is raised over time. Diabetes can be diagnosed based on elevated values in either of the three measures (fasting glucose, 2-hour glucose, or HbA_{1c}) (67;68). However, the derangements in glucose metabolism often starts long before the diagnosis of diabetes (69-71) and it has been suggested that individuals with different states of 'pre-diabetes' (67) have different pathophysiological derangements leading to disturbances in glucose homeostasis (72;73). As such, individuals can be grouped into five overall categories of glucose tolerance status, determined by an oral glucose tolerance test (OGTT)(67): 1) normal glucose tolerance (fasting plasma glucose <6.1 and 2-hours plasma glucose <7.8 mmol/l); 2) isolated Impaired Fasting Glycaemia (i-IFG), including individuals with elevated fasting plasma glucose (6.1 to 6.9 mmol/l) but normal plasma glucose levels two hours after glucose load (<7.8 mmol/l); 3) isolated Impaired Glucose Tolerance (i-IGT) including individuals with normal fasting plasma glucose values (<6.1 mmol/l) but elevated 2-hours plasma glucose levels (≥7.8 and < 11.1 mmol/l); 4) combined IFG/IGT including individuals with elevated fasting plasma glucose (6.1 to 6.9 mmol/l) *and* 2-hours plasma glucose concentration but not above the threshold values for defining type 2 diabetes (7.8 to 11.1 mmol/l); 5) type 2 diabetes (T2DM), including individuals with fasting plasma glucose ≥7.0 mmol/l *or* 2-hours plasma glucose concentration ≥11.1mmol/l.

Insulin sensitivity and Insulin resistance

In normal glucose metabolism, insulin binds to specific receptors on the cell surface and a cascade of reactions leading to translocation of the glucose receptor to the cell membrane results in increased

glucose uptake from the blood (66). Persons with glucose intolerance often suffer from decreased insulin sensitivity (66;73;74), which reflects the sensitivity of body tissues to insulin. Generally, insulin sensitivity can be divided into: 'whole-body insulin sensitivity', reflecting the insulin sensitivity in the peripheral tissues and the liver; 'peripheral insulin sensitivity', reflecting the sensitivity of the skeletal muscles to insulin (75); and 'hepatic insulin sensitivity', reflecting the glucose regulation in the liver. The gold standard method to determine whole-body insulin sensitivity is the euglyceamic hyperinsulinemic clamp test (76). This technique, however, is not possible to perform in large-scale clinical studies due to the complex test set-up and high costs. Furthermore, when using the euglyceamic hyperinsulinemic clamp test, only insulin sensitivity is measured, while insulin secretion has to be determined using another test, such as the hyperglyceamic glucose clamp test or an intra-venous glucose tolerance test (77). In epidemiological studies, the oral glucose tolerance test has proven useful to determine insulin sensitivity based on measures and calculated indices derived from different time points during the OGTT (78). 'Insulin resistance' is the inverse of insulin sensitivity, and thus, gives an estimate of the 'whole-body-', 'peripheral-', or the 'hepatic-'resistance towards insulin.

Insulin secretion & Beta cell function

In order to maintain normal glucose homeostasis, an adequate insulin secretion is necessary. Insulin is secreted from the Islets of Langerhans in the pancreas' beta-cells as a response to glucose concentration in the blood (66;79). The relationship between insulin sensitivity and insulin secretion is hyperbolic, with decreases in insulin sensitivity accompanied by increases in insulin secretion from the beta cells in persons with normal glucose homeostasis (80). In persons with impaired glucose tolerance, however, the beta cells are not capable of compensating sufficiently for the decrease in insulin sensitivity (Figure 4)(79). In epidemiological studies, beta cell function can be derived using measures from the OGTT (e.g. by using the disposition index) (77;81).

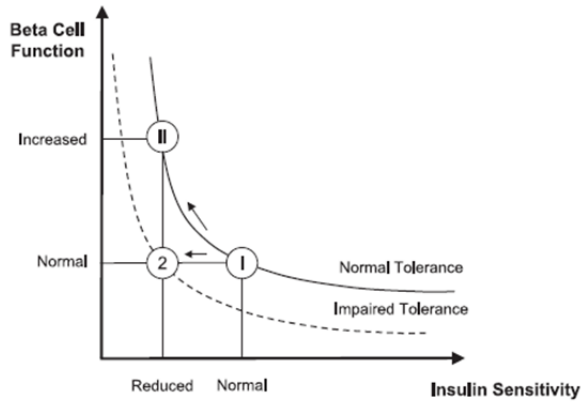

Figure 4 The Hyperbolic relationship between insulin sensitivity and insulin resistance and the importance of expressing beta cell insulin secretion in relation to insulin sensitivity by using the disposition index. A person with normal glucose tolerance (I) responds to decreased insulin sensitivity by increasing insulin secretion (II). In contrast, a person with imparied glucose tolerance does not increase insulin secretion above normal levels when insulin sensitivity is decreased (2). From Corbelli et al (79)

Long-term glycaemia

Glycated hemoglobin A_{1c} (HbA_{1c}), reflects the average glucose level over 2-3 months (82) and can thus be seen as a measure of long-term glycaemia often leading to micro- and macro-vascular complications in diabetes patients (66;83). Furthermore, during the last few years, HbA_{1c} has gained increasing interest as a diagnostic tool for type 2 diabetes due to standardized measurement methods and high feasibility in clinical practice (66;68). In 2011, a new recommendation to diagnose and treat type 2 diabetes based on HbA_{1c} values was published (84).

Physical activity, sedentary behavior, and glucose homeostasis

Physical activity & glucose homeostasis

In numerous studies, lower physical activity levels are found to be adversely associated with metabolic risk factors, including measures of glucose homeostasis (14). Furthermore, glycated hemoglobin A_{1c} (HbA_{1c}) has been suggested to be modifiable by exercise of moderate-to-vigorous intensity (85;86). In cross-sectional and prospective studies, physical activity of light intensity, as well as moderate-to-vigorous intensity, has been related to better glucose homeostasis (4;6;7), whereas other studies have found overall physical activity to be the main determinant of insulin sensitivity (4;5). Duration of the physical activity bouts is thought to be one of the primary factors that influences the response of insulin action to exercise training (87), but also the intensity, as well as the frequency of bouts, seems to influence insulin sensitivity (88;89). In experimental studies, the physiological pathways linking exercise bouts with increased insulin sensitivity and glucose uptake is suggested to include increased capillarisation, oxidative capacity of the mitochondria, and increased glucose transporter 4 protein, GLUT4 (90;91). After contraction of the skeletal muscle, a cascade reaction (Figure 5) leading to translocation of GLUT4 to the cell membrane (92) is started.

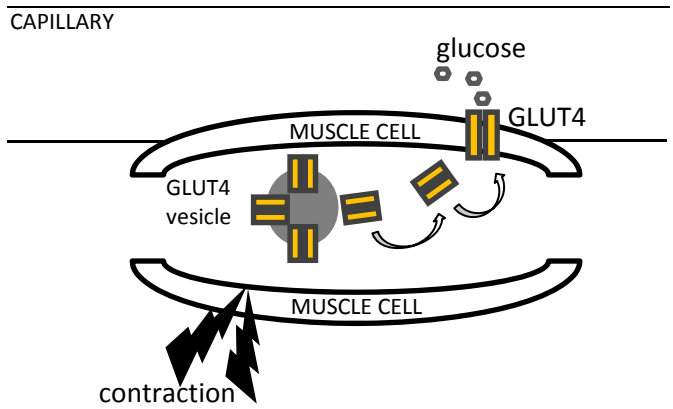

Figure 5. Muscle contractions causes translocation of glucose transporter protein 4 (GLUT4) to the cell membrane and stimulates glucose uptake

The effect of exercise on insulin sensitivity has been found to last up to 48 hours (93), which has been the background for recommending regularly physical activity to improve glucose homeostasis. In

addition to increasing insulin sensitivity, exercise training has been suggested to be associated with an increased insulin secretion in diabetes patients and with a decrease in healthy persons (94).

Since experimental laboratory studies and targeted interventions do not reflect 'real-life' settings, epidemiological studies with precise measures of physical activity and glucose homeostasis are important in order to highlight whether evidence found in laboratory settings is also valid in everyday life.

While most epidemiological studies, investigating the relationship between overall physical activity levels or different physical activity intensities and glucose metabolism, have obtained physical activity measures by self-report methods (6;14;26;58;95-99), recent studies have used activity monitors (4;5;57;59;100;101;101-106). However, only few studies have combined the use of self-report and objective measurements. Additionally, most studies have limited details of glucose homeostasis.

Sedentary behavior and glucose homeostasis

In modern societies, the time spent in sitting activities is increasing (107;108), and an increasing number of studies have focused on the deleterious health consequences of prolonged sitting (109). Even for persons meeting the recommended physical activity level of 30-minutes MVPA per day, sedentary behavior is adversely associated with the metabolic profile (110). Accelerometer-assessed sedentary behavior has been found to be adversely associated with insulin concentration and with hepatic insulin resistance (100;111), and questionnaire-assessed sedentary time with plasma glucose levels two hours after an OGTT (2-hour plasma glucose)(112). Additionally, breaking up sedentary time has been shown to be beneficially associated with 2-hour plasma glucose levels (27). Furthermore, television (TV) viewing time (a widely used marker of sedentary behavior) has been shown to be adversely associated with fasting and 2-hour plasma glucose levels (113), with cardiometabolic risk (114-116) and with death (115;117). Moreover, increases in TV viewing time have been found to be associated with an increased cardiometabolic risk (116). However, it is not known how changes in TV-viewing time are associated with changes in glucose homeostasis markers, independently of physical activity levels.

The underlying mechanisms of the deleterious associations of sedentary behavior with glucose homeostasis markers are to be elucidated (23). However, results from animal studies suggest that decreased contractile stimulation of skeletal muscles while being inactive results in a reduced glucose uptake through reduced translocation of GLUT4 to the cell surface (25). Accordingly, results from bed-rest studies find that bed-rest causes a severe whole-body insulin resistance in offspring of persons with type 2 diabetes, as well as in healthy controls (118). However, it is important to note that bed-rest represents inactivity in the most extreme form, and thus, should not be compared to

sitting activities, such as reading or watching TV, which relates to activities during daily life. In contrast, bed-rest is not considered a normal daily life activity, except among severely diseased persons.

Altogether, increasing evidence suggests higher physical activity levels to be associated with an improved glucose homeostasis. However, no studies have reported results from objectively measured physical activity performed in daily life and the associations with detailed glucose homeostasis markers, including different indices and measures of long-term glycaemia. It has been suggested that different subtypes of dysglycemia show different types of associations with physical activity (119). Therefore, the inclusion of specific indices of peripheral insulin sensitivity, hepatic insulin resistance, beta cell function, and the absolute insulin response to a glucose load, may provide a more detailed picture of the association of physical activity and glucose homeostasis. Furthermore, it still remains unclear how different physical activity patterns are associated with glucose metabolism. Thus, exploring whether subgroups of persons with different physical activity patterns exist and describing group-specific characteristics could provide important knowledge contributing to the design of successful physical activity interventions in diabetes prevention and treatment or environmental and policy actions for promoting physical activity in public health. Lastly, it is yet to be elucidated how changes in sedentary behavior, as measured by TV viewing time, is associated with changes in glucose homeostasis markers, when taking physical activity levels into account.

METHODS

Study population

In the present thesis, physical activity patterns were identified by using information derived from both self-report methods and by combined accelerometry and heart rate monitoring. The associations of different dimensions of physical activity with glucose metabolism were explored in three populations with different degrees of diabetes risk: the Health2008 study, the ADDITION-PRO study, and the AusDiab study.

The Health2008 study

The cross-sectional 'Health2008' study (in Danish: 'Helbred2008') on which *Paper I* is based, was set up to validate physiological measures used in a previous population based study: the 'Health2006' study (120;121). A random sample of 2,218 men and women, aged 30-60 years, living in the Western part of Copenhagen, was extracted from the Danish Civil Registration System and invited for a health

examination. Pregnant women, persons with known cardiovascular disease, diabetes, chronic obstructive pulmonary disease, hypertension or history of blood clots, or persons unable to perform physical activities such as bicycling and climbing stairs, were excluded from participating in the Health2008 study. A total of 795 eligible participants (36% of invited persons) accepted the invitation, underwent an extensive clinical examination, and responded to a standardized, self-administered questionnaire between September 2008 and December 2009 (120). The study was approved by the Ethics Committee of the Copenhagen Region (KA-20060011) and all participants provided written informed consent. Participants were asked to wear a direct physical activity monitor (ActiHeart®) for 7 days. In total, 463 participants (58% of attending participants) agreed to wear the monitor. For the analysis in *Paper I*, only participants with a minimum ActiHeart wear-time of 24 hours throughout the measurement period, with valid data from the physical activity questionnaire, and who had been fasting prior to examination were included (n=360).

The ADDITION-PRO study

The ADDITION-PRO study population (*Paper II)* was recruited from a stepwise screening program performed in the Danish arm of the '**A**nglo-**D**anish-**D**utch study of **I**ntensive **T**reatment **I**n pe**O**ple with scree**N** detected diabetes in primary care', aiming to identify individuals with type 2 diabetes (122). A detailed flowchart of the ADDITION Denmark screening procedure is included in Appendix I, Figure A1. At the beginning of the screening process, which took place in 2001 to 2006, 163,189 Danish men and women, aged 40-69 years, were mailed a risk score questionnaire (a modified version of the Danish diabetes risk questionnaire (123)), or completed the questionnaire while visiting their general practitioner. They were asked to indicate known risk factors: age, sex, BMI, known hypertension, family history of type 2 diabetes, gestational diabetes, and leisure time physical activity. Each answer was assigned a value (Appendix I, Figure A1). Persons with more than five points were considered at elevated risk of developing diabetes and were invited to continue in the stepwise program, which included random blood glucose and HbA1c testing, a fasting blood glucose test, and an OGTT.

Persons with different elevated diabetes risk profiles, but without diabetes, were invited to participate in a follow-up health examination (Appendix I, Figure A2) (see appendix IV for a full description of the ADDITION-PRO study) (124). Individuals who were eligible were: those still alive; those living in the regions of the four Danish research centres (Holstebro Hospital, Aarhus University Hospital, Hospital of South West Jutland, Esbjerg, and Steno Diabetes Center A/S, Gentofte); and those who had not withdrawn consent to study participation. In total 4,188 persons with different diabetes risk profiles (see Appendix I, Figure A2) were invited to participate in the ADDITION-PRO study. Of these, 2,082 completed the health examinations for ADDITION-PRO, which took place from

2009 to 2011 at the four research centers. The study was approved by the ethical committee of the Central Denmark Region (journal no. 20080229) and was conducted in accordance with the 1996 Helsinki Declaration. All participants provided written informed consent. For the analysis in *Paper II* persons with incident diabetes since screening (n=329) or persons who had not been fasting (n=11) prior to the health examinations were excluded. Of the 1,753 remaining participants, only participants with valid data in the outcome variables (markers of glucose homeostasis and long-term glycemia) were included (n=1,531, 87 % of ADDITION-PRO participants)(Figure 6).

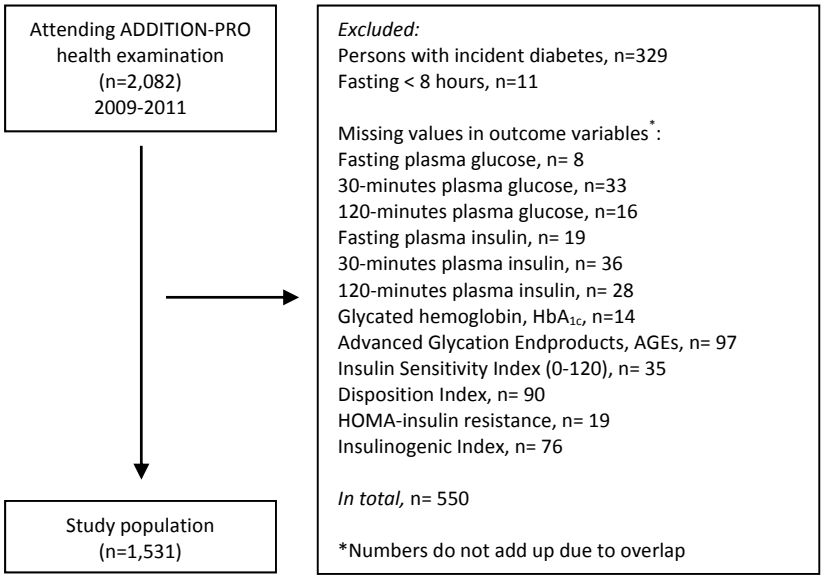

Figure 6. Flow chart of the ADDITION-PRO study population included in *Paper II*

The AusDiab study

The Australian Diabetes, Obesity and Lifestyle (AusDiab) study was carried out with the overall aim of determining the national prevalence of diabetes and other non-communicable diseases and their risk factors (125). The first phase of the study (baseline) took part from 1999 to 2000 and the second phase (follow-up) in 2004 to 2005 (125;126). The study consisted of a household interview and a biomedical examination. Eligible participants for the baseline examinations included all non-institutionalized adults ≥25 years of age, residing in each of the six states and the Northern Territory of Australia (a minimum of 6 months of dwelling at the address was required) (125). Based on a stratified cluster sampling method, with clusters based on 'census collector districts' (CDs) defined from the Australian Bureau of Statistics, 42 CDs were randomly selected to be enrolled in the study. In total, 20,347 persons were eligible (Appendix I, figure A3) and of these, 11,247 completed the household interview and the biomedical examination (55.3% of eligible participants). In 2004/2005, all surviving eligible participants were invited to the AusDiab five-year follow-up study. Non-eligible participants included: persons refusing further contact (n=128), deceased (n=310), persons who had

moved overseas or into a nursing home, or who had a terminal illness (n=21). In total, 10,788 persons were eligible to participate in the AusDiab follow-up study. Of these, 6,538 persons (60.6% of eligible) underwent the biomedical examination. These 6,538 participants form the eligible study population for the analysis in *Paper III*. Participants who had not fasted for ≥8 hours prior to the examination, those who were pregnant, those who had known diabetes, or who had missing data in the outcome variable of the analysis were excluded (Figure 7). In total, the study sample comprised 4,843 participants (73.9% of eligible study population).

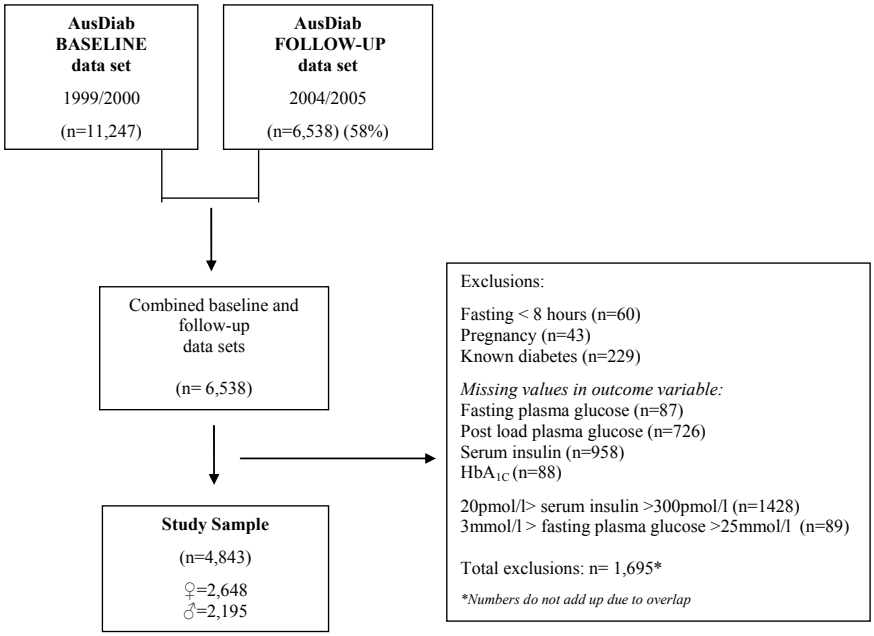

Figure 7. Flowchart of the study population in *Paper III* (the AusDiab study)

Physical activity measurements

Participants of the Health2008 (*Paper I*) study and the ADDITION-PRO study (*Paper II*) had their physical activity levels measured by the combined accelerometer and heart rate monitor (ActiHeart®, CamNTech Ltd., Cambridge, United Kingdom) (22). In addition, self-report physical activity questionnaires were used to obtain information on the type and context of the physical activity. Participants of the Health2008 study answered the 'Physical actvitiy scale II' (PAS2) (127;128), whereas participants of the ADDITION-PRO study answered the 'Recent Physical Activity Questionnaire (RPAQ)' (129). Participants of the ADDITION-PRO and Health2008 studies also answered questions on their typical leisure time category using the questions developed by Saltin and Grimby (130), and participants of the Health2008 study had their fitness level measured by a 'watt-max' test (34). In the AusDiab study, information on weekly TV viewing time was obtained using an interviewer-administered questionnaire and information on physical activity was obtained using the 'Active Australia' questionnaire (131).

Estimating fitness level (maximal oxygen consumption, VO_2max)

Participants of the Health2008 study (*Paper I*) had their fitness level, expressed as maximal oxygen consumption, measured using an indirect maximal cycle ergometer test based on work load (watt-max test)(120). Participants of the Health2008 study performed the watt-max test by cycling on an electronic 'Monark 839E Ergomedic'. The test-procedure was performed according to a standardized protocol (34) with a 5-minute warm-up period (50 Watts), a start load of 70 Watts followed by increments of 35 Watts every second minute. Measures of maximal oxygen consumption, VO_2max (mlo_2/kg/min), were derived using the manufacturer's software.

Combined heart rate and accelerometry sensing

The ActiHeart activity monitor was placed horizontally on the participants' chest on two standard electrocardiogram electrodes, one at the lower part of the sternum and the other one on the same horizontal level, on the left side, as laterally as possible. The monitor was set to store heart rate and movement activity every 60 seconds (60 second 'epochs'). Participants from the Health2008 study (*Paper I*) and the ADDITION-PRO study (*Paper II*) were asked to wear the monitor for seven days and to maintain their usual physical activity pattern during the period.

Individual calibration of heart rate to physical activity intensity

Due to large variations in heart rate from person to person, the heart rate measured with the ActiHeart monitor should be calibrated. In the ADDITION-PRO study (*Paper II*), this was done by performing a sub-maximal step test where heart rate was measured for a known activity intensity (stepping up and down a step bench at a given speed). The sub-maximal step test was performed on the day of the health examination. The eight-minute step test was administered from the ActiHeart software to indicate the cycles of stepping up and down a 20.5 cm step bench (Rucanor Europe B.V., Nieuwerkerk, The Netherlands). The stepping frequency ranged from 15 to 33 step cycles per minute over the duration of the test, followed by a two-minute recovery period (sitting). Exclusion criteria's for the step test were: persons who had ever been told by their doctor that they had angina pectoris; blood clots in heart or brain; or those who had ever had a stent or by-pass operation. Additionally, persons who were disabled were excluded from the step test. After participants had completed the step test and data were downloaded, the monitor was placed on the ECG electrodes for long-term recording. The participants of the Health2008 study did not perform an individual calibration. Thus, the derived heart rate to physical activity intensity relationship was established using a previously calculated group calibration derived from the 'INTERACT' study (132) (Appendix II, Table A1).

Deriving group calibration from individual calibration levels

In the study population for *Paper II* (ADDITION-PRO), the heart rate to physical activity intensity relationship, for those participants who did not perform the step test, was derived based on a group calibration. The group calibration was based on the regression estimates from the heart rate to physical activity intensity relationship from all the ADDITION-PRO participants (including participants excluded for the analysis in *Paper II*) with a valid individual calibration (n=1,046). For the analysis in *Paper II*, a total of 733 participants had a valid individual calibration, whereas for 451 participants the group calibration was applied. The equations for the individual and group calibrations are shown in Appendix II, Table A1.

Processing of data from the ActiHeart Monitor

Records from the activity monitor were downloaded and pre-processed using the manufacturer's software (ActiHeart Software version 4.0.70) (www.camntech.com). Data from the monitor consists of raw files with information on movement (accelerometer counts) and heart rate. These are converted to estimates of PAEE. However, prior to that, extensive file- and data processing had to be carried out in order to deal with noise, non-wear time and un-physiological heart rate measures. First of all, files were checked for presence and corrupted data. Secondly, periods in which the monitor was worn or not worn were checked and the files were trimmed according to start and end time (according to logs)(see flowchart of the procedure in Appendix 2, figure A6)(133). This procedure was done using the manufacturer's software and according to standard operating procedures. After processing of files, they were converted to comma-separated 'csv'-files for further data processing using the Java 'Physical Activity Data Viewer' program (133). While importing files into the program, they were double-checked for quality and any corrupted files were 'flagged' to avoid including completely corrupted heart rate or activity data into the analysis (since noise reduction is only possible to a certain limit). With the Java 'Physical Activity Data Viewer' program, noisy heart rate measures were reduced and periods of non-wear were inferred from the combination of non-physiological heart rate and prolonged periods of inactivity (to minimize diurnal information bias when summarizing the intensity time-series into PAEE measures), using the procedure published by Stegle et al (134). After the data processing, data were exported as csv-files to STATA version 11 (135) for calculation of the physical activity estimates.

Estimating energy expenditure based on heart rate and accelerometry data

For all time points, two estimates of physical activity intensity (PAI) are calculated. One estimate is based on accelerometry data (PAI_{ACC}) and one on heart rate data (PAI_{HR}). The PAI_{ACC} is calculated using a segmented linear accelerometry equation (Appendix II, Table A2) (136). The PAI_{HR} is based on the individual or group calibrations of heart rate to physical activity intensity (Appendix II, Table A1).

These physical activity intensities are then combined using the 'branched equation model' (39)(Appendix II, Figure A5), providing minute-by-minute measurements of (among others): sleeping heart rate, SHR, in beats per minute (bpm); heart rate above sleep, HRaS (bpm); and physical activity energy expenditure, PAEE (J/kg/min). Measures of PAEE were summarized to hourly (J/kg/min) and daily measures (kJ/kg/day). From these, fraction of time (per hour) spent in physical activity intensity groups, expressed as multiples of predicted resting metabolic rate (METs), were derived. Estimated basal metabolic rate was derived using the 2005 Oxford Model (21). The fraction of time spent in MET-intensities was summarized to daily measures. The branched equation modeling was performed in STATA version 11 (135).

Physical activity questionnaires

Recent Physical Activity Questionnaire (RPAQ)

Participants of the ADDITIO-PRO study (*Paper II*) completed a modified Danish version of the Recent Physical Activity Questionnaire (RPAQ) (129), asking about type, frequency, intensity, and context of physical activity performed in the last four weeks prior to the health examination. The RPAQ was completed on the health examination day and help was provided from the staff if participants had any questions or difficulties in filling out the questionnaire. The questionnaires were checked for completeness before the participants finished their health examination visit. From the RPAQ, time (hours per week) spent in different activities was computed.

Physical Activity Scale II (PAS2)

Participants of the Health2008 study (*Paper I*) completed a physical activity questionnaire: the 'Physical Activity Scale II' (PAS2), which inquires about 'usual' time spent in various daily and weekly physical activities, including occupational and leisure time sitting, active commuting and moderate-to-vigorous physical activity (127;128). The PAS2 was completed prior to or at the health examination day. From the self-report PAS2, time spent in different PA domains (hours per day) and time spent in moderate-to-vigorous PA (minutes per week) were computed.

TV viewing time

As part of an interviewer-administered questionnaire, AusDiab participants (*Paper III*) reported time (hours and minutes) spent watching TV or DVDs, or playing games on the TV, for workdays and non-workdays (separately), during the last week.

The question was, *'Please estimate the total time during the last week that you spent sitting for watching TV or DVDs or playing games on TV. This is when it was the main activity that you were doing'.*

Total TV viewing time (hours/week) was calculated as the sum of weekday TV viewing time and non-weekday TV viewing time. This measure has been shown to have reasonable reliability and validity for estimating TV viewing time in adults (137). Measures of leisure time moderate-to-vigorous physical activity were obtained using the 'Active Australia' questionnaire (131), which has previously been found to provide reliable and valid estimates of leisure time physical activity among adults (138).

Glucose homeostasis markers and derived indices

Venous blood samples were collected after an overnight fast (≥8 hours of fasting) in all three studies (*Paper I-III*) by trained staff and according to standard operating procedures. All participants then underwent a standardized oral glucose tolerance test, OGTT (67). After intake of the glucose drink (75g glucose dissolved in 2.5dl water), venous blood samples were drawn at following time points: 30 minutes (*Paper I-II*), and 120 minutes (*Paper I-III*).

Plasma glucose and plasma/serum insulin levels

In *Paper I* and *Paper II*, plasma glucose and plasma insulin levels were determined for all three time points (fasting and 30, 120 minutes after the OGTT). For the analyses in *Paper I*, however, only the fasting and 2-hour measures were utilized, since the focus of this paper was on deriving physical activity patterns, and hence, only the more crude glucose indices were applied. In *Paper III*, fasting- and 120-minutes plasma glucose, and fasting serum insulin, was determined at both baseline and follow-up examination. For a detailed description of the assessment methods, see *Paper I-III*.

Insulin sensitivity and Insulin resistance

Homeostasis model assessment (HOMA and HOMA2)

HOMA implies deriving measures of insulin resistance and beta cell function from fasting plasma measurements of glucose and insulin by utilizing a mathematical algorithm (139). In *Paper I-III*, measures of insulin resistance (IR) were derived from homeostasis model assessment. In *Paper II*, the standard equation of HOMA-IR was applied: HOMA-IR (mmol/l x mU/L) = fasting plasma glucose (mmol/l) x (fasting plasma insulin (pmol/l)/6.945)/22.5 (27), whereas in *Paper I* and *Paper III*, this was performed using the HOMA-calculator version 2 (http://www.dtu.ox.ac.uk/homacalculator/index.php)(140).

Since, HOMA-insulin resistance or HOMA-insulin sensitivity is based on fasting values, these indices mainly represent the hepatic contribution to insulin sensitivity. In contrast, measures of the peripheral insulin sensitivity can be obtained by deriving the 'Insulin Sensitivity Index$_{(0, 120)}$.

Insulin sensitivity index$_{(0, 120)}$ (Paper II)

As the name implies (0, 120), this index is based on fasting and 120-minutes values of glucose and insulin. The insulin sensitivity index ($ISI_{0,120}$) was calculated according to Gutt et al. (141) to give an estimate of insulin sensitivity in the peripheral tissues. In brief, insulin sensitivity is expressed as the ratio between glucose uptake rate and the logarithmic transformation of serum insulin concentration.

$$ISI_{0,120} = \frac{(75000+(\text{glucose}_{\text{t0min}}\times 18-\text{glucose}_{\text{t120min}}\times 18)\times 0.19\times \text{weight}_{\text{kg}})/120}{(\text{glucose}_{\text{t0min}}+\text{glucose}_{\text{t120min}})/2} / \log\left(\frac{(\text{insulin}_{\text{t0min}}/6.945)+(\text{insulin}_{\text{t120min}}/6.945)}{2}\right)$$

Beta cell function

Beta cell function was, in *Paper I* and *Paper III,* determined by using the HOMA2 beta cell function as determined by the HOMA2-calculator. In *Paper II*, beta cell function was determined by calculating the disposition index (29), which reflects the ability of the beta cells to compensate for decreased insulin action. To do this, first phase insulin release was calculated as described by Stumvoll et al (first phase insulin release$_{Stumvoll}$ = 1283 + 1.829*insulin$_{t30min}$ - 138.7*glucose$_{t30min}$ +3.772*insulin$_{t0min}$) (30). Disposition index (DI) was then calculated using the following formula: DI = first phase insulin release$_{Stumvoll}$ x $ISI_{0,120}$. Furthermore, in *Paper II*, the absolute insulin response to the glucose load was determined by calculating the insulinogenic index$_{t30min}$ by using the formula: (Insulin$_{t30min}$- Insulin$_{t0min}$)/ (Glucose$_{t30min}$- Glucose$_{t0min}$) (31).

Long-term glycaemia

In all three populations (*Paper I-III*), measures of long-term glycaemia were determined by measuring the proportion of glycated hemoglobin A_{1c} (HbA_{1c}) in the blood. Additionally, in *Paper II*, another measure of the even longer term load of protein glycation, the skin accumulation of 'Advanced Glycation Endproducts' (AGE_{skin}), was assessed using skin auto-fluorescence (142).

Statistical methods

Investigating physical activity patterns using latent class analysis (Paper I)

In order to examine if subgroups with different physical activity patterns existed in a demographically homogenous Danish population, a latent class analyses approach was applied. In combination with objectively measured PAEE and self-reported active transportation and sitting time, self-reported MVPA was included in the analysis to obtain information on participants' perceived physical activity level, as compared to the current Danish physical activity recommendations (2).

Latent class analysis identifies subgroups in empirical data based on patterns of observed categorical variables (*indicators*)(143). Five binary indicator variables were defined according to the threshold values given in Figure 8. These 5 variables were used as input for a latent class analysis.

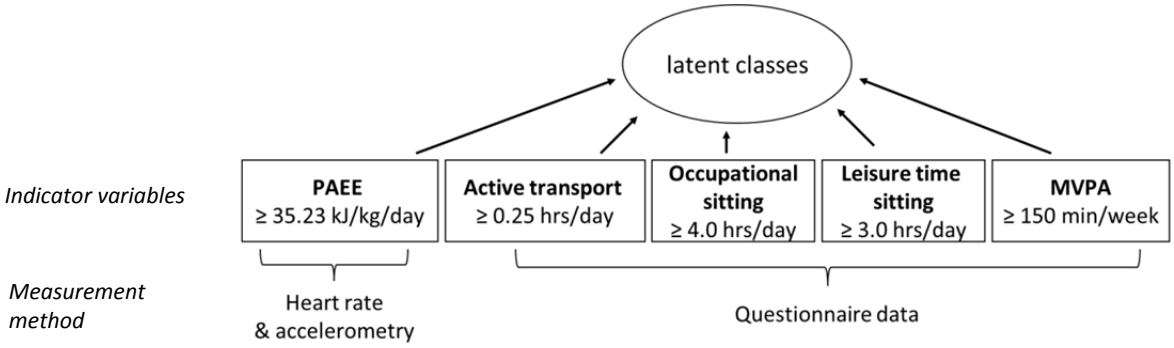

Figure 8. Latent class model used to classify the study population in groups based on 5 binary indicator variables (*Paper I*). PAEE = physical activity energy expenditure; MVPA = moderate-to-vigorous physical activity

The 32 groups (2^5 potential subgroups in the data) defined from the 5 binary variables were reduced to fewer groups, where a group was characterized by 5 posterior probabilities of a positive score for each binary variable. These posterior probabilities were reported as indicators of the group characteristics. Some persons will fit perfectly into one of the derived classes, whereas other persons will be just in the middle of two classes – that is, if a person's physical activity pattern, to some extent, fits into more classes. Therefore, each person was assigned a posterior probability of belonging to each of the classes. Subsequently, each person was assigned to the class in which they had the largest posterior probability. Since the number of classes must be chosen a priori for a particular analysis, models were fitted with 1, 2, 3, and 4 classes, comparing the fit of the models using the Akaike Information Criterion (144). The model with two classes was found to be most appropriate. The model was fitted using PROC LCA (145) in SAS version 9.2 (SAS Institute, Cary, NC).

Multiple imputation of missing data (Paper II/III)

Missing data on all determinants for the participants included in *Paper II*, and missing data on baseline and follow-up determinants for all participants included in *Paper III* were imputed using the Multivariate Imputation by Chained Equations (MICE) (146) method in R software (147) with missing-at-random assumptions. Fifty copies of the data, each with missing values suitably imputed, were independently assessed in the linear regression analysis. Fifty imputations were chosen to be sufficient to obtain valid inference (148). Estimates of parameters of interest were averaged across the copies to give a single mean estimate. Standard errors and p-values were adjusted according to Rubin's rules (149). Parameter estimates and 95% confidence intervals from the analyses using imputed data are presented as main results.

Linear regression analysis (Paper II/III)

In the analysis for *Paper II*, the association of daily PAEE (kJ/kg/day) (exposure)with the different glucose homeostasis markers and long-term glycaemia (outcomes) were examined using multiple linear regression analyses. Analyses were adjusted for baseline diabetes risk group based on the results of the ADDITION-Denmark stepwise screening procedure performed in 2001 to 2006, to control for differences in the invitation procedure and as an indicator of participants' clinical history (see Appendix I, figure A1 for the different diabetes risk groups). Further adjustments included age, sex, employment status, smoking, and alcohol consumption. Furthermore, due to the potential mediating role of obesity on the link between PA and glucose homeostasis (104), waist circumference were included in the full model in a separate level.

In *Paper III*, multiple linear regression analyses with five-year change in TV viewing time (exposure) and five-year change in glucose homeostasis markers (outcome) were applied. Covariates included in the regression models were: Model A: baseline age, baseline TV viewing time, baseline glucose homeostasis markers under study; Model B additionally adjusted for baseline and five-year change in educational attainment, employment status, income level, smoking status, alcohol consumption, diet quality, energy intake, and parental history of diabetes at follow-up; Model C: Model B covariates and adjustments for baseline and five-year change in time spent in MVPA; Model D: Model C covariates and baseline and five-year change in waist circumference. These four levels of adjustments were chosen to identify the crude associations (Model A) and to isolate the effect of adding physical activity to model B (Model C), and abdominal obesity to model C (Model D). Analyses were stratified by gender given previous AusDiab study findings showing differences between men and women in associations of TV viewing time with biomarkers (113;150).

RESULTS

Physical activity patterns

In a generally healthy Danish adult population (*Paper I*), with a median physical activity energy expenditure of 35 kJ/kg/day or approximately 2,748kJ/day for a person weighing 78.5 kg (median weight of the study population in *Paper II*, for comparison purposes), latent class analysis revealed two latent classes with different physical activity patterns: 'Inactive occupational sitters' (n=49, [14%]) were characterized by the highest probability of low PAEE and high level of occupational sitting; 'Overall active exercisers' (n=311, [86%]) were characterized by a high probability of high PAEE, more than four hours of occupational sitting per day, and more than 150 self-reported minutes

per week of moderate-to-vigorous physical activity. The probability of active transportation and leisure time sitting was not different between the two groups (Figure 9).

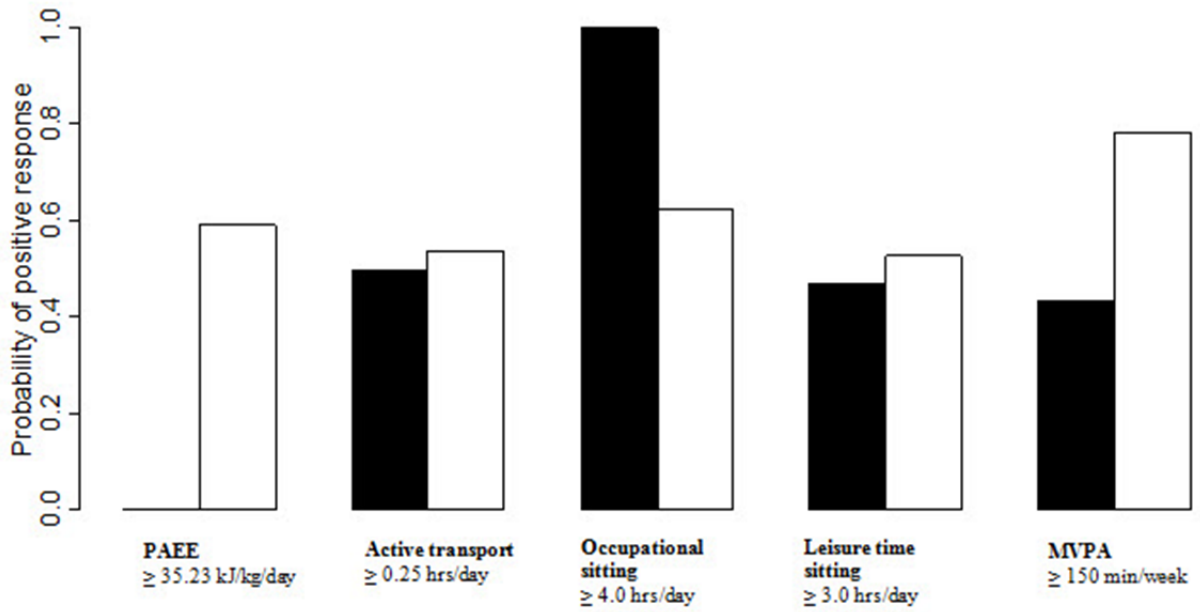

Figure 9. Class-specific physical activity patterns. Bars indicate item-response probabilities for the different classes: Black ='inactive occupational sitters'; White='overall active exercisers'. PAEE = physical activity energy expenditure. MVPA =Moderate-to-vigorous physical activity.

When plotting the PAEE (J/kg/min) as a function of time of day, for each class, the same diurnal shape of PAEE emerged, albeit the specific levels of PAEE were different (Figure 10).

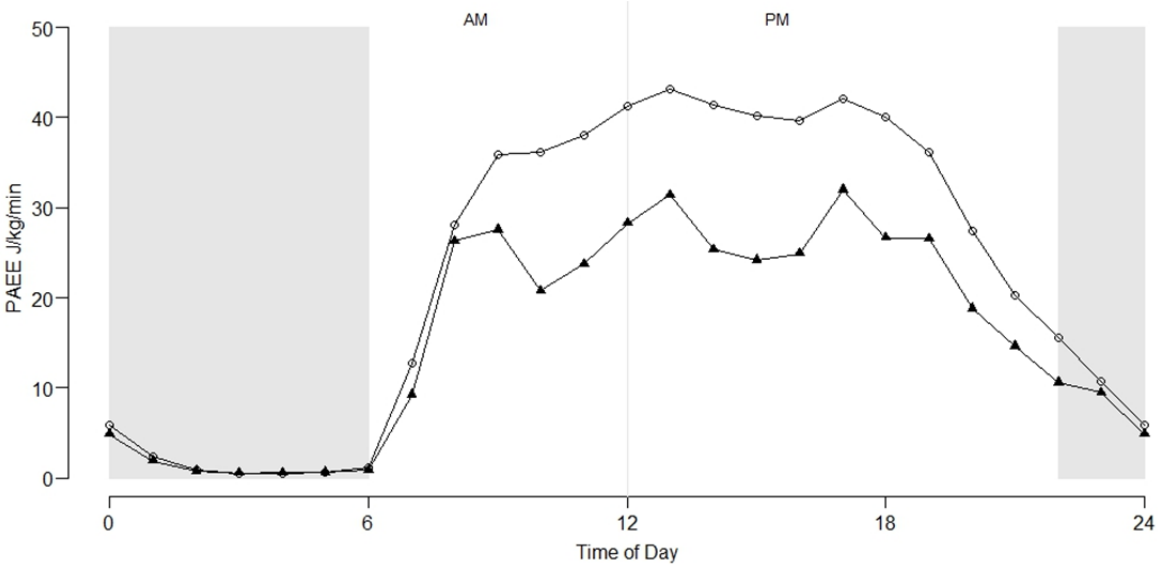

Figure 10. Class-specific patterns of median daily physical activity energy expenditure (PAEE J/kg/min) per time of day. Symbols represent classes: black triangles = 'inactive occupational sitters'; white circles= 'overall active exercisers'

In an elderly population at low-to-high risk of developing diabetes (*Paper II*), median PAEE was 33 (23.5; 46.1) kJ/kg/day, amounting to 2591kJ/day for a person weighing 78.5 kg (median weight of the study population). Figure 11 illustrates the fraction of time spent with different physical activity intensities (per 24 hours) and Table 1 shows the top ten most frequent leisure time physical activities in the ADDITION-PRO study population (*Paper II*)

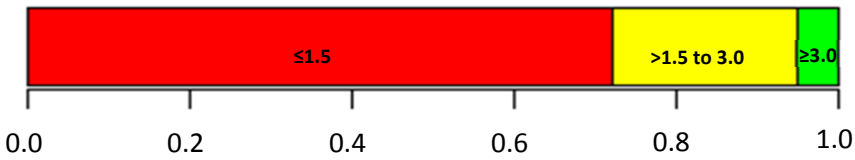

Figure 11. Fraction of time (per 24 hour) spent in different MET-intensities in the ADDITION-PRO study (*Paper II*).

***Table 1**. Top 10 self-report typical leisure time activity in the ADDITION-PRO study population (Paper II) (% reporting at least once the last 4 weeks)**

Leisurely walking	89.5
Do-it-yourself	66.4
Gardening	66.3
Leisurely cycling	46.0
Floor exercises	36.9
Aerobic exercise	22.6
Playing musical instruments/singing	13.2
Leisurely swimming	13.1
Jogging	11.1
Weight lifting	7.0

*of participants with available data (n=1,481)

Physical activity and glucose homeostasis

The two sub-groups defined in *Paper I* had differences in glucose homeostasis markers – compared to 'inactive occupational sitters', 'overall active exercisers' had significantly lower mean [SD] levels of fasting plasma insulin (32.1 [20.8] vs. 40.1 [26.6] pmol/l, *P*<0.017) and median [Q1; Q3] insulin resistance (0.6 [0.4; 0.9] vs. 0.7 [0.5; 1.1], P<0.030). In the population at low-to-high risk of developing diabetes (*Paper II*), when adjusting for age, sex, diabetes risk group, occupation, alcohol intake, and smoking status, PAEE was positively associated with insulin sensitivity index$_{0,120}$ (% increment per 10 kJ/kg/day increment in PAEE: 1.6 [95% CI: 0.6; 2.6]). It was negatively associated with: 2-hour plasma glucose (difference per 10 kJ/kg/day increment in PAEE: -0.01 [95% CI: -0.02; 0.01] mmol/); plasma insulin (% decrement per 10 kJ/kg/day increment in PAEE [95% CI]): fasting plasma insulin (2.1 [0.7; 3.4]); 30-minute plasma insulin (1.9 [0.7; 3.2]); 120-minute plasma insulin (3.4[1.5; 5.5]); and with HOMA-insulin resistance (2.2 [0.5; 3.9] % decrement per 10 kJ/kg/day increment in PAEE). After additionally including waist circumference in the model, these associations remained significant for 120-minute plasma insulin and insulin sensitivity index$_{0,120}$ only (standardized estimates in Figure 12). Per every 10kJ/kg/day increment in PAEE, a 2.5 (95%CI: 0.8; 4.1) percent

decrement in 120-minute plasma insulin was found (*P*<0.05), and a 1.0 (0.2; 1.9) percent increment in insulin sensitivity index (*P*<0.05).

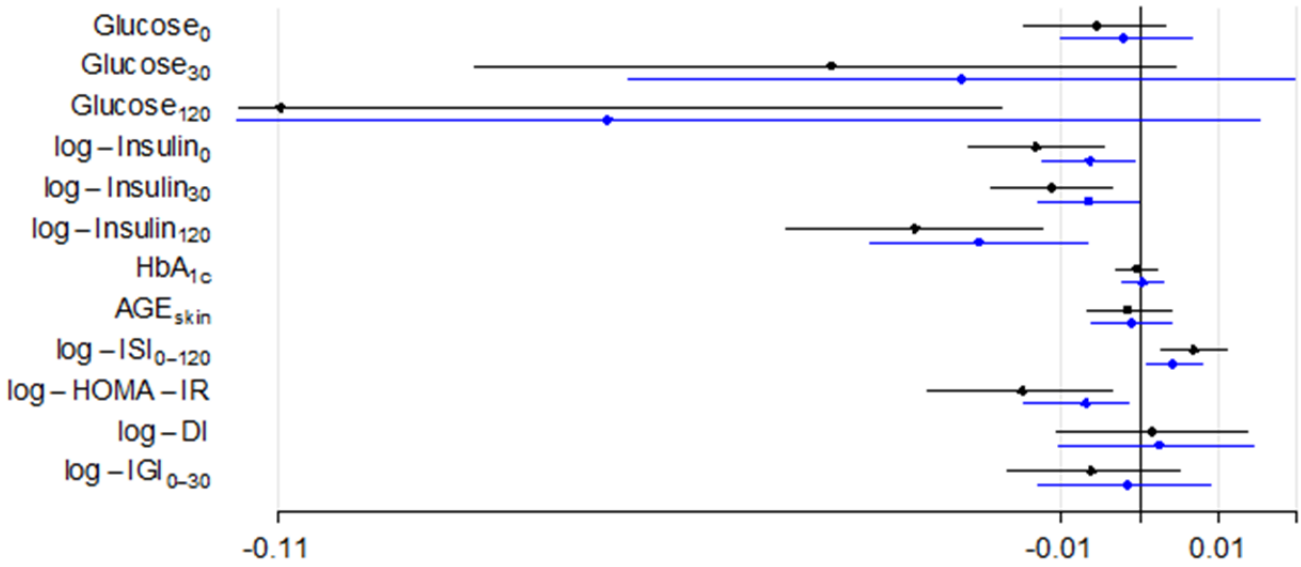

Figure 12. Effect of 10 kJ/kg/day increment in physical activity energy expenditure on the population SD difference in glucose homeostasis markers (standardized estimates and 95% CI).
Black, Model 2: adjusted for age, sex, diabetes risk group at baseline screening, occupation, alcohol intake, smoking. Blue, Model 3: Model 2 plus waist circumference. Due to large variations in 120-minute glucose levels (high population SD) the lower end of the x-axis is cut right after the standardized estimate for 120-minute glucose. $Glucose_0$= fasting plasma glucose; $Glucose_{30}$=30-minute plasma glucose; $Glucose_{120}$= 120-minute plasma glucose; $Insulin_0$= fasting plasma insulin; $Insulin_{30}$= 30-minute plasma insulin; $Insulin_{120}$= 120-minute plasma insulin; HbA_{1c}= Glycated hemoglobin; AGE_{skin}= advanced glycation endproducts (skin autoflourescence); $ISI_{0\text{-}120}$: Insulin sensitivity index (0-120); HOMA-IR= homeostasis model assessed insulin resistance; DI= disposition index; $IGI_{0\text{-}30}$= Insulinogenic index (0-30).

Figure 13 illustrates the effects shown in Figure 12 in an example situation of a 66 year-old man at high risk of developing diabetes, but with normal glucose tolerance (as determined by an OGTT). The modeled values indicate a more rapid glucose uptake with higher PAEE level (Figure 13.a), while the log-insulin response to the glucose load is lower the higher PAEE levels (Figure 13.b).

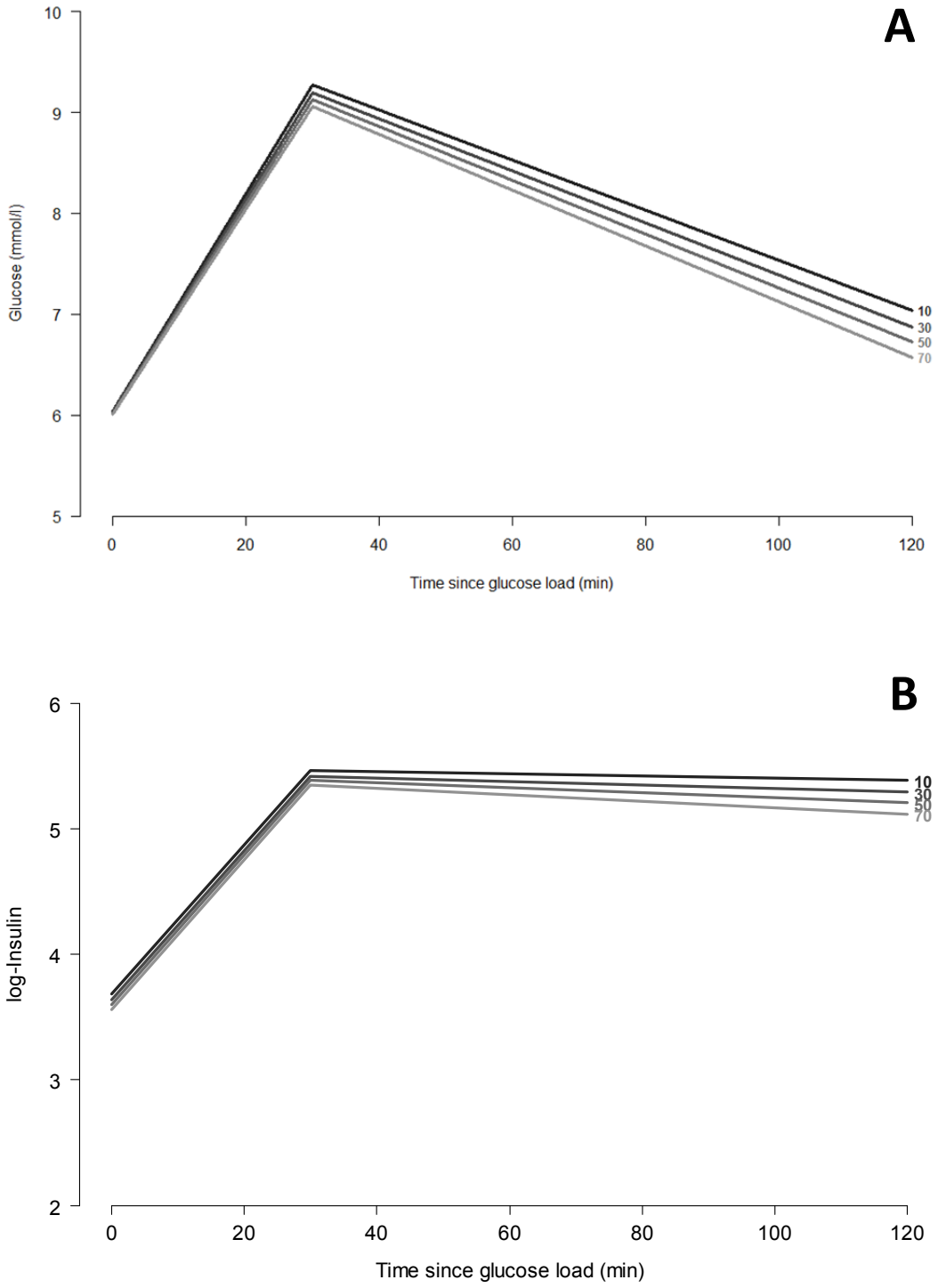

Figure 13. Glucose concentration (mmol/l) (A) and log-insulin concentration (B) per time since glucose load for a 66 year-old man, with baseline high diabetes risk but normal glucose tolerance, by different physical activity levels (10-70 kJ/kg/day)(black=10 kJ/kg/day, light grey=70kJ/kg/day).

TV viewing time and glucose homeostasis

Average five-year changes in TV viewing time, leisure-time moderate-to-vigorous physical activity, and glucose homeostasis markers in a representative sample of Australian adults (*Paper III*) is shown in Table 2.

Table 2. *Change in TV viewing time, leisure time MVPA and glucose homeostasis markers from baseline (1999/2000) to follow-up (2004/2005) in the AusDiab study (Paper III)*

	Men	Women
TV viewing (hours per week)	0.9 (±8.8)	1.2 (±9.3)
MVPA (minutes per week)	-8.0 (±378.4)	28.8 (±323.4)
Fasting plasma glucose (mmol/l)	-0.1 (±0.6)	-0.0 (±0.5)
2-hour plasma glucose (mmol/l)	0.1 (±2.0)	-0.2 (±1.8)
Fasting serum insulin (pmol/l)	-10.8 (±36.3)	-10.6 (±33.7)
HOMA2-Insulin resistance	-0.2 (±0.8)	-0.2 (±0.7)
HOMA2-Beta cell function (%)	-8.8 (±33.7)	-11.2 (±33.8)
Glycated hemoglobin (mmol/mol)	4 (±3)	4 (±3)

Mean (SD)
MVPA= moderate-to-vigorous physical activity; HOMA2=homeostasis model assessment (version2)

For every five-hour increase in TV viewing from baseline to follow-up, there was an increase in the five-year change in 2-hour plasma glucose (ß-estimates and 95% CI: 0.063 [0.011; 0.114] mmol/l) and insulin levels (1.195 [0.302; 2.088] pmol/l), HOMA2-insulin resistance (0.024 [0.001; 0.047]), and HOMA2-beta cell function (1.109 [0.038; 2.179] %) in men. In women, for every five hour per week increase in TV viewing time from baseline to follow-up, there was an increase in the five-year change in fasting plasma glucose (0.012 [0.001; 0.024] mmol/l), HOMA2-insulin resistance (0.027 [0.005; 0.049]), HOMA2-beta cell function (1.073 [0.023; 2.122] %), and fasting serum insulin (1.060 [0.320; 1.800] pmol/l). These associations remained statistically significant in models adjusted for baseline age, baseline TV viewing, baseline glycemic marker under study, and baseline and change in education levels, employment status, income, smoking status, alcohol consumption, diet quality, energy intake, MVPA, and parental history of diabetes at follow-up (Model C in table 2, *Paper III*). However, after additionally including baseline and change in waist circumference in the models (Model D in table 2, *Paper III*), only the association with fasting serum insulin (0.712 [0.027; 1.398] pmol/l) in women, and 2-hour plasma glucose (0.052 [0.000; 0.103] mmol/l) in men remained significant.

DISCUSSION

This thesis aims to identify patterns of physical activity in a Danish population, and to quantify and delineate the associations of objectively measured physical activity energy expenditure and a measure of sedentary behavior (TV viewing time) with different glucose homeostasis markers in persons at low-to-high risk of developing diabetes and in a general population. Two subgroups with

different physical activity patterns were found, using 'latent class analysis', in a generally healthy Danish sample (*Paper I*): 'overall active exercisers' and 'inactive occupational sitters'. 'Overall active exercisers' had significantly lower levels of fasting insulin and insulin resistance than 'inactive occupational sitters', suggesting an unhealthier glucose metabolic profile when being less physically active and having higher levels of prolonged sitting. In an adult population at low-to-high diabetes risk (*Paper II*), objectively assessed physical activity levels were generally of light intensity, but nonetheless positively associated with insulin sensitivity and negatively associated with insulin concentration two hours after glucose load, indicating that even small increments in physical activity levels are associated with a better glucose metabolic profile. Increased TV viewing time over five years was adversely associated with five-year changes in glucose homeostasis markers in a representative sample of Australian adults (*Paper III*), suggesting that the initial pathophysiological changes that contribute to the development of type 2 diabetes may be adversely associated with sedentarism.

This thesis elucidates on how the different dimensions of the 'movement continuum' acts to bring about the beneficial effects on glucose metabolism. As such, this thesis clarifies some aspects of the health consequences of a physical activity pattern like the case study of 'Paul' (presented in the introduction), characterized by prolonged sitting at work and during leisure time, albeit being moderately-to-vigorously active a minimum of 45 minutes per day.

Methodological considerations

Measuring physical activity by combined heart rate and accelerometry

In *Paper I* and *Paper II*, PAEE was measured using the combined heart rate and activity monitor, the ActiHeart. The overall advantage of this monitor includes the possibility to combine PAEE derived from measures of heart rate with PAEE derived from accelerometry measures. In several studies, the combination of measures from heart rate and accelerometry has shown valid and more precise measures of PAEE in comparison to measures obtained by heart rate and accelerometry alone (39;136;151;152). The validity of the ActiHeart monitor has been tested in several studies (151-153). In general, there is good agreement when comparing with calorimetry (R^2=0.78) (39), and acceptable agreement with the doubly labeled water method when using a group calibration (mean bias: -7.6 [SD: 20.2] kJ/kg/day) and with significantly improved agreement when using individual calibration (mean bias: -4.6 [SD: 13.1] kJ/kg/day) (151).

Calibration of heart rate to physical activity intensity

As with any heart rate monitor used for estimating PAEE, the heart rate for each level of physical activity intensity must be calibrated in order to take individual variation into account. Including individual calibration of the heart rate to physical activity relationship in the calculation of the PAEE considerably improves the validity of the estimates (136;151). Group calibration has shown only modest improvement in PAEE validity (151;154). However, using a combined heart rate and movement monitor with group calibration still provides more accurate PAEE results than when using, for example, an accelerometer alone (151;155). ADDITION-PRO participants who did not have a valid step test included: those who had been excluded from the test according to the exclusion criteria; those who did not have the possibility to perform the step test due to station shut down (due to lack of staff or monitors); those with invalid test results due to bad signal and download problems. In total, 50.2% (1046/2082) of the ADDITION-PRO participants had a valid step test. The group calibration was derived from these step tests. Compared to the group of participants without a valid step test, the group of participants with a valid step test was more likely to: be working (42 vs. 35 % [*P*=0.002]); have a higher alcohol intake (7.0 vs. 6.0 units per week [*P*=0.005]); have a lower proportion of smokers (16.2 vs. 18.8% [*P* =0.049]); to have a lower proportion of participants with a self-reported leisure time characterized by mainly sitting activities (7.6 vs. 12.3%) and slightly higher proportions with a leisure time characterized by low (68.2 vs. 65.3 %) and moderate (24.0 vs. 22.2%) physical activity level (*P* =0.006). Furthermore, the distribution of diabetes risk at baseline screening was different among participants with and without a valid step test (*P* =0.001), with more participants in the 'low risk' group among those with a valid step test, albeit also more participants in the impaired glucose tolerance groups. In contrast, the group of participants without a valid step test was more likely to have a higher proportion of participants in the baseline screening groups classified as having a high risk of developing diabetes but with normoglycaemia. As such, given the above mentioned differences, the group calibration derived from the valid step tests might not be exactly representative for the entire ADDITION-PRO population. However, using a group calibration derived from the ADDITION-PRO population should be a more appropriate approach than using a group calibration derived from another study population. Among the study population for *Paper II*, 733 had a valid individual calibration, whereas for 451 participants, the group calibration was applied. For those with a valid step test, the median PAEE derived by the group calibration and individual calibration was 35.2 (26.0; 47.2) kJ/kg/day and 35.3 (IQR: 26.0; 49.6) kJ/kg/day, respectively (*P* =0.623). Characteristics of the participants with individual or group calibration are shown in Appendix II, table A3. In the present thesis, individual calibration was used for those who had valid data. Another approach could have been to apply the group based calibration to all participants, thus minimizing potential bias (since all participants would have the same equation), albeit generating

more inaccurate PAEE estimates. However, there was no statistically significant difference between estimates derived from the individual and group calibration for the participants in *Paper II.* Hence, there is a limited risk of biased results.

In Health2008 (*Paper I*), the participants did not perform a step test calibration. Although participants did perform a watt-max test, the raw data from this test (including mechanical workload, physical activity intensity and the observed heart rate) was not available for inclusion in a calibration of the ActiHeart monitor (the calibration can be derived from all kinds of tests examining the heart rate to physical activity intensity relationship). Accessibility to that data would have improved the validity of the derived PAEE measures. However, in the analyses of the Health2008 study, a group calibration from the 'INTERACT' study population was applied (132). The INTERACT group calibration was derived from individual calibrations performed in 1,941 persons (among these, 200 Danish men and women). The INTERACT study population was similar to the participants of the Health2008 study in terms of mean [SD] age (53.8 [9.4] vs. 46.9 [8.1] years) and BMI distribution (25.8 [4.1] vs. 25.7 [4.1] kg/m^2) but included a slightly higher proportion of women (70% vs. 57%) (132). As such, although the group calibration does take age and sex of the individual into account, using a group calibration derived from another population might have slightly biased the PAEE estimates in *Paper I*. Unfortunately, it is not possible to rule out the direction of this potential bias – whether the PAEE estimates generated based on the INTERACT group calibration would be slightly *higher* or *lower* as compared to individually calibrated estimates.

Quality Assurance

During the pre-processing and processing of physical activity data, all long-term heart rate and accelerometry traces were manually checked as part of quality assurance. First, they were checked in the manufacturer's software (while stopping and trimming the files, see flowchart of data processing in appendix II, Figure A6). Later on, they were double checked when imported into the Java Physical Activity Data Viewer program (133). All corrupt files were logged and either completely excluded from analysis (if both heart rate and accelerometry were corrupted) or 'flagged' (thus excluding the corrupted heart rate *or* accelerometry measures), including only sufficiently good quality files in the analysis. Completely corrupted or flagged (incl. those with < 24 hours of ActiHeart wear time) files would result in missing PAEE data in both the Health2008 and ADDITION-PRO study populations. For the Health2008 study, this constituted 15% of the observations. As such, one could speculate if the results might be biased. However, since the log revealed that the bad data were mostly due to monitor problems, the risk of bias is relatively low since the missing data was randomly missing. For the participants in *Paper II*, missing data were imputed, and as such, the results should not be biased.

Even though the ActiHeart monitor provides valid and reliable results of PAEE, when sufficiently calibrated and when data are cleaned and processed properly to avoid estimates being biased from: noise; outliers; or periods with insufficient data, some disadvantages of the monitor exist. As such, given the precise measures that are obtained from the monitor, the drawbacks of using it in large-scale epidemiological studies includes the relatively 'low feasibility', since performing individual calibrations is time consuming in a large-scale clinical setting and requires extensive training of the staff in order to obtain good quality measures. The subsequent process of data cleaning and processing requires large computational power and manpower in order to keep to a satisfactory time frame. Lastly, the monitor is somewhat costly, limiting the use in studies with a limited budget. Altogether, due to the stepwise generating and processing of data, there is a potential risk of introducing bias throughout the work-process. Ideally, procedures should be standardized and quality assurance should be performed. When generating and processing the data for the present thesis, standardized operation procedures were followed and quality assurance was performed by trained personnel, in order to limit the risk of introducing errors.

Other objective monitors to estimate physical activity and sedentary behavior

The activity monitor used in the present thesis is only one of several available monitors. There is an increasing range of wearable objective physical activity monitors that can be used to derive PAEE, time spent in different physical activity intensities, and to determine time spent in a certain position: accelerometers and inclinometers, heart rate monitors, pedometers, and combination monitors (51). While accelerometers and heart rate monitors have traditionally been developed to measure physical activity, some challenges emerge when these monitors are used to obtain measures of sedentary behavior. For example, the thresholds by which activity obtained from, for example, accelerometers are classified into 'sedentary', 'light', 'moderate', or 'vigorous' physical activity intensities, are based on somewhat arbitrary cut-points, and there is some scientific discussion regarding the accuracy of these cut-points (156). Some monitors have the ability to indicate the position of the body, i.e. sitting/lying, standing, and walking. One such monitor is the ActivPAL monitor (157). However, while position measures of sitting/lying, standing, and walking have been successfully validated according to direct observation (157;158), the derived MET-intensities are based on calculations based on step cadence and activity intensities from the MET-compendium (22). In contrast, activity monitors such as the Actigraph (models without inclinometer) or ActiHeart cannot define the position, but solely rely on the accelerometry and heart rate measures to define intensity.

Altogether, the validity of the physical activity outcome relies on how well data are obtained and generated, if physiological variables that affect the estimates (e.g. heart rate, movement speed, or

step length) are calibrated or not, and if the derived measures are validated using 'gold standard' methods (33). Some monitors come with software programs to derive the specific physical activity measures. However, only recently, equations used to derive these measures are starting to be published rather than being 'black box' formulas. Together with increased storage capacity, longer living batteries, and access to 'raw' data, the new generation of monitors enables researchers to obtain large amounts of data that can be processed using varying formulas in force at the time of question (159). As such, storing raw data is 'future proof'. However, the massive amount of raw data is a challenge when summarizing the data and generating individual physical activity measures (160). Lastly, when using wearable activity monitors there is always the risk that study participants do not wear them for the prescribed period (the more monitors they wear the worse compliance). Thus cleaning of data and dealing with 'non-wear' time is a critical issue (160). The use of ActiHeart in the Health2008 and ADDITION-PRO studies was based on decisions to obtain precise measures of PAEE, during daily living, in many participants. These decisions were taken four-to-five years ago when planning the studies. In the meantime, other monitors have been developed that have high feasibility in large-scale epidemiological studies (e.g. tri-axial accelerometers) and which are relatively precise in measuring PAEE. However, recent findings confirms the ability of the ActiHeart monitor to provide more precise measures of PAEE, as compared to, for example, using tri-axial accelerometry (151).

Self-report methods to asses TV-viewing time and time spent sitting

Even though novel activity monitors have the capability to objectively measure dimensions of physical activity and sedentary behavior, self-report methods are necessary when information on the context of the behavior is required. Furthermore, self-report methods are feasible and inexpensive to use in large-scale clinical and epidemiological studies. Moreover, although the validity of questionnaires is often modest (31), they tend to provide reliable results (depending on the dimension investigated). There is a range of physical activity questionnaires asking about time spent sitting in various domains (49). One of the main challenges when using questionnaires to obtain information on physical activity and sedentary behavior is the risk of recall bias.

It is often difficult to remember shorter activity periods, or, in general, periods with non-exercise activity (49). As a consequence, results from self-report methods are often reported in 'bouts' (e.g. rounded, up or down, to ten minutes bouts or one-hour bouts). This is in contrast to objective measurements often providing minute-by-minute results of a given position, acceleration, or heart rate (or even more detailed time units). However, periods in which a specific activity takes place, for example, watching TV or DVD's or time spent sitting at work, are often easier to recall, due to, for example, specific TV programs or movies, or due to specific work tasks (47;49). In general, questionnaires have to be culturally adjusted to the target-population, since the answers to the

questions rely on the subjective interpretation of the questions and the perception of the behavior of the individuals itself (161). Furthermore, they have to be validated according to 'gold-standard' (or criterion) methods of the given questions. In general, questionnaires which obtain measures of sedentary behavior have acceptable to good reliability (29;49), whereas they tend to have poorer validity (as measured according to direct observation or accelerometry)(49), and in general, with a large variability depending on the criterion method (29).

Although the ability of the PAS2 questionnaire (*Paper I*) to rate physical activity has been validated against objectively measured physical fitness (maximal oxygen consumption) with acceptable results (127), the time spent sitting at work or during leisure time has not yet been validated against criterion methods. However, the measures have been validated in terms of construct validity, defining the degree to which the questionnaire observed the construct it was designed to measure (128). This was done by interviews, clarifying whether the response given by respondents was in accordance with the intentions (128). As such, the questions should provide reliable results, however, it is somewhat uncertain whether the reported time spent sitting in *Paper I* is subject to under- or over-reporting. This should be explored further in the near future, using appropriate criterion methods. The RPAQ, which was used in the ADDITION-PRO study (*Paper II*) has previously been validated against the doubly labeled water (DLW) method (for total energy expenditure) and against combined heart rate and accelerometry (using the ActiHeart monitor to assess the intensity of activities). The total energy expenditure and PAEE, as measured by the questionnaire, was found to be significantly associated with results of the DLW method (129). Furthermore, self-reported vigorous physical activity was significantly correlated with measures obtained by the ActiHeart monitor. The correlation of self-reported and objectively measured sedentary time, however, was marginally insignificant, and there were no significant correlations between self-reported and objectively measured light- and moderate-physical activity (129). Thus, this questionnaire can be used for ranking individuals' physical activity level. However, in the present thesis, only information on preferred leisure time activity was used.

The TV viewing measure used in *Paper III* (the AusDiab study) has previously been found to be a reliable and valid measure of time spent watching TV (137). In spite of this, the measure might have limited validity in terms of determining overall sedentary behavior, since it only reflects some of the time spent sitting during the day (44). Moreover, modern technology has resulted in increased time spent with 'screen-based' activities other than watching TV (such as playing computers, using tablets, e-readers, smartphones etc.) (162). Therefore, questionnaires asking about screen-based activities are continually evolving. However, time spent with these devices are not always considered as 'sedentary'; for example, playing with computer game consoles can involve physical activity, and tablets and smartphones are often used 'on-the-go'. Moreover, while TV viewing has traditionally

been a 'sitting' activity, it is now also carried out as a secondary activity (e.g. while cooking, jogging on the treadmill in the fitness center, etc.). Nevertheless, the questions asking about time spent watching TV in the AusDiab study (*Paper III*) exclusively asked about periods where TV viewing was the primary activity and which took place while sitting (see methods section for specific phrasing of the question). As such, the TV viewing time reported in *Paper III* may be considered as a surrogate measure of sitting time, albeit not reflecting all time spent sitting throughout the day. In general, self-report measures of TV viewing are known to be subject to under-reporting when compared to objective measures of TV viewing time (163). As such, given the underestimation of time spent sedentary by using the TV viewing measure, the associations observed in *Paper III* are likely to be stronger if including measures of sedentary time obtained by other methods.

All in all, the choice of method used to obtain measures of physical activity and sedentary behavior relies on the research question that is to be answered. However, in practice, it is certainly a trade-off between feasibility and precision of the measurement methods. In the present thesis, this trade-off resulted in using the ActiHeart monitor to obtain precise measures of PAEE during daily living in the Health2008 study and the ADDITION-PRO study. However, due to practical considerations; individual calibration was performed in only one of the studies. In all three studies questionnaires were used to obtain information regarding the context and type of activity performed.

Measures of glucose homeostasis

This thesis utilized different indices of the glucose homeostasis, based on the oral glucose tolerance test (OGTT). Since deriving glucose homeostasis indices from the OGTT is not considered the gold standard method, there are some limitations associated to deriving these indices. Two different approaches were used to derive the measures of HOMA-insulin resistance: the standard HOMA-model, and the HOMA2-model. The standard HOMA-model consists of a simple mathematical model derived from experimental data obtained from only a few persons (139). Furthermore, this model is based on a linear approach, whereas the HOMA2-model has non-linear solutions, and thus, allows for increases in insulin due to higher plasma glucose levels (140). The HOMA2-model has been found to have improved estimates of beta cell function, as compared to the standard HOMA-model (140). However, both the standard HOMA-model and the HOMA2-model provide robust measures of insulin sensitivity, and hence, insulin resistance (140). A major drawback of using the HOMA2-model, however, is the fact, that the specific equations used in the HOMA2-calculator are not published. As such, this is a 'black box' method. HOMA-IR measures are validated against clamp techniques, though it is known to reflect hepatic rather than peripheral insulin resistance (75). For this purpose, the insulin sensitivity index$_{0,120}$ (ISI$_{0,120}$) has proven to be more valid (141). In *Paper I* and *Paper III*,

utilizing the $ISI_{0,120}$ could potentially have resulted in slightly stronger associations. However, in *Paper I*, only more crude measures of glucose homeostasis were used, due to the different scope of deriving physical activity patterns. In *Paper III*, it was not possible to derive $ISI_{0,120}$, as no measures of 120-minute insulin were available.

The use of the HOMA-derived index to determine beta cell function has proven less valid (140). In contrast, the disposition index is a more valid and robust measure of beta cell function, since the insulin secretion is expressed in relation to insulin sensitivity (79). Due to the differences in the models used in the present thesis, results of the different papers are not directly comparable. The measures performed in *Paper II* might be more robust than the measures of beta cell function in *Paper I* and *Paper III*. As such, given these limitations, the results of the present thesis should be interpreted in the light of the methods used. However, this thesis sought to obtain the most precise measures of glucose homeostasis achievable in an epidemiological setting, and since these indices are generally accepted as valid measures, results should at least be comparable to those of other studies utilizing the same methods. The inclusion of the various indices was done in order to obtain a deeper insight into the pathophysiological derangements which precede and lead to diabetes.

Study populations

The Health2008 study population (*Paper I*) consisted of a generally healthy Danish population. Therefore, the findings of this paper are not necessarily generalizable to the general Danish population. However, the overall aim of *Paper I* was to identify if subgroups with different physical activity patterns could be found even in a homogenous population. For this purpose, the study population was suitable. Since the study population was included according to strict inclusion criteria based on the participant's physical capacity, the included population is likely to be more physically active than the general Danish population. As such, the proportion of the population found with an inactive lifestyle is likely to be even higher in the general population. Only 360 participants of the 795 eligible participants wore the activity monitor, and thus, were included in the analysis. Although this was only 45% of the total study population, the sub-sample of people did not differ from the overall study population in terms of gender distribution, age, time spent at moderate-to-vigorous physical activity, occupational sitting time, leisure time sitting, or active transportation. As such, the sub-sample can be considered representative for the total Health2008 study population.

Participants of the ADDITION-PRO study (*Paper III*) were recruited by a pragmatic stepwise screening program. The screening program identified persons at low-to-high risk of developing diabetes, and as such, the findings are generalizable to other people in the Danish population with low-to-high risk of developing diabetes, and who have the overall same characteristics as the ADDITION-PRO

population. The participation rate of the ADDITION-PRO study was 50% (2,082/4,188) and non-attenders did not differ substantially from attenders (see Appendix IV, ADDITION-PRO protocol).
The study population in *Paper III* (the AusDiab study) comprised a national, population-based sample of Australian men and women participating in the AusDiab baseline (1999/2000) and follow-up (2004/2005) health examinations. The response rate was 55% at the baseline examination and of these, 60% attended the follow-up examination five years later, which, in practice, is an acceptable response rate for a large-scale clinical study. Furthermore, the selection procedure to recruit households and people to be included in the AusDiab study sought to minimize selection bias in order to obtain an appropriately representative sample of Australian adult men and women.

In conclusion, the findings of the present thesis can be generalized to other populations with similar characteristics as the ones included in this thesis. For example, since none of the three studies in the present thesis included children or adolescents, the observed associations might not be generalizable to such populations. Additionally, the observed associations might not be generalizable to other 'non-westernized' cultures with other habitual lifestyles, societal structures, or with specific racial differences.

Latent class analysis

In *Paper I*, a latent class analysis approach was used to identify if subgroups with different physical activity patterns could be found in a rather homogenous Danish population. This method was chosen because of its ability to identify subtypes of individuals that exhibit similar patterns of individual characteristics (here: physical activity patterns). Other methods can be used for that purpose, such as factor analysis and cluster analysis. Overall, the different methods aim for the same thing – data reduction. The latent classes or factors (derived from latent class analysis and factor analysis, respectively) are unobserved constructs inferred from observed data. However, whereas factors analysis is often concerned about the correlations of structures of variables in the data set, latent class analysis is concerned about the structure of cases, that is, the patterns of the persons included in the data (143;164) . Cluster analysis is another method used to derive subgroups in data. However, cluster analysis is based on a mathematical rather than a statistical model (164). Therefore, it provides information on how the cases are clustered into groups, but it does not provide information on the probability that a given person is in one or the other group derived, something which is provided with the latent class analysis (143;164). In latent class analysis, the latent variable is categorical, comprised of a set of 'latent classes'. Likewise are the observed variables (or *indicator* variables). The categorization of the indicator variables enables researchers to define binary exposure variables with a 'yes' or 'no' response for, for example, having a physical activity level, or

sitting time, below or above a given threshold value. The definition of these thresholds has to be meaningful and justified, since the use of other threshold values could result in other latent class structures. In the analysis for *Paper I*, five binary indicator variables were created. The thresholds values were defined according to the median values of the studied population (PAEE, active transportation, and leisure time sitting) or according to the literature. As such, threshold values for 'lower' occupational sitting time were based on results from previous studies, with participants reporting an average occupational sitting time of 3.5 hours and 4.2 hours (165;166). Threshold for self-reported MVPA were defined according to current Danish physical activity recommendations (2). The fit of the models with different number of latent classes (1, 2, 3, or 4) was compared using the Akaike Information Criterion (144). When comparing different models within the same set of data, models with lower values are preferred (143). In addition, choice of the optimal number of classes was also based on the distinctiveness and interpretability of the classes. For example, the modest participation number in *Paper I* (n=360) did not justify a higher number of latent classes than four, since this would have resulted in very low numbers in each group.

Although there may be some loss of sensitivity from categorizing data, dichotomizing variables may help in the communication and applications of findings, and is an approach that is commonly applied in latent class analysis methods (143). Furthermore, because the probability of membership in a particular class did not necessarily equal 1 for each individual, there is some uncertainty associated with assigning individuals to their respective latent class. Hence, it is important that the results are interpreted as such. The given names of the latent classes are collective names for the individuals in that specific group and do not necessarily fit every individual one-hundred percent, due to the uncertainty associated with assigning individuals to their respective classes as mentioned before. However, the different latent classes are given names that, to some degree, describe the most prevalent characteristics of the group. Latent class analysis is to a large degree 'data-driven'. Therefore, the latent classes identified are unique for the population studied. Hence, taking the population into account is an important issue when interpreting the data.

Despite the abovementioned methodological points, latent class analysis is favorable to cluster- or factor analysis in grouping individuals into mutually exclusive groups due to the person-oriented approach. The knowledge of potential population specific subgroups with different patterns of physical activity, provides important information, that can be used when planning intervention studies, for example, when stratifying persons into appropriate groups and when framing and tailoring specific communication messages.

Multiple imputation of missing data

Missing data is an un-avoidable problem in epidemiological studies. The type of missing data varies, and hence, it is important to evaluate the structure of missing data. Generally, data can be missing in three ways (167). Number 1) 'missing completely at random' (MCAR), which is when individuals who have missing data are a random subset of the complete sample of subjects (examples: breakdown of activity monitor, accidentally loss of questionnaire, etc.). If MCAR, complete cases analysis give unbiased results, but excludes part of the available data, and hence, are less efficient. Number 2) 'Missing not at random' (MNAR), which is when the probability that an observation is missing depends on information that is un-observed (example: when asking about income level in a survey, missing data are likely to occur if income level is particularly high or low). Number 3) 'missing at random' (MAR), which implies that the missing data are considered random, conditional to other characteristics of the individual with missing data (the reason for missing is based on other patient characteristics). For example, when measuring abdominal obesity by ultrasonography more data might be missing in overweight and obese persons. However, if there is no selection in the missing values from overweight or obese persons (i.e. the missing values from overweight or obese persons are a random sample of the overweight or obese population), the MAR assumption is valid.

Often, the problem with missing data is handled by excluding observations with missing data in variable of interest, and thus, analyzing data using only 'complete' data. If data are missing completely at random, opting for a complete case analysis will not bias results but carries the disadvantage of reducing the power of the analysis to demonstrate a true association at a statistically significant level. However, analyses based on complete data may be biased when it is plausible that missing data are not missing completely at random. This is due to the fact that a relatively modest amount of missing data can lead to the exclusion of a large and biased selection of data records, and hence, the analysis are no longer based on a random sample of the source population.

Imputation of missing data is a way to diminish this bias. Simple imputation techniques involve, for example, replacing missing values with the mean value of the population or with the last measured value (168). However, more sophisticated methods such as 'multiple imputation' are known to provide more valid estimates (167). One of the underlying assumptions prior to performing multiple imputation is that missing data are MAR (or MCAR). When imputing the data in *Paper II* and *Paper III,* by the 'Multivariate Imputation by Chained Equations' (MICE) in the statistical software package 'R', all available variables predicting the missing variables were included. Thus, the missing at random assumption should be reasonable (168). Furthermore, in *Paper III*, where a larger fraction of the data were missing, analysis was done on both complete cases (supplemental material *Paper III*) and on imputed data (*Paper III*) for comparison purposes. Lastly, in *Paper II,* basic characteristics were

presented for the number of participants with available data in order to be able to see the missing structure of the data. The participants (*Paper II*) with missing physical activity data did not differ substantially from persons with physical activity data in terms of demographical and behavioral determinants (Appendix III, Table A4).

The multiple imputation procedure included in this thesis involved three steps: 1) generating fifty imputations for missing values in the data (resulting in fifty copies of the datasets that were complete); 2) analyzing each imputed data set by linear regression analysis; and 3) pooling the fifty estimates into one estimate, combining the variation within and across the fifty imputed datasets. In the literature, more than twenty imputations are generally considered a valid number of imputations (148). However, since computational power has increased, more imputations can be done within a reasonable time frame, and generally, the number of imputations needed relies on the analyses that are to be performed. In *Paper II* and *Paper III*, fifty imputations was considered sufficient, based on the literature and based on the number of variables to be included in the imputation (the more variables to be included and the more missing data, the more time consuming the procedure). Thus, missing data in *Paper II* and *Paper III* should be suitably imputed (168).

Potential confounding factors

Potential confounding factors for the analysis in *Paper II* and *Paper III* were identified from the literature (115;169;170): age, sex, employment status, alcohol consumption, smoking status (and moderate-to-vigorous physical activity for the analysis in *Paper III*). Furthermore, measures of abdominal obesity (waist circumference) were included in the full models at a separate level. This was done based on the consideration that obesity can be seen as a potential confounding factor as well as a mediator of the link between physical activity and glucose homeostasis. It is well-known that central adiposity and obesity are strongly associated with insulin resistance (171). Thus, if one considers that decreased physical activity leads to increased overweight or obesity and hence to insulin resistance, obesity can be regarded as a mediator. It has been proposed that sedentary behavior and obesity should be viewed as separate entities in relation to certain health outcomes (172), and that increased levels of physical activity may protect against metabolic derangements even without reduction in fat mass (104). In contrast, if one considers that increased obesity may lead to decreased physical activity levels and hence to insulin resistance, obesity can be considered a confounder. Thus, obesity can be seen as either a potential mediator or confounder. In the present thesis, waist circumference was taken into account in separate models in order to be able to observe its impact on the relation between PA and glucose homeostasis in isolation. If waist circumference is considered a confounder, the waist-adjusted effect estimate can be regarded as the best approximation of the true effect of PA on glucose homeostasis, regardless of obesity levels. If waist

circumference is considered a potential mediator, as for example in the case of the analysis of TV viewing time and glucose homeostasis in Paper III, including waist circumference in the models represents a statistical over-adjustment (173) if the aim is to approximate the true effect of PA on glucose homeostasis. In this case the effect estimates from the waist-adjusted model should be interpreted in combination with the effect estimates from the models without waist adjustment. The degree of attenuation of the effect caused by waist adjustment can then be tentatively interpreted as the proportion of the PA-glucose homeostasis relation which is mediated by central obesity. Multivariate statistical models cannot separate a mediating from a confounding effect. Indeed, in all likelihood both effects are at play simultaneously in the case of the relation between PA and glucose homeostasis.

In the analysis for *Paper II*, diabetes risk status at baseline screening was included as a confounding factor. This was done in order to adjust for participants' clinical history, since participants are likely to have received different types or intensities of lifestyle advice by their general practitioners (GP) and depending on the level of risk observed at baseline. On one hand, people at highest risk may have received the strongest advice to exercise, but at the other hand, this group may be the one least likely to follow this advice. Because it is not possible to assess and adjust for the details of the health advice and other GP interactions during the follow-up period, adjustments for the present analysis included the categories as classified at baseline. Although the analysis in *Paper II* and *Paper III* did consider a substantial number of confounding factors, there is always a risk of residual confounding due to other, un-measured factors. A consequence of this would be biased associations.

Even though the methods used in the present PhD thesis might have some limitations, the thesis is based on three populations with specific measures of physical activity energy expenditure (*Paper I & II*), with detailed measures of glucose homeostasis (*Paper I, II, III*), and with prospective data on TV viewing time (*Paper III*) in large populations. Altogether, this thesis elaborates on the associations of PAEE and a measure of sedentary behavior with glucose homeostasis markers by: using robust measures of objectively measured PAEE in an epidemiological setting; by exploring potential patterns of physical activity, using specific statistical methods; suitably imputing missing data to avoid bias; and including prospective data on TV viewing time to identify changes over time and exploring associations with changes in glucose homeostasis markers.

Discussion of findings

Physical activity patterns

In *Paper I*, the study sample consisted of a relatively homogenous group of people in terms of age, health status, and physical capacity. Therefore, the finding of two sub-groups with different physical

activity pattern was surprising. Moreover, the finding that 14% of the study population had a lifestyle characterized by low levels of PAEE and with prolonged occupational sitting was unexpected in this generally healthy population, where strict exclusion criteria for physical capacity had been applied. There are a limited number of studies investigating physical activity patterns using latent class analysis in physical activity research, although the method has long been used to identify other social or behavioral patterns, for example drinking patterns (145). The studies utilizing the latent class analysis approach in physical activity research have found that sub-groups of individuals with internally similar physical activity patterns are likely to exist, in both heterogeneous (54-57) and more homogenous populations (174). As such, subgroup analyses are relevant to perform if knowledge on specific patterns of physical activity is desired (for example, information could be used when planning strategies to promote physical activity).

In contrast to the expected high PAEE levels in the generally healthy study sample, low levels of PAEE were expected in the sample of low-to-high risk of developing diabetes (*Paper II*) due to their higher age, and, because initial screening included physical activity questions (with low physical activity levels giving more points) (123). As expected, a high fraction of time was spent in MET-intensities below 3 METs (figure 11). In comparison to the PAEE levels found in *Paper I & II*, Vaughan et al. (1991) found slightly lower mean PAEE (2,190kJ/day) in a slightly older (mean age: 71 years) American population as measured by a respiratory chamber method (175). These findings are consistent with others, showing that physical activity levels decrease with increasing age (176). In different European populations, PAEE levels differ markedly according to the assessment method. Adult populations where PAEE has been assessed by heart rate monitors alone (59;105) show higher PAEE levels than adult populations where PAEE have been assessed by accelerometry alone (177). This is consistent with the known disadvantages of heart rate and accelerometer monitors. Thus, PAEE levels should be interpreted in the light of the measurement method used.

Some laboratory and intervention studies have studied the role of different accumulation of physical activity for overall PAEE. While some find exercise bouts of moderate-to-vigorous intensity to increase overall PAEE in adults and younger adults (178;179), others find middle-aged persons to have unchanged overall PAEE in periods with exercise bouts of moderate-to-vigorous intensities, thus indicating lower PAEE related to non-exercise activities on days with exercise bouts (180;181). These results highlight the need for physical activity promotion strategies that do not only focus on meeting physical activity recommendations, but which also motivate people to increase light physical activity or, in general, energy expenditure associated with everyday activities (known as non-exercise activity thermogenesis, 'NEAT' (182) through reducing time in sedentary activities. As such, when looking at the physical activity levels from the Health2008 study sample (Figure 10 in 'results' section), focus should not only be on increasing the number of peaks at different time points, but also on increasing

the overall PAEE level throughout the day. In figure 10, the PAEE patterns of the two groups are similar throughout the day, albeit the PAEE levels are different. Thus, by increasing the overall PAEE level throughout the day, the inactive group could potentially reach the PAEE level of the active group.

The patterns of the inactive group in *Paper I* exemplify the fact that energy expenditure associated with everyday activities has diminished over time concurrently with increased sedentary behavior. In spite of this, current physical activity recommendations only focus on motivating increases in moderate-to-vigorous physical activity and do not include recommendations on minimizing sedentary behavior, or increasing overall physical activity level irrespectively of the intensity. As a consequence, the 30 minutes of recommended moderate-to-vigorous physical activity might substitute some of the everyday physical activity, rather than *add* to the everyday physical activity (Figure 14 (183;184)).

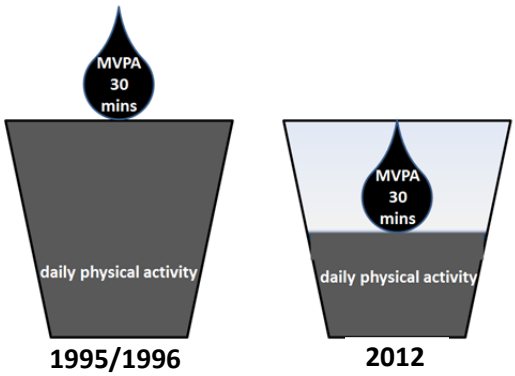

Figure 14. Relationship of the diminishing 'everyday physical activity energy expenditure' and recommended daily moderate-to-vigorous physical activity (MVPA) level (30 minutes). In 1995/1996: Publication of the physical activity recommendations from the Centers for Disease Control and Prevention and the American College of Sports Medicine (183) and publication on Surgeon General's Report on Physical Activity and Health (US department of Health and Human Services, 1996). 2012: Current state (modified from Dunstan 2012 (184))

The top ten leisure time activities of the elderly population in *Paper II* revealed that activities such as leisurely walking, do-it-yourself projects, gardening and leisurely cycling were most prevalent (Table 1). Since environmental factors are known to impact the physical activity level (185), structural changes (e.g increasing walkability or biking possibility) in addition to motivational support could potentially increase the frequency with which these activities are performed.

Physical activity and glucose homeostasis

In *Paper I,* the 'overall active exercisers' had significantly lower levels of fasting insulin and insulin resistance than 'inactive occupational sitters', suggesting an unhealthier glucose metabolic profile when being less physically active and having higher levels of prolonged sitting. Thus, physical activity level and the accumulation of physical activity might have joint contributions to the beneficial effects on glucose homeostasis. In a representative sample of American adults it was found, that both MVPA in bouts of <10 minutes or ≥10 minutes was associated with lower BMI and waist circumference (186), albeit the association was stronger with MVPA accumulated in bouts of 10 minutes. These

results indicate that even accumulation of non-bout MVPA may be a beneficial starting point to increase physical activity levels and to decrease overweight and obesity. Recent studies also suggest, that the context in which physical activity is performed is important for specific health outcomes (e.g. during leisure time or at work). While most studies suggest leisure time physical activity to be beneficial for health outcomes, some discrepancy in determining the direction of the association of occupational physical activity with cardio-metabolic risk factors exists (187). The hypothesis of a u-shaped curve between occupational physical activity and metabolic risk factors is somewhat supported by recent findings from a large Scandinavian study (97). Compared to men with an occupational physical activity level characterized as 'sedentary', men with an occupational physical activity level characterized as 'moderate' had lower insulin resistance (as compared to men with sedentary levels), whereas those that had a 'high' occupational physical activity level had slightly higher levels of HOMA-insulin resistance (although not statistically significant) compared to those in the sedentary category. Among women, high occupational physical activity was associated with higher HOMA-insulin resistance values (as compared to women in the sedentary group) (97). Noteworthy, these associations were seen even after adjusting for educational attainment. As such, the accumulation of PAEE throughout the day might be an important factor in relation to specific health outcomes.

The physical activity levels of the adult population at low-to-high diabetes risk (*Paper II*) were generally of light intensity, but nonetheless positively associated with insulin sensitivity and negatively associated with insulin concentration two hours after glucose load. This indicates that even small increments in physical activity levels are associated with a better glucose metabolic profile. These findings are consistent with those of other studies using accelerometer-(5), and questionnaire-(95;99) based estimates of PAEE. Others have found higher physical activity levels (as measured by questionnaire as well as by heart rate monitors and accelerometry) to be associated with lower HOMA-insulin resistance (97), fasting serum insulin (4;95), and 2-hours plasma glucose levels (6;95), even when adjusting for body composition measures. In fully adjusted models, the associations with fasting-, and 30-minute plasma insulin levels, HOMA-insulin resistance, and 2-hours plasma glucose levels observed in *Paper II,* were attenuated and lost statistical significance. This is probably due to the small effect size in this elderly population performing mainly light intensity physical activity and spending a large amount of time with sedentary activities. In *Paper II*, there was no association of PAEE with indices of the beta cell function (disposition index and insulinogenic index) or with measures of long-term glycemia (HbA_{1c} and $AGEs_{skin}$), indicating peripheral glucose uptake to be most important with regards to explaining the association of PAEE with glucose homeostasis in persons performing mainly light intensity physical activity (Figure 12). A recent study found persons with high self-reported physical activity levels to have higher disposition index,

indicating improved ability of the beta cells to compensate for insulin resistance (95). The overall mechanisms, as introduced by exercise, are hypothesized to include (from rat studies) stimulation of beta cell proliferation and prevention of apoptosis, resulting in an expanded beta cell mass (188).
However, the conflicting findings (as according to *Paper II*) might be due to differences in population-specific characteristics, since the population of the afore-mentioned study were markedly younger (median age: 34.5 [95% CI: 29.1; 39.8] years) than the population in *Paper II,* and had significantly higher levels of physical activity (although self-reported).The failure to show any association of PAEE with HbA_{1c} might be due to the fact that HbA_{1c} is a more stable measure of the glucose homeostasis in contrast to glucose or insulin, since it is a measure of long-term glycaemia (68). This could indicate that physical activity must be performed on a regularly basis, in a longer period, or with higher intensity to be able to show any associations with HbA_{1c}. This hypothesis is supported by studies suggesting high volume but not low volume exercise is associated with a decrease in HbA_{1c} (85;86).

Glucose uptake and insulin response during the OGTT

Although the analysis in *Paper II* was cross-sectional, the findings indicate that an increment in PAEE level would result in a more rapid decline in glucose concentrations from 30- to 120-minutes after the OGTT (Figure 13), rather than in differences in fasting or 30-minute glucose levels. This finding is in line with laboratory studies showing that persons with high physical activity have better glucose uptake than persons with lower physical activity level (87), due to the increased glucose transport activity in skeletal muscles as a response to muscle contraction (189). Furthermore, the effects of physical activity on peripheral insulin sensitivity have, in laboratory studies and exercise interventions, been suggested to be mainly due to an increased oxidative capacity and mitochondrial function in muscles (189;190). As such, despite starting at almost the same fasting plasma glucose levels and ending with slightly different 2-hour plasma glucose levels by different PAEE levels, the results in *Paper II* suggest that persons with a higher PAEE level spend less time at the highest concentrations of circulating glucose. The initial plasma insulin response to glucose load was almost the same (equally steep slope) for all PAEE levels (Figure 13). However, 2-hours plasma insulin seemed to be lower for higher PAEE levels and with a slightly steeper slope from 30-minute to 120-minute, probably due to a higher insulin action in persons with a high PAEE level, since they are more likely to have higher peripheral insulin sensitivity (Figure 12) (90;189).

Dimensions of sedentary behavior and associations with glucose homeostasis markers

TV viewing time and glucose homeostasis

In the cohort of Australian adults (*Paper III*), TV viewing time increased on average by approximately one hour per week from 1999/2000 to 2004/2005. This is consistent with findings from the 2006

Australian Time Use Survey which reported a one-hour per week increase in TV viewing time for adults from 1997 to 2006 (191) and with results from the US (192). Change in TV viewing time over the five-year period was associated with significant increases in continuous measures of insulin resistance, beta cell function, fasting serum insulin and plasma glucose levels in women; and, with increased levels of 2-hours plasma glucose and fasting serum insulin, insulin resistance, and beta cell function in men. Importantly, the associations were shown to be independent of MVPA time and other confounders including dietary quality and energy intake. As expected, the inclusion of waist circumference in the analyses attenuated the associations of five-year change in TV viewing time with five-year change in fasting plasma glucose, insulin resistance, and insulin secretion among women and with insulin resistance, insulin secretion, and fasting serum insulin among men.

The observed associations are consistent with previous studies that have shown cross-sectional associations of TV viewing time with glucose homeostasis markers, impaired glucose tolerance, and diabetes (115;193). An elevation in 2-hour plasma glucose levels (such as the one observed in men in the present study) is consistent with the hypothesized later stages of the pathophysiological changes leading to diabetes (70), characterized by raised glucose levels due to decompensation (due to dysfunction of the beta cells). Moreover, the associations of five-year increases in TV viewing time with increased insulin levels are supported by recent physiological insights into the acute response of one day of prolonged sitting on insulin action in healthy men and women (194). In a counterbalanced, cross-over trial, Stephens and colleagues found that, compared to a condition in which sitting was minimized, prolonged sitting was associated with a substantial reduction (18%) in insulin action over 24-hours, even when taking into account the lower energy expenditure associated with prolonged sitting (194). Furthermore, recent findings from bed-rest studies (a more extreme form of sedentary behavior as described in the background section) confirms that GLUT4 and glycogen synthase activity is decreased after 7-days of inactivity (189;195) and the consequence of these mechanisms may be even more deleterious in persons at high risk of developing diabetes (189;196;197).

Prolonged sitting and 'breaks' in sitting time

In *Paper I,* the 'inactive occupational sitters' group had a higher fraction of persons reporting spending above four hours of occupational sitting as compared to the 'overall active exercisers' and the 'inactive occupational sitters' reported lower levels of walking or standing time at work. The level for occupational sitting found in *Paper I* was consistent with findings from an Australian study of workers, suggesting that occupational sitting time contributes with more than half of the overall sitting time during a day (198). Although this thesis did not examine 'breaks' in sitting time, a higher amount of time spent walking or standing could potentially indicate more breaks in occupational

sitting time among the 'overall active exercisers'. Breaks in sedentary time are found to be associated with lower 2-hour plasma glucose levels in middle-aged persons (110). Furthermore, in elderly persons, those with more breaks in sitting time had a (non-statistically significant) trend towards a lower odds ratio for a cluster of metabolic risk factors as compared to persons with less breaks in sitting time (199). These findings are irrespective of increased leisure time physical activity, since an increased physical activity during leisure time does not seem to compensate for the metabolic derangements associated with prolonged sitting (110). However, a recent study failed to find any associations of breaks in sitting time with insulin levels or HOMA2-insulin resistance in persons with newly diagnosed diabetes, although it found total sedentary time to be positively associated with insulin levels and HOMA2-insulin resistance (111). The potential harmful effects of prolonged sitting might be even more pronounced in populations with metabolic disturbances, and in a large-scale prospective study, persons with metabolic disturbances and high amounts of occupational sitting had a 63% higher hazard ratio for death from all causes, as compared to persons with metabolic disturbances and occupational physical activity characterized by some or much walking and lifting (200). In conclusion, these studies show, that prolonged sitting has health consequences that are independent to those that can be ascribed to lack of physical activity. The results of *Paper I* somewhat support this hypothesis with inactive occupational sitters having an unhealthier glucose metabolic profile.

Clinical relevance of findings

Although the associations observed in this thesis are modest in magnitude, they nevertheless may be important from a public-health perspective (64). In *Paper II*, the reported 10kJ/kg/day increment in PAEE level (which would approximate one hour of walking with a pace of 3.22 km per hour for a person weighing 73 kg) would result in a 1% increment in peripheral insulin sensitivity. Accordingly, a five-year increase in serum insulin of 1.0pmol/l per five-hour increase in TV viewing time (*Paper III*) could be viewed as a small increase. However, in conjunction with other metabolic changes, the observed differences could be significant for cardiovascular risk. Furthermore, on the individual level, for persons with 2-hour fasting plasma glucose levels near the cut-off point for the diagnostic criteria for impaired glucose tolerance or diabetes, an additional increase of 0.05mmol/l would be of potential significance.

It is encouraging that the associations of PAEE with insulin sensitivity and 2-hour insulin levels are observable even within populations with only low-to-modest physical activity levels and that a decrement in TV viewing time over time is associated with improvements in fasting serum insulin (women), and 2-hour plasma glucose levels (men), irrespective of the physical activity level. This indicates that, even without high intensity exercise, aiming to increase the overall level of PAEE, and

to decrease the time spent with sedentary activities by small but reasonable amounts in an entire population at risk of developing type 2 diabetes, may be a realistic and worthwhile goal to aim for from a public health perspective.

PERSPECTIVES

The findings of the present thesis indicate that even small increments in physical activity levels are associated with a better glucose metabolic profile. Moreover, the findings of the same diurnal PAEE pattern in groups with different PAEE levels, suggests that the structural and societal settings which allow and encourage people to make these small changes are also of importance when motivating individuals to engage in a more active lifestyle. As mentioned earlier, even the modest effect sizes found in this thesis might have a large effect at a population level. Results from a recent cohort study somewhat confirms this. The study, which included more than 400,000 persons, found that as compared to being inactive, even 15 minutes per day of self-reported leisure time physical activity (moderate intensity) was associated with a 14% decrease in death of all causes (201). Moreover, subgroup analysis found this reduction to be even more pronounced in persons with diabetes (22%) or who had 'pre-diabetes' (21%)(201). Increasing the overall physical activity level by a low volume of moderate physical activity intensity (rather than meeting the recommendations of a minimum of 30 minutes MVPA) might be a realistic approach for some people, when going from a sedentary lifestyle towards a more physically active lifestyle.

The remaining question is – *how do we motivate a person like Paul (presented in the introduction) to be more physically active and to avoid periods with prolonged sitting throughout the day?* There is not just one answer to that question. However, some studies suggest that adults' physical activity levels are highly influenced by other people, such as peers (202). As such, including an individual's entire network could potentially be a successful and sustainable strategy when aiming to promote physical activity and decrease time spent in prolonged sitting. Additionally, different persons have various preferences in terms of lifestyle. Thus, different preferences or patterns of physical activity could be explored by using, for example, latent variable analysis (or other ways to obtain information on physical activity patterns and preferences). This knowledge would be beneficial prior to designing interventions or communicating campaigns, in order to successfully reach the right recipients with the right interventions.

In everyday life, persons could be motivated to increase their overall physical activity level, that is, choose activities with higher energy expenditure when exercising, and, try to build even small increases in physical activity into everyday life. Increasing the energy expenditure related to everyday physical activities (or NEAT activation) has been found to have a potential role in minimizing obesity

and weight gain (182), and as such, can be important for glucose metabolism as well. NEAT-activation is an easy and inexpensive strategy to implement in a large population – given that individuals are motivated to change. Other ways to increase the overall physical activity in large populations could involve dissemination of exercise programs. For example, Nose et al established a health promotion exercise program for people above the age of 40 years, including: interval walking training; the use of a portable accelerometer and heart rate monitor; and an internet-based health promotion system (203). More than 4,000 have been targeted through that specific program. The interval walking program consists of low volume high intensity walking, e.g. three minutes of high intensity walking, five sets per day, and four days per week. By doing this, persons who have problems with continuous high intensity walking can better accomplish the shorter exercise bouts. This approach of high intensity training (called 'HIT') in short bouts has gained increasing interest and has been shown to be able to increase muscle oxidative capacity (204), to reduce diastolic and systolic blood pressure (203), and, to reduce post-prandial blood glucose and proportion of time spent in hyperglycemia in diabetes patients (205). Moreover, walking (or cycling for that matter) is an activity that most people can relate to (walking was the most preferred activity in the ADDITION-PRO study), and it is possible to do the training together with others. The idea of increasing awareness of an individual's physical activity level through the use of objective monitors and, for example, by graphical outputs (on a web-based platform) function as a motivator and as a possibility for researchers to gather information on physical activity at the same time. Altogether, introducing HIT and NEAT-activation seems like an applicable strategy to increase the overall physical activity in large adult populations – with the aim of shifting the entire population level of PAEE to the right, and thus, potentially, gaining huge beneficial health effects.

CONCLUSIONS

In conclusion, this thesis shows how the relationship between physical activity and glucose metabolism is shaped by dimensions far beyond just the amount of energy spent during exercise. Even in a relatively homogenous and healthy population of Danish men and women, sub-groups of individuals can be identified who have different physical activity patterns. Although patterns of PAEE during the day were similar between the two groups, the physical activity levels differed. A healthier glucose metabolic profile was seen in men and women who performed regular physical activity and avoided prolonged occupational sitting. The beneficial associations of physical activity and glucose homeostasis markers were furthermore found in a population at low-to-high risk of developing diabetes. The physical activity level of this population was generally of light intensity, but nonetheless positively associated with insulin sensitivity and negatively associated with insulin

concentration two hours after glucose load. This indicates that even small increments in physical activity levels are associated with a better glucose metabolic profile. Moreover, a five-year increment in sedentary behavior, measured as TV viewing time, was associated with the initial pathophysiological changes which contribute to the development of diabetes. In summary, these findings show that relatively small differences in activity patterns and levels across multiple domains may impact glucose metabolism. They also suggest that physical activity and sedentary behavior play a parallel role of as determinants of dysglycemia.

SUMMARY

Regular physical activity (PA) has several beneficial health effects, including protection against diabetes and cardiovascular disease. A person's physical activity pattern is defined by a mix of PA dimensions: the type and total volume of PA (including the frequency, intensity, and duration of PA bouts) and the context in which PA is performed. This multidimensional concept of PA suggests that it is possible to accumulate PA in many ways. However, it is uncertain whether subgroups with different PA patterns exist in a homogenous Danish population, and if different PA patterns are associated with glucose metabolism in different ways. Furthermore, little research investigating the associations of different dimensions of everyday PA and sedentary behavior (SB) with detailed measures of glucose homeostasis has been done across groups with different diabetes risk profiles. This thesis aims to identify patterns of PA in a Danish population, and, to quantify and delineate the relationship between the different dimensions of PA and SB with glucose homeostasis markers in a general population and in persons at low-to-high risk of developing diabetes. Three populations were examined in order to address this overall aim: the Health2008 study; the ADDITION-PRO study; and, the AusDiab study.

In a demographically homogenous Danish population (the Health2008 study, n=360), patterns of PA were examined by using latent class analysis. Based on measures of PA obtained by a self-report method and by a combined accelerometer and heart rate monitor (ActiHeart), two sub-groups were identified: an active (86%) and an inactive (14%) group. The diurnal pattern of PAEE accumulation was similar between the groups, with only a difference in the PAEE level. Compared to the inactive group, the active group had significantly lower fasting insulin levels and insulin resistance. From a stepwise screening program 1,531 men and women with low-to-high risk of developing diabetes were recruited (the ADDITION-PRO study) and the associations of ActiHeart-assessed PAEE with glucose homeostasis markers were quantified. Even in this elderly population with low to moderate PAEE (33 kJ/kg/day), higher PAEE was associated with higher peripheral insulin sensitivity and with lower plasma insulin levels two hours after an oral glucose tolerance test. In a large Australian cohort (the AusDiab study) consisting of 4,870 men and women, a five-year increase in self-reported TV-viewing time (a widely used marker of sedentary behavior) were found to be associated with an increase in five-year changes in fasting serum insulin (women) and with plasma glucose levels two hours after an OGTT (men). In conclusion, this thesis shows how the relationship between dimensions of physical activity, sedentary behavior, and glucose metabolism is shaped by dimensions far beyond just the amount of energy spent during exercise. Furthermore, the findings of this thesis, suggest that relatively small differences in activity patterns and levels across multiple domains may impact glucose metabolism.

DANSK RESUMÈ

En persons fysiske aktivitetsmønster udgøres af flere forskellige dimensioner af fysisk aktivitet (FA): type og volumen (frekvens, intensitet og varighed) samt af sammenhængen, hvori FA udføres. Det er således muligt at sammensætte fysisk aktivitet på mange måder. For eksempel er der i en amerikansk befolkning tidligere identificeret en "weekend warrior" gruppe, som overvejende bestod af personer, der udførte en stor del af deres fysiske aktivitet i løbet af en meget kort periode – typisk løbet af weekenden. Potentielt kan andre mønstre identificeres og det er muligt at forskellige FA-mønstre påvirker glukose stofskiftet forskelligt. Fysisk aktivitet har vist sig at være positivt relateret til en række faktorer med betydning for glukose stofskiftet. Omvendt er stillesiddende adfærd (f.eks. sidde og se TV) tilsyneladende ugunstigt associeret med markører for glukose stofskiftet. Der er dog kun få studier som, med detaljerede mål for FA og glukose stofskiftet, har undersøgt, hvordan forskellige dimensioner af FA og stillesiddende adfærd i hverdagen er associerede med glukose stofskiftet. Denne ph.d.-afhandling søger at identificere FA-mønstre i en dansk befolkning, samt at kvantificere og beskrive sammenhængen mellem forskellige dimensioner af FA, stillesiddende adfærd og glukose stofskiftet. Ved brug af "latente klasse-analyser" undersøgtes hvorvidt der fandtes grupper med forskellige FA-mønstre i en ellers homogen Dansk normalbefolkning ("Helbred2008"-studiet, n=360). Baseret på FA målt ved selv-rapportering samt ved brug af en bevægelses- og hjertefrekvens-måler (ActiHeart), blev der i denne homogene stikprøve fundet to undergrupper med forskellige FA-mønstre: en aktiv gruppe (86%) og en inaktiv gruppe (14%). Der var stor forskel på FA-energiforbruget (FAE) i de to grupper, men FAE fordelte sig på samme måde over døgnets 24 timer. Sammenlignet med den inaktive gruppe havde den aktive gruppe signifikant lavere faste insulin niveauer og grad af insulin resistens. I et andet studie, som havde til formål at opspore personer med type 2 diabetes, blev 1531 personer med lav til høj risiko for at udvikle diabetes inviteret til at deltage i et opfølgningsstudie ("ADDITION-PRO"-studiet). Sammenhængen mellem FA målt med ActiHeart og glukose stofskiftet blev undersøgt. Selv i denne undersøgelsesgruppe som havde et lavt FA-niveau fandtes: jo højere FAE-niveau, desto højere insulinfølsomhed og desto lavere koncentration af plasma insulin to timer efter en glukosebelastningstest. I det sidste studie "AusDiab", et Australsk kohortestudie (n=4870) fandtes at en øgning i TV-kiggeri over fem år var relateret til en øgning i faste serum insulin koncentrationen blandt kvinder og til en øgning i to-timers plasma glukose koncentration blandt mænd, selv når der var taget højde for personernes FA-niveau. Således viser denne afhandling, hvordan forholdet mellem FA, stillesiddende adfærd og glukose stofskiftet ikke bare afhænger af energiforbruget ved fysisk træning, men også af, hvordan FA sammensættes. Resultaterne viser desuden, at selv relativt små ændringer i FA-mønstre og FA-niveauer over flere domæner kan have en gunstig påvirkning på glukose stofskiftet.

ACKNOWLEDGEMENTS

Writing this thesis would not have been possible without the help of a number of persons and institutions. First of all, I would like to thank my supervisors *Mette Aadahl*, *Jørn W. Helge*, and *Daniel R. Witte*. A special thanks to you, Daniel, for your enthusiasm and extensive knowledge within epidemiology, and for your creative mindset. Thanks to you, Mette, for always being available, for sharing your interests in and knowledge of activity monitors with me, and especially, for introducing me to the research group in Australia. To you, Jørn, my deepest thank you for being such an experienced supervisor and for always putting things into perspective.

In Australia, I would like to thank *David Dunstan* and *Neville Owen,* at the Physical Activity Lab, Baker IDI Heart and Diabetes Institute, for hosting me for four months and for letting me work on the AusDiab data. I am grateful for your skilled supervision, and for teaching me Australian lingo. Furthermore, during the work included in the present thesis, I have learned to use several statistical methods. I could not have done this without the guidance from my two colleagues and statistical supervisors *Dorte Vistisen* and *Bendix Carstensen*.

A huge thank you to my fellow study coordinators of the ADDITION-PRO study: *Nanna B. Johansen*, *Troels M. Jensen*, and *Annelotte Phillipsen* and the rest of the ADDITION-PRO team: *Carol V.T. Simonsen*, *Lars Kinnunen*, *Anne K. Eriksen*, *Michael Budde*, and *Frederik T. Maindal*. Especially to Nanna, who did all the initial work on planning and setting up the study, and besides that was the best officemate I could ever imagine. Thanks to the ADDITION-Denmark steering commitee: *Torsten Lauritsen*, *Annelli Sandbæk*, *Knut Borch-Johnsen*, *Marit E. Jørgensen* and *Daniel R. Witte* for initiating the study, and to *Ynna M. Nielsen*, *Else-Marie Dalsgaard*, *Søren B. Morsing*, and *Marianne Pedersen* for administrational and data management support. I acknowledge the essential contributions of the dedicated staff at the ADDITION-PRO research centres headed by *Lise Tarnow* (Clinical Research unit, Steno Diabetes Center), *Jens S. Christiansen* (Aarhus University Hospital), *Erling B. Pedersen* (Holstebro Hospital), and *Jeppe Gram* (Hospital of South West Jutland, Esbjerg). I furthermore acknowledge the clinical biochemistry department at Steno Diabetes Center, headed by *Merete Frandsen*, for analyses and management of blood and urine samples.

I am grateful to the Research Centre for Prevention and Health for kindly letting me work on their data from the Health2008 study and to all staff who were involved in the data collection.

Moreover, I owe a special thanks to *Søren Brage* and *Kate Westgate* at the MRC Epidemiology Unit, Cambridge, UK, for support when deriving the physical activity data from the ActiHeart monitor, and to *Daniel F. Jepsen* for consultancy support on the process.

Finally, I would like to thank all the *participants* of the Health2008, the ADDITION-PRO and the AusDiab studies for contributing to the studies.

During the work with this PhD thesis, I was funded by the *Capital Region of Denmark* (financing my PhD study program), the *Danish Cardiovascular Research Academy* (research stay in Australia), the *Lundbeck Foundation* (travel grants), *Carpenter Sophus Jacobsen and wife Astrid Jacobsen's Foundation*, and the *Danish Medical Laboratory Technicians' Research and Development Foundation*.

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APPENDICES

Appendix I: Study populations

Appendix II: Deriving physical activity estimates from the ActiHeart activity monitor

Appendix III: Missing data structure in *Paper II*

Appendix IV: Protocol for ADDITION-PRO: a longitudinal cohort study of the cardiovascular experience of individuals at high risk for diabetes recruited from Danish primary care

Paper I: Physical activity patterns and glucose metabolism in an adults Danish population: the Health2008 study

Paper II: Combined heart rate- and accelerometer- assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes. The ADDITION-PRO study

Paper III: Adverse associations of increases in television viewing time with 5-year changes in glucose homeostasis: the AusDiab study

APPENDIX I – STUDY POPULATIONS

Screening procedure in ADDITION Denmark 2001-2006 and selection of study participants for ADDITION-PRO

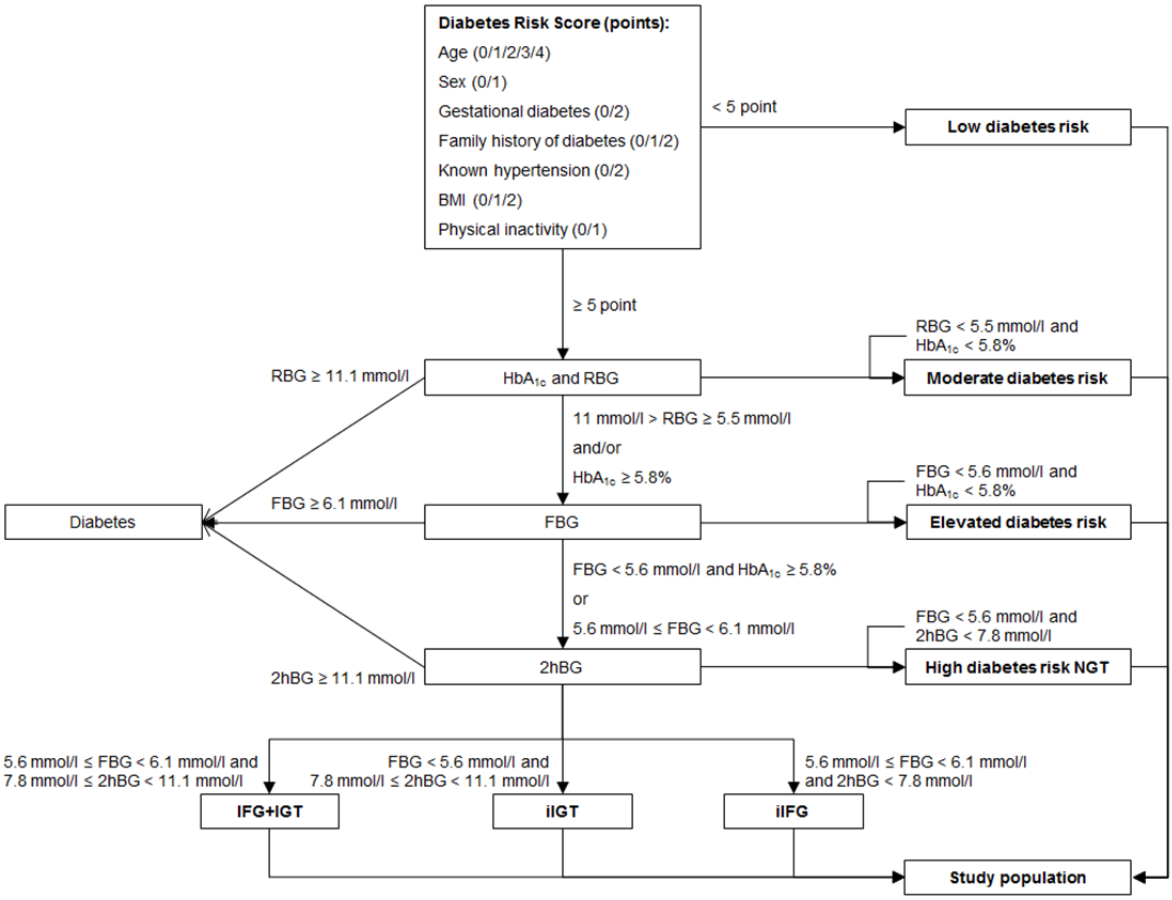

Figure A1. Stepwise screening procedure ADDITION-Denmark 2001-2006.
Glucose was measured in capillary blood. RBG=random blood glucose, FBG=fasting blood glucose, 2hBG=2-hour blood glucose, NGT=normal glucose tolerance, iIFG=isolated impaired fasting glucose, iIGT=isolated impaired glucose tolerance, IFG+IGT=impaired fasting glucose and impaired glucose tolerance. Study population= eligible study population for the ADDITION-PRO study

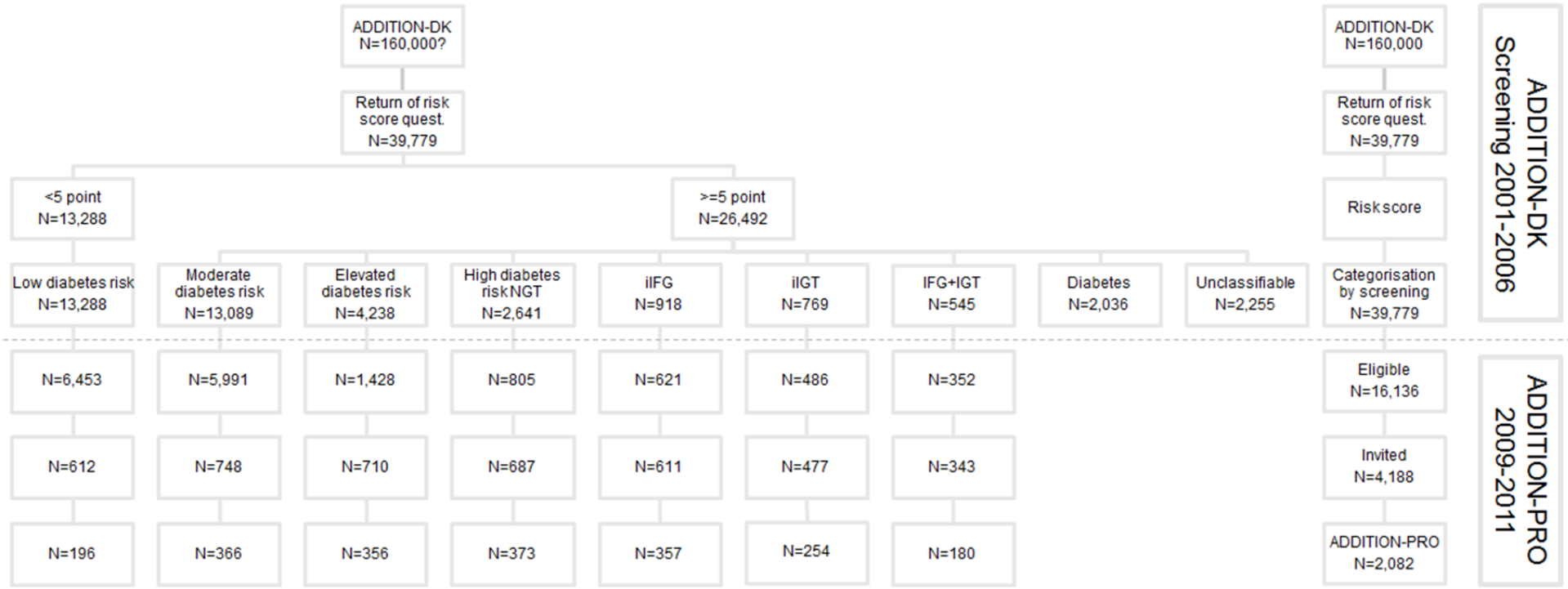

Figure A2. The ADDITION-PRO study population.
In 2009-2011, a follow-up examination (The ADDITION-PRO study) of a subset of the diabetes risk-stratified population was performed at four out of five ADDITION-Denmark study centers. Individuals who were still alive, who lived in the region of the four study centers, and who had not withdrawn consents to participate constitute the target population of the follow-up study (Eligible, n=16,136). From the target population, all individuals with impaired glucose regulation at screening and those who were diagnosed with diabetes during the follow-up period before time of invitation were invited (n=1,483), as well as a random subset of individuals distributed across the lower risk strata (2,705 out of 14,677). In total, 2,082 out of 4188 (50%) agreed to participate in the ADDITION-PRO study.

Selection procedure of the households and participants included in the AusDiab study

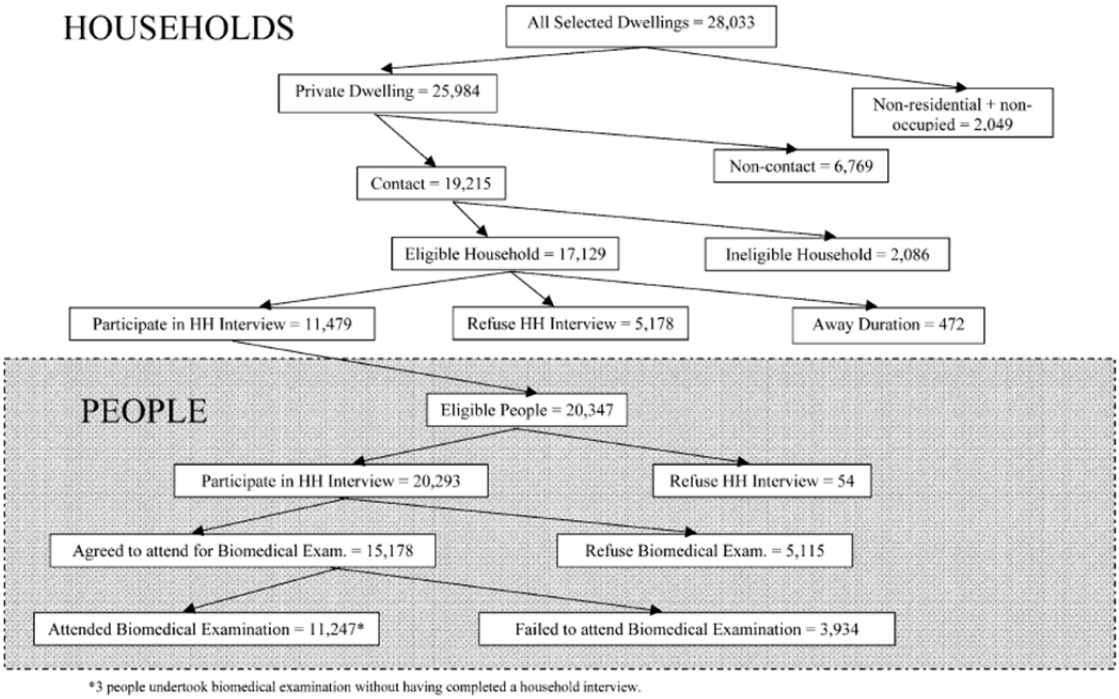

Figure A3. Flowchart of households and persons selected to participate in AusDiab (From D.W. Dunstan et al. (125))

APPENDIX II – DERIVING PHYSICAL ACTIVITY MEASURES FROM THE ACTIHEART ACTIVITY MONITOR

Relationship of heart rate to physical activity

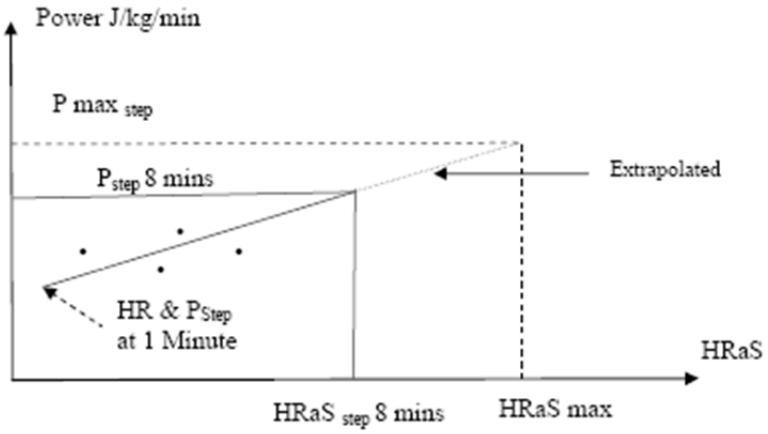

Figure A4 The heart rate (HR) to physical activity intensity (PAI) relationship. The HR-PAI relationship is established using linear regression. The regression line is extrapolated to the predicted $HRaS_{max}$ (defined by the Tanaka equation). HRaS= heart rate above sleeping heart rate; P_{step} = the power generated during stepping (J/kg/min = $9.81 m/s^2$ x height on step bench x number of elevations [up]).

Individual and group calibration of heart rate to physical activity intensity

Table A1. *Individual and group calibration of heart rate to physical activity intensity*

The ADDITION-PRO study

Calibration type	Equation
Individual (n=733)	PAI_{HR}=3.33*HRaS+0.524*HRaS*$beta_{step}$+0.302*$alpha_{step}$+4.90*test duration-1.3*$HRaS_{recovery}$-0.850*SHR-13.2
Group (n= 451)	PAI_{HR}=(5.93-0.003*age+0.233*sex+0.0007*SHR+0.12*betablocker)*HRaS -0.10*age+20.48*sex-0.07*SHR-0.08*SHR*sex+2.87*betablocker-70.05

The Health2008 study

Calibration type	Equation
Group* (n= 360)	PAI_{HR}=(6.22-0.0003*age+0.28*sex-0.0062*SHR+0.00*betablocker)*HRaS +0.21*age+3.9*sex-0.97*SHR-0.00*SHR*sex+0.00*betablocker-31.8

PAI_{HR} = Physical activity intensity as estimated by heart rate; HRaS= heart rate above sleeping heart rate; $beta_{step}$=slope, and $alpha_{step}$= intercept from regression between individually observed HRaS during the step test and PAI for the particular step test protocol (136); $HRaS_{recovery}$= HRaS during recovery period (90 seconds after end of step test); test duration = step test duration; betablocker= on betablocking agents (yes/no)
*Based on the Interact population (132)

Accelerometry equations

Table A2. Segmented linear equations for accelerometry to physical activity intensity (PAI_{ACC})*

PAI_{ACC}= 248*acceleration	for acceleration ≤0.5 m/s^2
PAI_{ACC}= 68*acceleration+90	for acceleration >0.5 m/s^2 and acceleration <2.5 m/s^2
PAI_{ACC}=64*acceleration+99	for acceleration ≥2.5 m/s^2 and acceleration <5.0 m/s^2
PAI_{ACC}=78*acceleration+31	for acceleration >5.0 m/s^2

*As published by Brage et al (136)
Acceleration (m/s^2) is calculated as 0.003*ActiHeart counts per minute

The Branched equation model – figure A5

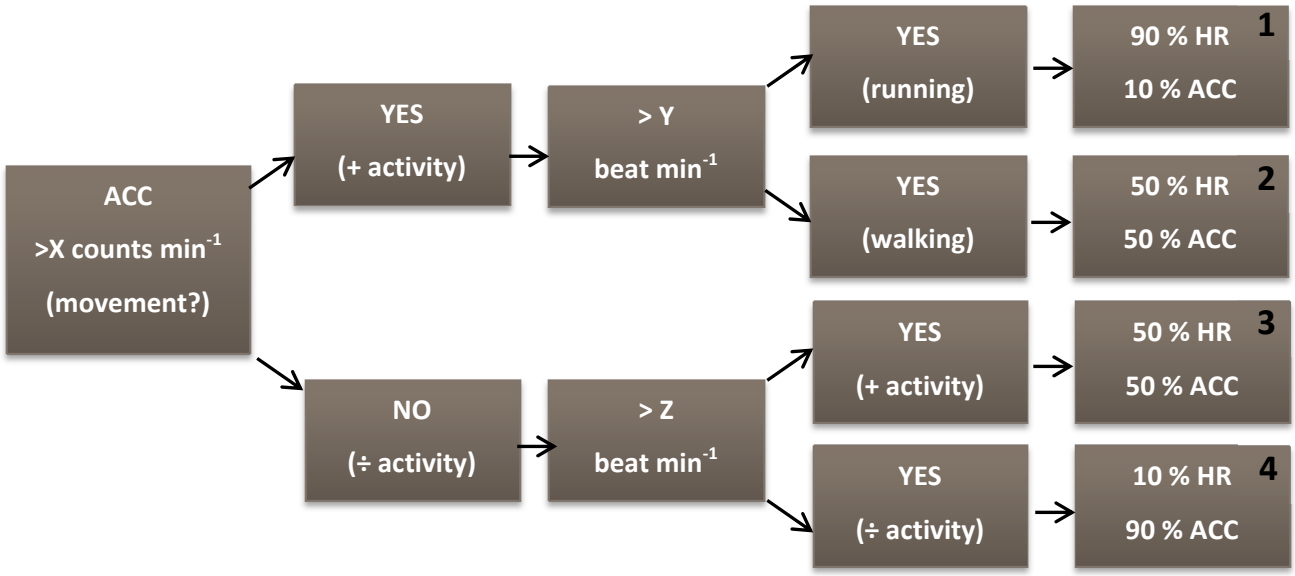

Figure A5. Principle of the 'branched equation modeling' (modified from Brage et al (39))

Box 1 – 4 shows the weighting factors for accelerometry- and heart rate-based physical activity intensity.

X is used to discriminate between 'activity' and 'no activity'.

Y and Z are used to apply heart rate thresholds in the presence and absence of activity, respectively.

Y is used to discriminate between walking and running. At running speeds heart rate is a very reliable measure of energy expenditure, whereas activity as measured by vertical acceleration is less reliable, since during running, the latter does not increase linearly with speed. This is reflected by the weighting in Box 1 where heart rate weighting is high.

At the other end of the spectrum heart rate is a poor measure of intensity, whereas movement registration is more reliable, and this is reflected by a relatively low weighting of the energy expenditure from heart rate, i.e. heart rate weighting in box 4 is low.

Z is used to discriminate between raised heart rate due to some true activity in the presence of 'no activity' (as set by X) and raised heart rate due to other factors. In boxes 2 and 3, energy expenditure from movement and heart rate are equally weighted.

Table A3. Characteristics of participants[**] with individual step test calibration and group calibration in ADDITION-PRO (*Paper II*)

	Participants with individual (step test) calibration (n=733)	Participants with group calibration (n=451)	P-value[*] for differences between participants with individual and group based calibration
Men, n (%)	400 (54.6)	236 (52.3)	0.489
Age (years)	66.2 (61.4; 71.0)	66.9 (62.7; 72.3)	0.003
Occupation, n (% working)	305 (41.6)	152 (33.7)	0.007
Alcohol consumption (units per week)	7.0 (3.0; 10.2)	6.0 (2.0; 14.0)	0.164
Smoking status, n (%yes)	110 (15.0)	77 (17.1)	0.078
Weight (kg)	78.9 (67.9; 88.6)	78.7 (69.2; 88.0)	0.818
BMI (kg/m^2)	26.5 (23.9; 29.3)	27.2 (24.6; 30.1)	0.021
Waist circumference (cm)	94.7 (85.5; 103.5)	95.8 (88.0; 104.0)	0.237
Self-report leisure time category, n (%)			0.027
mainly sitting activities	49 (6.7)	50 (11.3)	
low physical activity level	502 (68.5)	294 (66.5)	
moderate physical activity level	175 (23.9)	97 (22.0)	
elite	0	1 (0.0)	
Diabetes risk status at screening n (%)			0.007
low risk	96 (13.1)	29 (6.4)	
Moderate risk	143 (19.5)	91 (20.2)	
Elevated risk	145 (19.8)	91 (20.2)	
NGT	140 (19.1)	91 (20.2)	
iIFG	109 (14.9)	64 (14.2)	
iIGT	71 (9.7)	65 (14.4)	
IFG+IGT	29 (4.0)	20 (4.4)	

** Of participants with complete data (n=1184 in total)

*Non-parametric test for non-normally distributed values, t-test for normally distributed. Chi-square for proportions

Median values and interquartile ranges if not stated elsewhere. PA=physical activity

Pre-processing procedure of ActiHeart data

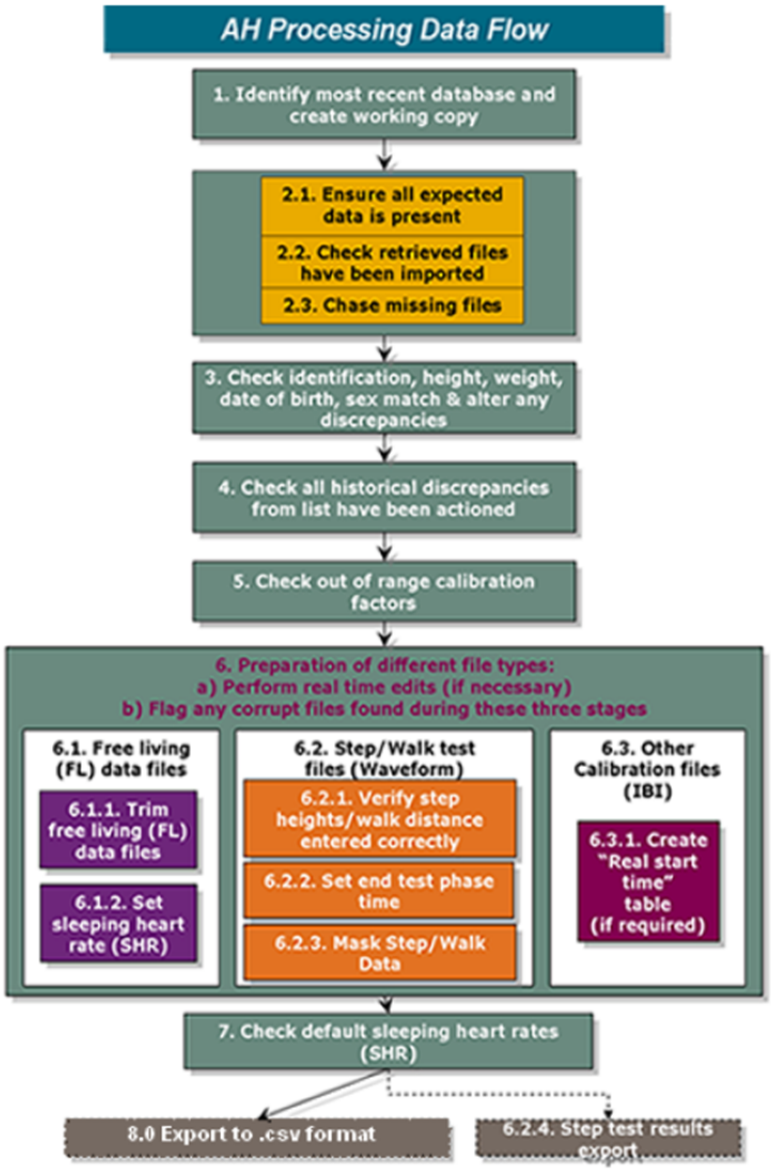

Figure A6. Procedure for pre-processing ActiHeart data (from MRC Epidemiology unit, Cambridge, UK (133))

APPENDIX III – MISSING DATA STRUCTURE IN *PAPER II*

Table A4. Characteristics of participants with and without ActiHeart-assessed physical activity energy expenditure in ADDITION-PRO (*Paper II*)

	Participants without ActiHeart assessed PAEE (n=347)	Participants with ActiHeart assessed PAEE (n=1184)	P-value* for differences between participants with and without ActiHeart assessed PAEE
Men, n (%)	189 (54.4)	636 (53.7)	0.853
Age (years)	66.6 (62.6; 72.1)	66.6 (62.0; 71.5)	0.592
Occupation, n (% working)	132 (38.0)	457 (38.6)	0.913
Alcohol consumption (units per week)	7.0 (2.0; 14.0)	7.0 (3.0; 14.0)	0.192
Smoking status, n (%yes)	67 (19.3)	187 (15.8)	0.199
Weight (kg)	77.6 (67.5; 88.0)	78.8 (68.3; 88.4)	0.320
BMI (kg/m^2)	26.4 (23.8; 29.5)	26.7 (24.2; 29.7)	0.357
Waist circumference (cm)	93.8 (85.6; 102.9)	95.0 (86.3; 103.9)	0.364
Self-report leisure time category, n (%)			
mainly sitting activities	37 (10.7)	99 (8.4)	0.093
low PA level	212 (61.1)	796 (67.2)	
moderate PA level	98 (28.2)	272 (23.0)	
elite	0	1 (0.0)	
Diabetes risk status at screening n (%)			
low risk	44 (12.7)	125 (10.6)	<0.05
Moderate risk	76 (21.9)	234 (19.8)	
Elevated risk	71 (20.5)	236 (19.9)	
NGT	90 (25.9)	231 (19.5)	
iIFG	34 (9.8)	173 (14.6)	
iIGT	22 (6.3)	136 (11.5)	
IFG+IGT	10 (2.9)	49 (4.1)	

*Non-parametric test for non-normally distributed values, t-test for normally distributed. Chi-square for proportions
Median values and interquartile ranges if not stated elsewhere.

APPENDIX IV

Protocol for ADDITION-PRO: a longitudinal cohort study of the cardiovascular experience of individuals at high risk for diabetes recruited from Danish primary care

(*BMC Public Health. 2012 Dec 14;12(1):1078. [Epub ahead of print]*)

BMC Public Health

BioMed Central
The Open Access Publisher

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BMC Public Health 2012, **12**:1078 doi:10.1186/1471-2458-12-1078

Nanna B Johansen (nabj@steno.dk)
Anne-Louise S Hansen (asih@steno.dk)
Troels Mygind Jensen (tmyj@steno.dk)
Annelotte Philipsen (aphi@steno.dk)
Signe S Rasmussen (s_saetre@live.dk)
Marit E Jørgensen (maej@steno.dk)
Rebecca K Simmons (rebecca.simmons@mrc-epid.cam.ac.uk)
Torsten Lauritzen (tl@alm.au.dk)
Annelli Sandbæk (annelli.sandbaek@alm.au.dk)
Daniel R Witte (daniel.witte@crp-sante.lu)

ISSN 1471-2458

Article type Study protocol

Submission date 20 November 2012

Acceptance date 6 December 2012

Publication date 14 December 2012

Article URL http://www.biomedcentral.com/1471-2458/12/1078

Protocol for *ADDITION-PRO*: a longitudinal cohort study of the cardiovascular experience of individuals at high risk for diabetes recruited from Danish primary care

Nanna B Johansen[1]
Email: nabj@steno.dk

Anne-Louise S Hansen[1]
Email: asih@steno.dk

Troels Mygind Jensen[1]
Email: tmyj@steno.dk

Annelotte Philipsen[1]
Email: aphi@steno.dk

Signe S Rasmussen[2]
Email: s_saetre@live.dk

Marit E Jørgensen[1]
Email: maej@steno.dk

Rebecca K Simmons[1,3,4]
Email: rebecca.simmons@mrc-epid.cam.ac.uk

Torsten Lauritzen[4]
Email: tl@alm.au.dk

Annelli Sandbæk[4]
Email: annelli.sandbaek@alm.au.dk

Daniel R Witte[1,5,*]
Email: daniel.witte@crp-sante.lu

[1] Steno Diabetes Center A/S, Gentofte, Denmark

[2] Department of Endocrinology-Gastroenterology, Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark

[3] MRC Epidemiology Unit, Cambridge, UK

[4] Department of Public Health, Section of General Practice, Faculty of Health Sciences, Aarhus University, Aarhus, Denmark

[5] Centre de Recherche Public de la Santé, Luxembourg, UK

* Corresponding author. Centre de Recherche Public de la Santé, Luxembourg, UK

Abstract

Background

Screening programmes for type 2 diabetes inevitably find more individuals at high risk for diabetes than people with undiagnosed prevalent disease. While well established guidelines for the treatment of diabetes exist, less is known about treatment or prevention strategies for individuals found at high risk following screening. In order to make better use of the opportunities for primary prevention of diabetes and its complications among this high risk group, it is important to quantify diabetes progression rates and to examine the development of early markers of cardiovascular disease and microvascular diabetic complications. We also require a better understanding of the mechanisms that underlie and drive early changes in cardiometabolic physiology. The *ADDITION-PRO* study was designed to address these issues among individuals at different levels of diabetes risk recruited from Danish primary care.

Methods/Design

ADDITION-PRO is a population-based, longitudinal cohort study of individuals at high risk for diabetes. 16,136 eligible individuals were identified at high risk following participation in a stepwise screening programme in Danish general practice between 2001 and 2006. All individuals with impaired glucose regulation at screening, those who developed diabetes following screening, and a random sub-sample of those at lower levels of diabetes risk were invited to attend a follow-up health assessment in 2009–2011 (n = 4,188), of whom 2,082 (50%) attended. The health assessment included detailed measurement of anthropometry, body composition, biochemistry, physical activity and cardiovascular risk factors including aortic stiffness and central blood pressure. All *ADDITION-PRO* participants are being followed for incident cardiovascular disease and death.

Discussion

The *ADDITION-PRO* study is designed to increase understanding of cardiovascular risk and its underlying mechanisms among individuals at high risk of diabetes. Key features of this study include (i) a carefully characterised cohort at different levels of diabetes risk; (ii) detailed measurement of cardiovascular and metabolic risk factors; (iii) objective measurement of physical activity behaviour; and (iv) long-term follow-up of hard clinical outcomes including mortality and cardiovascular disease. Results will inform policy recommendations concerning cardiovascular risk reduction and treatment among individuals at high risk for diabetes. The detailed phenotyping of this cohort will also allow a number of research questions concerning early changes in cardiometabolic physiology to be addressed.

Keywords

Diabetes, Cardiovascular disease, Primary care, Complications, Microvascular, Impaired fasting glucose, Impaired glucose intolerance, Aortic stiffness, Physical activity, Body composition

Background

The increasing global prevalence of diabetes has led several countries to propose or introduce screening programmes for diabetes in the past decade [1,2]. These screening programmes, which generally combine a structured assessment of risk factors for diabetes with a measure of glycaemia, will inevitably find more individuals at high risk for diabetes than those with undiagnosed prevalent disease. The *ADDITION-Europe* study [3] included a stepwise screening programme for diabetes in general practice in Denmark, the UK and the Netherlands, and identified more individuals with impaired fasting glycaemia (IFG) or impaired glucose tolerance (IGT) than those with screen-detected diabetes [4,5]. The screening programme also identified large numbers with normal glucose levels despite having one or more risk factors for diabetes or cardiovascular disease (CVD) [4]. While established guidelines for treating individuals with diagnosed diabetes are available [6], it remains unclear which treatment or prevention strategies should be introduced among those found to be at high risk of diabetes following screening. Development of diabetes can be prevented by very intensive lifestyle intervention among motivated individuals with IGT [7] but no trials of diabetes prevention have been performed in groups at lower absolute risk. Furthermore, there are no large-scale studies of the association between diabetes risk, dysglycaemia, and the development of the initial stages of diabetic micro- and macrovascular complications.

In order to make better use of the opportunities for primary prevention of diabetes and its complications afforded by the identification of large groups of at-risk individuals, there are a number of research questions that require investigation. It is necessary to accurately assess progression to diabetes in groups at different levels of risk and to examine the development of early markers of CVD and diabetic microvascular complications. A better understanding of the mechanisms that underlie and drive early changes in cardiometabolic physiology is also required.

To investigate these issues, we invited individuals at different levels of diabetes risk identified from the stepwise screening programme of the Danish arm of the *ADDITION-Europe* study [3] to take part in a longitudinal cohort study (*ADDITION-PRO*). The overall aim of *ADDITION-PRO* is to increase understanding of CVD risk and its underlying mechanisms among individuals at different levels of diabetes risk. We will quantify the incidence of diabetes and CVD in high risk individuals, and examine diabetes and CVD progression using detailed measures of anthropometry, body composition, biochemistry, aortic stiffness and lifestyle behaviours. Results will inform policy recommendations concerning CVD risk reduction and treatment among individuals at high risk for diabetes.

Specific objectives of the *ADDITION-PRO* study include:

- To quantify progression rates from groups at different levels of diabetes risk to IFG, IGT and diabetes, and to examine the determinants of glycaemic state transition
- To establish whether initial levels of glycaemia and subsequent transitions from one glycaemic state to another affect the initial stages of micro- and macrovascular complications, and the risk of incident CVD and mortality
- To examine the association between:
  - objectively measured physical activity and markers of glucose homeostasis
  - anthropometric and body fat distribution measures, markers of glucose homeostasis and the initial stages of micro- and macrovascular complications
  - deterioration in glucose metabolism, long-term glycaemia and the development of initial stages of micro- and macrovascular complications

Methods/Design

The *ADDITION-PRO* study is nested within the Danish arm (*ADDITION-DK*) of the *ADDITION-Europe* study [3,8]. *ADDITION-DK* consists of two phases: a population-based stepwise screening programme for type 2 diabetes and a randomised controlled trial of early intensive treatment among those found to have diabetes. A subset of individuals identified at high risk for diabetes following the screening phase of the study were invited to take part in the longitudinal *ADDITION-PRO* cohort study. Ethical approval was obtained from the scientific ethics committee in the Central Denmark Region (no: 20000183). Participants gave written informed consent to take part in the study and for linkage of their data with National Registers for the purposes of the *ADDITION-PRO* study.

ADDITION-DK stepwise screening programme

Full details of the screening programme are reported elsewhere [9,10]. In brief, a population-based, stepwise high-risk screening programme was performed in 190 family practices in five counties covering urban, suburban and rural areas of Denmark (Copenhagen, Århus, Ringkøbing, Ribe and South Jutland) from 2001 to 2006. Individuals eligible for invitation to screening were people registered with one of the participating general practices, aged 40 to 69 years, and not known to have diabetes. Exclusion criteria were assessed by the general practitioners and included pregnancy or lactation, being housebound, having a psychological or psychiatric problem likely to invalidate informed consent, or having an illness with a likely prognosis for life expectancy of less than one year. Eligible individuals received a slightly modified version of the Danish diabetes risk questionnaire by post or were asked to complete the questionnaire opportunistically while visiting their GP [11,12]. Recipients were asked to indicate known risk factors for diabetes including age, sex, BMI, known hypertension, family history of type 2 diabetes, gestational diabetes and leisure time physical activity. Those with a risk score ≥ 5 points were invited to continue in the stepwise screening programme, which included random blood glucose and HbA_{1c} testing, a fasting blood glucose test, and an oral glucose tolerance test (OGTT). World Health Organisation criteria were used to diagnose diabetes [13].

In *ADDITION-DK*, 163,189 individuals aged 40–69 years were mailed the risk score questionnaire or filled it in opportunistically at their GP. 26,491 had a diabetes risk score ≥ 5

points and attended for a random blood glucose and/or HbA_{1c} measurement. Participants who completed the screening programme and who were not found to have diabetes (n = 22,200) were classified into groups of increasing diabetes risk: (i) high diabetes risk (≥5 points on diabetes risk score) with normoglycaemia (n = 19,968); (ii) isolated IFG (n = 918); (iii) isolated IGT (n = 769); and (iv) IFG and IGT (n = 545). In order to establish a group at low risk of diabetes, between 2001 and 2002, a sub-group of participants in Aarhus and Copenhagen county from both the postal and opportunistic screening programmes (n = 32,894) were asked to return their risk questionnaire regardless of their score. 13,288 individuals with a low diabetes risk (<5 points on diabetes risk score) were identified [12]. These stratified risk groups constitute the sampling frame for the *ADDITION-PRO* study (Figure 1).

Figure 1 *ADDITION-DK* screening programme

ADDITION-PRO study

In 2009–2011, a follow-up health examination of a subset of the stratified high risk population was performed at four out of the five original *ADDITION-DK* study centres to establish a longitudinal cohort. Individuals who were eligible to be invited included those (i) who were still alive, (ii) who lived in the regions of the four research centres (Steno Diabetes Center, Aarhus University Hospital, Holstebro Hospital, and Hospital of South West Jutland, Esbjerg), and (iii) who had not withdrawn consent to study participation. Of these 16,136 people, all individuals with impaired glucose regulation at the time of screening and those who were diagnosed with diabetes during the follow-up period before the time of invitation were invited (n = 1,483) as well as a 19% random sample of individuals from the low and high risk groups (n = 2,705). In total, 2,082/4,188 (50%) people agreed to participate and attended the *ADDITION-PRO* health assessment (Figure 2). Compared to attenders, non-attenders were more likely to be women and to have a normal weight, and were less likely to have a family history of diabetes (Table 1). Participation did not differ in terms of age, hypertension, history of gestational diabetes, or physical activity.

Figure 2 Design and participant flow in the *ADDITION-PRO* study

Table 1 Difference in characteristics at screening between attenders and non-attenders to the *ADDITION-PRO* study

Characteristics at screening		Attenders n = 2,082	Non-attenders n = 2,106	p-value[1]
Men (%)		53.4	42.8	< 0.001
Median age (years)		58.5	59.3	0.551
Gestational diabetes (% of women)		2.2	1.4	0.185
Family history of diabetes (%)	No relatives	67.7	73.8	< 0.001
	1 relative	24.3	19.6	
	2 relatives	6.1	5.0	
Hypertension[2] (%)		36.7	36.5	0.838
Weight (%)	Normal weight	36.0	40.2	0.006
	Overweight	45.0	40.3	
	Obese	17.8	18.1	
Physically inactive[3] (%)		78.1	78.1	0.856

[1] p-value from t-tests or chi-squared test for the difference between attendees and non-attendees
[2] Hypertension defined as "has a doctor ever told you that you have high blood pressure" in the risk score questionnaire [12]
[3] Classified as inactive from four questions pertaining to leisure time physical activity in the risk score questionnaire [14]

ADDITION-PRO health assessment

Health assessments were performed by trained staff following standard operating procedures. See Table 2 for a full list of measures taken during the screening programme and at the *ADDITION-PRO* health assessment.

Table 2 Measures used at the screening phase (2001–06) and at the health assessment (2009–11) in the *ADDITION-PRO* study

	Screening phase: low risk[1] participants	**Screening phase: high risk[2] participants**	***ADDITION-PRO* health assessment**
Danish risk score questionnaire	X	X	X
Socio-demographic variables			
Age	X	X	X
Sex	X	X	X
Biochemistry			
Random capillary blood glucose		X	
Fasting capillary blood glucose[3]		X	
Venous glycated haemoglobin (HbA_{1c})		X	X
75 g oral glucose tolerance test[3,4]		X	X
Insulin			X
Total cholesterol		X	X
LDL-cholesterol		X	X
HDL-cholesterol		X	X
Triglycerides		X	X
Plasma and urine creatinine[3]		X	X
Urine albumin[3]		X	X
DNA[3]		X	X
Alanine transaminase			X
Alkaline phosphatase			X
Biobank: plasma, serum, spot urine[3]		X	X
Whole saliva[5]			X
Clinical measures			
Electrocardiogram			X
Heart rate			X
Brachial blood pressure			X
Central blood pressure			X
Aortic pulse wave velocity			X
Advanced glycation end-products			X
Anthropometric variables			
Height		X	X
Weight		X	X
Waist circumference		X	X
Hip circumference		X	X
Body fat percentage			X
Abdominal fat distribution by ultrasound[6]			X
Hepatic fat content by ultrasound[6]			X
Physical activity			
Combined accelerometer and heart rate monitor (ActiHeart)			X
Recent physical activity questionnaire (RPAQ)			X
Sleep questionnaire[7]			X
Questionnaire measures			
Ethnicity / nationality		X	
Education		X	
Occupation		X	X
Personal medical history		X	X
Family history of diabetes	X	X	X
Family history of CVD		X	X
Current medication			X
Recent hospital admissions			X
Gestational diabetes (women only)	X	X	X

Lifestyle behaviours (smoking, alcohol consumption, physical activity)		X	X
Current weight, birth weight, weight loss/gain, perceived body image			X
EuroQol 5-D (health utility)			X
SF-36 (functional status)			X
Registry information			X
Cardiovascular disease	X	X	X
Type 2 diabetes	X	X	X
Medication use	X	X	X
Health service use	X	X	X
Mortality			X

[1] Low diabetes risk <5 points on the diabetes risk score
[2] High diabetes risk ≥5 points on the diabetes risk score
[3] At screening, only for individuals with elevated RBG/FBG levels who progressed through to the later stages of the screening programme
[4] At *ADDITION-PRO*, only for individuals without incident diabetes since screening
[5] Only collected at the Steno Diabetes Center
[6] Only collected at the Steno Diabetes Center and in Aarhus
[7] Collected at the Steno Diabetes Center, Esbjerg and Aarhus from July 2010

Clinical measures

A ten second 12-lead electrocardiogram (ECG) was taken with the participant in a supine position. Brachial blood pressure and heart rate was measured three times after a 10 minute rest (Omron M6 comfort, Omron Healthcare, Milton Keynes, UK) with the participant in a sitting position. The average of the three measurements for each parameter constitutes the values for brachial systolic and diastolic blood pressure and heart rate.

Central haemodynamics (aortic pulse wave velocity (aPWV) and central blood pressure) were assessed by applanation tonometry using a SphygmoCor device (version 8, Atcor Medical, West Ryde, NSW, Australia) and a high fidelity tonometer. With the participant in supine position after 10 minutes of rest, the velocity of the pulse wave was assessed between the right carotid and femoral artery. This is a validated method of assessing aortic stiffness [15], an assessment of subclinical organ damage. The tonometer was used to capture wave forms at the carotid and subsequently at the femoral artery simultaneously with an ECG recording using the intersecting tangent [16]. The transit time was based on the mean of ten pulse waves. The distance from the suprasternal notch to the carotid artery was measured with a tape measure and from the suprasternal notch to the femoral artery with an anthropometer (Seca, Medical Scales and Measuring Systems, Hamburg, Germany). The anthropometer was used to avoid overestimation of the distance and consequently the velocity in obese individuals. The path length was determined by subtracting the carotid-sternal notch distance from the femoral-sternal notch distance. In each participant, aPWV was measured twice. If the difference in aPWV between the two measurements was larger than 0.5 m/s, a third measurement was taken. The average of the two closest measurements in each participant constitutes the value of aPWV.

Central blood pressure, augmented pressure and augmentation index were calculated from the peripheral pressure waveforms recorded at the radial artery with the participant in the supine position. The radial waveforms were calibrated by the supine brachial systolic and diastolic blood pressure based on a built-in generalised transfer function. Supine brachial blood pressure was measured after a 10-minutes rest with an automated oscillometric blood pressure recorder (Omron M6 comfort). From the central waveforms, central systolic and diastolic blood pressure, pulse pressure, augmented pressure and augmentation index were estimated. At least two measurements were taken, and the average of each pressure index

constitutes the values of central systolic blood pressure, diastolic blood pressure, pulse pressure, augmentation index and augmented pressure.

Advanced glycation end-products (AGE) were measured by skin autofluorescence using an AGE-reader (Diagnoptics Technologies B.V, Groningen, The Netherlands). The measurement is based on illumination of a ~ 4 cm^2 area of skin on the volar side of the forearm with light (wavelength of 300–420 nm), which excites fluorescent moieties in the skin. Autoflourescence, the fluorescent light subsequently emitted from the skin (wavelengths of 420–600 nm), is measured by an integrated spectrometer and expressed as the ratio between the intensity of the emitted fluorescent light and the excitation light [17]. AGE measurements were taken on the right forearm preceded by the removal of dead skin cells with alcohol preparation pads. One measurement session consisted of three AGE measures, and in each participant three sessions were completed. The average of the three sessions is regarded as the AGE value.

Anthropometric measures

Height was measured to the nearest millimetre using a fixed rigid stadiometer (Seca, Medical Scales and Measuring Systems, Hamburg, Germany). Weight was measured in light indoor clothing without shoes to the nearest 0.1 kg with a Tanita Body Composition Analyser (Tokyo, Japan). Clothes were estimated to weigh 0.5 kg and this weight was deducted from the total weight. Waist and hip circumference were measured with the participant in a standing position using a D-loop tape. Waist circumference was measured at the mid-point between the lower costal margin and the level of the anterior superior iliac crest to the nearest millimetre and hip circumference was measured at the widest level of the hips. Waist and hip measurements were completed twice. If the difference between two consecutive measurements was more than 3 cm, a third measurement was taken. The mean of the two closest measurements constitutes the values of waist and hip circumference. Body fat percentage was assessed by bio-electrical impedance using the TANITA analyser. Measurements were registered with the participant standing barefoot on the weighing platform. Participants with pacemakers or other internal medical devices were measured only using SECA scales.

Body fat distribution

Abdominal fat distribution and hepatic fat content were assessed by ultrasonography (Logiq9 ultrasound machine, GE Healthcare, Waukesha, WI, USA) at two study centres (Steno Diabetes Center and Aarhus) [18,19]. With the participant lying down, the transducer was placed on the abdomen where the xiphoid line crosses the waist circumference (described above). Measurements were performed at the end of a quiet expiration using minimal pressure on the transducer. Subcutaneous fat was recorded as the vertical distance from the skin to the linea alba with a 9 L (2.5-8.0 MHz) transducer in the transverse position. Visceral fat was recorded at the same location, with a 4C (1.5-4.5 MHz) transducer placed longitudinally, as the vertical distance from the peritoneum to the front edge of the vertebra. In order to quantify hepatic fat content an image of the liver was captured intercostally with the participant in the supine position, with the right arm held above the head. A curved array 4C transducer (3 MHz) was used. All ultrasound machine settings were standardised to allow subsequent analysis of image characteristics to quantify hepatic fat content.

Objectively measured physical activity

Physical activity was measured using a combined accelerometer and heart rate monitor (ActiHeart®, CamNTech Ltd., Cambridge, United Kingdom) [20]. The monitor was placed horizontally on the participants' chest on two standard electrocardiogram electrodes (Maxensor, Alton, United Kingdom), one at the lower part of the sternum and the other one on the same horizontal level, on the left side, as laterally as possible. On the day of the health examination, a sub-maximal step test was performed to allow individual calibration of the heart rate to physical activity intensity [21]. The eight-minute step test was administered from the Actiheart software to indicate the cycles of stepping up and down a 20.5 cm step bench (Rucanor Europe B.V., Nieuwerkerk, The Netherlands). The stepping frequency ranged from 15 to 33 step cycles per minute over the duration of the test (8-minutes), followed by a two-minute recovery period (sitting). After the participant had completed the step test, the monitor was set up to record free-living physical activity, registering movement and heart rate every 60 seconds. Participants were asked to wear the monitor for seven days and nights and to maintain their usual physical activity pattern during the period. Alongside wearing the monitor, participants were asked to fill in a log to register any non-wear time and comments during the measuring period. Heart rate and accelerometry measures from the Actiheart monitor were downloaded using the manufacturer's software (www.camntech.com). These measures were then cleaned and processed to reduce noise, outliers, and incomplete heart rate measures [22]. Physical activity measures were derived by combining minute-to-minute heart rate and accelerometry measures using a branched equation model [23].

Biochemical measures

Spot urine for analysis of albumin and creatinine was collected in plastic containers. Venous blood samples were drawn after an overnight fast (≥8 hours). Participants without known diabetes underwent a standard 75 g OGTT with blood samples drawn at 30 and 120 minutes. Plasma for analysis of glucose was prepared immediately upon collection in fluoride-heparin coated tubes. Samples were placed on ice before centrifugation at 3000 rpm for 10 minutes at 4 °C. Plasma for analysis of creatinine, total cholesterol, HDL-cholesterol, and triglycerides was prepared upon collection in lithium-heparin coated tubes by incubating for 0.5-1.5 hours at room temperature with subsequent centrifugation at 3000 rpm for 10 minutes without cooling. Serum for analysis of insulin was prepared by incubating whole blood for 0.5-1.5 hours at room temperature with subsequent centrifugation for 10 minutes at 3000 rpm without cooling. Whole blood for analysis of HbA_{1c} and DNA was collected in EDTA coated tubes. Additionally, aliquots of plasma (0, 30, 120 min), serum (0, 30, 120) and spot urine were collected for the *ADDITION-PRO* biobank. Plasma for the biobank was collected in chilled EDTA coated tubes and centrifuged within 30 minutes at 3000 rpm for 10 minutes at 4 °C. Biobank samples were subsequently stored at −80 °C.

All biochemical measures were analysed at the Clinical Chemistry Department at the Steno Diabetes Center in Gentofte, Denmark. Serum insulin was measured by immunoassay (AutoDELFIA, Perkin Elmer, Massachusetts, United States). Glycated haemoglobin A_{1c} (HbA_{1c}) was measured by HPLC (TOSOH G7, Tokyo, Japan). Between 2009 and 2010, plasma glucose, alanine transaminase, alkaline phosphatase, total cholesterol, HDL-cholesterol, triglycerides, plasma creatinine, urine creatinine and urine albumin were measured using the Hitachi 912 system (Roche Diagnostics, Mannheim, Germany). During 2010, the study laboratory gradually implemented the Vitros 5600 Integrated System (Ortho Clinical Diagnostics, Illkirch Cedex, France). There was modest agreement between the

Hitachi 912 and Vitros 5600 instruments. Thus, all 'Vitros' values were converted to correspond to 'Hitachi' values, using regression equations from validation analyses performed by the study laboratory (Table 3).

Table 3 Conversion of biochemical measures in the *ADDITION-PRO* study

Biochemical measure	Equation
Glucose	x = (y + 0.2637) / 0.983
Alkaline phosphatase	x = (y-8.7108) / 1.0465
Alanine transaminase	x = (y + 0.7823) / 0.9761
Plasma creatinine	x = (y-2.7403) / 1.0147
HDL cholesterol	x = (y + 0.2141) / 1.1254
Triglycerides	x = (y + 0.0196) / 1.1017
Total cholesterol	x = (1.0303*y-0.2362)
Urine albumin	x = (0.8861*y-0.6412)
Urine creatinine	x = (y-111.72) / 1.0087

x = value measured by Hitachi 912 machine
y = value measured by Vitros 5600 machine

Albumin creatinine ratio was calculated using the formula: U-albumin mg/l x 8.84)/(U-creatinine(μmol/l)/1000). VLDL cholesterol (VLDL-C) was calculated using the formula: VLDL-C = triglycerides (mmol/l)/2.2). VLDL-C was calculated only for triglyceride values ≤ 5.05 mmol/l. LDL cholesterol was calculated using Friedewald's equation (LDL-C = TC – VLDL-C – HDL-C mmol/l) [24]. LDL-cholesterol was calculated only for triglyceride values ≤ 4.55 mmol/l.

Collection of whole saliva was performed at one of the study centres (Steno Diabetes Centre). Participants were asked not to brush teeth on the day of the health assessment. Upon arrival, participants were instructed to chew on paraffin wax for approximately one minute and then to empty their mouth of saliva. The participants were then instructed to chew on the paraffin wax for a further three minutes whilst spitting into a collection container whenever needed. The collected saliva was divided into two cryotubes. One tube was stored immediately at −80 °C. RNAlater (Ambion, Austin, TX) was added to the second tube in a 1:3 ratio (saliva:RNAlater) and then refrigerated for approximately 24 hours before being transferred to −80 °C storage.

Questionnaires

A general health questionnaire was mailed to each participant in advance of their measurement visit. The questionnaire included sections on personal medical history, family history of diabetes / CVD, and lifestyle behaviours (smoking status, weekly alcohol consumption, and physical activity). There were questions on current weight, birth weight, weight loss / gain and perceived body image, as well as commuting, occupational and leisure time physical activity habits throughout the life course. The questionnaire included the EuroQol (EQ-5D) health utility measure [25] and the SF-36 functional status measure [26]. Socio-demographic questions included age, occupation, nationality, and ethnicity. Data on current medication and recent hospital admittance were also collected. Medication was coded by a supervised nurse according to the Danish formulary (http://medicin.dk). Female participants were asked for details on the number of pregnancies and live births they had experienced, and for the number and birth weight of their children. They reported if they had developed gestational diabetes.

Physical activity during the last four weeks prior to filling out the questionnaire was assessed using a modified Danish version of the validated "recent physical activity questionnaire" (RPAQ) [27]. From July 2010 onwards a detailed sleep questionnaire was completed alongside combined heart rate and accelerometry measurement. All questionnaires were checked for completeness before the participant finished their measurement visit. The clinical research forms, general health questionnaire and RPAQ were scanned using a Teleform reader and verified manually. Double data entry of the sleep questionnaire was undertaken by experienced research assistants. All data were checked for outliers and nonsense values and cleaned before being uploaded to the study database.

Registry data

Data will be drawn from the unique register system in Denmark. The Danish National Civil Registry assigns a personal Civil Registration Number to all citizens in Denmark. All Danish citizens have National Health Insurance and are entitled to free access to medical care from general practice and hospitals. Data from different sources can be combined using the civil registration number. We will access the following registries to collect information on incident CVD, incident diabetes, mortality, medication and health service usage: the National Patient Registry (covering admissions and outpatient contacts to the hospitals), the National Health Service Registry (contacts in general practice), the Medical Prescription Registry, the Diabetes Registry, and the Death Cause Registry (based on death certificates, 100% coverage).

Sample size

Based on prior experience in the use of Statistics Denmark linkage for event follow up, we expect to achieve a 90% completeness of follow up for CVD events and a 100% completeness of follow up for mortality. Table 4 shows the power calculations performed prior to the start of the clinical examinations. The expected number of events in the *ADDITION-PRO* population was based on incidence rates and hazard ratios reported by the Hoorn study [28]. Based on expected mortality and attrition due to loss to follow up, we aimed to examine 1,800 participants who had IFG/IGT at screening, over half of whom were expected to progress to diabetes during follow-up. Adding a random subset of 900 participants who had normoglycaemia at screening yielded ample power to detect differences in CVD risk between the normoglycaemic group and the stable IFG/IGT and incident diabetes group, respectively. We examined 77% of the target population. One of the original *ADDITION-DK* study centres did not participate in the *ADDITION-PRO* health assessment and there was a lower than expected participation rate (50% rather than the expected 75%) amongst the group of participants with IFG/IGT. While the difference in CVD incidence between the stable IFG/IGT group and the incident diabetes group is expected to be smaller, comparison of the continuous measures of atherosclerosis, renal function and AGE accumulation will be sufficiently powered to highlight even small differences between all study groups.

Table 4 Power calculations for main outcomes in the *ADDITION-PRO* study

	Expected CVD events (n)	Expected incidence rate (per 1000 person years)	Power
Difference in CVD incidence between:			
NGT and stable IFG/IGT	53	7.4	0.97
NGT and incident DM	100	13.8	0.99
Stable IFG/IGT and incident diabetes	132	18.3	0.59
	Detectable differences	**Expected mean (SD) in reference group (NGT)**	**Power**
Aortic stiffness (pulse wave velocity)	0.4 m/s	7.5 m/s (2.5)	0.94
Renal function (eGFR)	5 ml/min/1.73 m^2	100 ml/min/1.73 m^2 (20)	0.98
AGE – (skin autofluorescence)	0.2 AU	2.0 AU (0.5)	0.94

AGE = advanced glycation end-products; *CVD* = cardiovascular disease; *eGFR* = estimated glomerular filtration rate; *IFG* = impaired fasting glucose; *IGT* = impaired glucose tolerance; *NGT* = normal glucose tolerance

Discussion

The *ADDITION-PRO* study is designed to increase understanding of CVD risk and its underlying mechanisms among individuals at high risk of diabetes recruited from a stepwise screening programme in Danish primary care. Key features of this study include (i) a carefully characterised cohort at different levels of diabetes risk; (ii) detailed measurement of anthropometric, body composition, biochemical and cardiovascular risk factors; (iii) objective measurement of physical activity behaviour; (iv) examination of the initial stages of micro- and macrovascular complications; and (v) long-term follow-up of hard clinical outcomes including mortality and CVD. Results will inform policy recommendations concerning CVD risk reduction and treatment among individuals at high risk for diabetes. The detailed phenotyping of this cohort will also allow a number of research questions concerning early changes in cardiometabolic physiology to be addressed.

Abbreviations

AGE, Advanced glycation end-products; aPWV, Aortic pulse wave velocity; BG, Blood glucose; CVD, Cardiovascular disease; ECG, Electrocardiogram; EQ-5D, EuroQol health utility measure; FPG, Fasting plasma glucose; GP, General practice; HbA_{1c}, Glycated haemoglobin; IFG, Impaired fasting glycaemia; IGT, Impaired glucose tolerance; NGT, Normal glucose tolerance; OGTT, Oral glucose tolerance test; RPAQ, Recent physical activity questionnaire

Competing interests

NBJ, ASH, AP, TMJ, and MEJ are employed by Steno Diabetes Center A/S, which is a research and teaching hospital collaborating with the Danish National Health Service and owned by Novo Nordisk A/S. NBJ, ASH, TMJ, AP, MEJ, TL and DRW hold shares in Novo Nordisk A/S. All other authors declare that they have no competing interests.

Authors' contributions

DRW, MEJ, TL and AS are principal investigators for the *ADDITION-PRO* study. SSR and DRW initiated and designed the study. NBJ, DRW, ASH, TMJ and AP were the study coordinators. NBJ, ASH, AP and TMJ trained the clinic staff in measurement techniques. ASH was responsible for physical activity measurements, AP for ultrasound measures, TMJ for biochemical measures and NBJ for all other clinical measurements. NBJ, DRW, ASH, TMJ, AP and RKS drafted the manuscript. All authors read and approved the final manuscript. DRW is the paper guarantor.

Acknowledgements

We would like to thank the *ADDITION-PRO* participants and the participating general practitioners for their contribution to the study. We acknowledge the very important contributions of the teams at the four clinical research centres led by Lise Tarnow (Steno Clinical Research Unit), Jens Sandahl Christiansen (Aarhus University Hospital), Erling Bjerregaard Pedersen (Holstebro Hospital) and Jeppe Gram (Hospital of South West Jutland, Esbjerg). We thank Merete Frandsen and her team at the Steno Laboratory for the management of analyses and the biobank; Torben Hansen at the NNF Center for Basic Metabolic Research for his input in the genetic and saliva protocols; our student helpers at Steno Diabetes Center (Anne Kirstine Eriksen, Michael Budde, Stine Krogsgaard, Frederik Terkildsen Løwenstein); Lars Kinnunen, Carol Vi Trang Simonsen, Ynna Margot Nielsen and Else-Marie Dalsgaard for administrative support; and Marianne Pedersen and Søren Bech Morsing for data management support at Aarhus University. We also thank Prof Knut Borch-Johnsen for his help setting up *ADDITION-PRO*. The *ADDITION-DK* stage of the study was supported by the National Health Service in the counties of Copenhagen, Aarhus, Ringkoebing, Ribe, and South Jutland in Denmark; the Danish Council for Strategic Research; the Danish Research Foundation for General Practice; Novo Nordisk Foundation; the Danish Center for Evaluation and Health Technology Assessment; the Diabetes Fund of the National Board of Health; the Danish Medical Research Council; and the Aarhus University Research Foundation. *ADDITION-DK* has been given unrestricted grants from Novo Nordisk A/S, Novo Nordisk Scandinavia AB, Novo Nordisk UK, ASTRA Denmark, Pfizer Denmark, GlaxoSmithKline Pharma Denmark, Servier Denmark A/S, and HemoCue Denmark A/S. Parts of the grants from Novo Nordisk Foundation, Danish Council for Strategic Research, and Novo Nordisk were transferred to the other European centres. The *ADDITION-PRO* study was funded by an unrestricted grant from the European Foundation for the Study of Diabetes/Pfizer for Research into Cardiovascular Disease Risk Reduction in Patients with Diabetes (74550801), by the Danish Council for Strategic Research and by internal research and equipment funds from Steno Diabetes Center.
The funding bodies played no role in the design, collection, analysis and interpretation of the data, in the writing of the manuscript, or the decision to submit for publication.
The *ADDITION-PRO* study is managed by the *ADDITION-DK* steering committee (Torsten Lauritzen, Knut Borch-Johnsen, Annelli Sandbæk, Marit E. Jørgensen and Daniel R. Witte). Requests for collaboration can be sent to any of the *ADDITION-DK* steering committee members.

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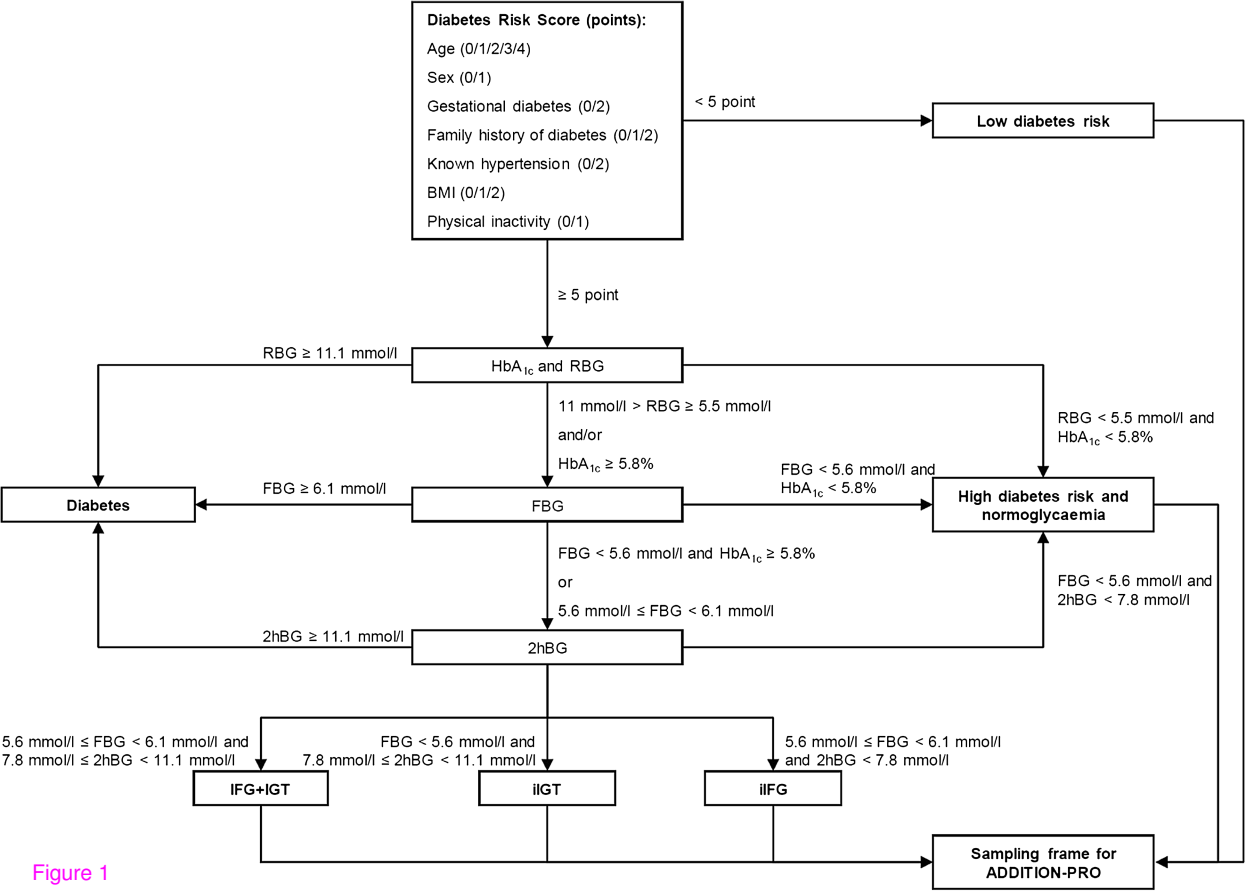
Diabetes Risk Score (points):
Age (0/1/2/3/4)
Sex (0/1)
Gestational diabetes (0/2)
Family history of diabetes (0/1/2)
Known hypertension (0/2)
BMI (0/1/2)
Physical inactivity (0/1)
< 5 point
Low diabetes risk
≥ 5 point
RBG ≥ 11.1 mmol/l
HbA1c and RBG
11 mmol/l > RBG ≥ 5.5 mmol/l
and/or
HbA1c ≥ 5.8%
RBG < 5.5 mmol/l and
HbA1c < 5.8%
Diabetes
FBG ≥ 6.1 mmol/l
FBG
FBG < 5.6 mmol/l and
HbA1c < 5.8%
High diabetes risk and
normoglycaemia
FBG < 5.6 mmol/l and HbA1c ≥ 5.8%
or
5.6 mmol/l ≤ FBG < 6.1 mmol/l
FBG < 5.6 mmol/l and
2hBG < 7.8 mmol/l
2hBG ≥ 11.1 mmol/l
2hBG
5.6 mmol/l ≤ FBG < 6.1 mmol/l and
7.8 mmol/l ≤ 2hBG < 11.1 mmol/l
FBG < 5.6 mmol/l and
7.8 mmol/l ≤ 2hBG < 11.1 mmol/l
5.6 mmol/l ≤ FBG < 6.1 mmol/l
and 2hBG < 7.8 mmol/l
IFG+IGT
iIGT
iIFG
Sampling frame for
ADDITION-PRO

Figure 1

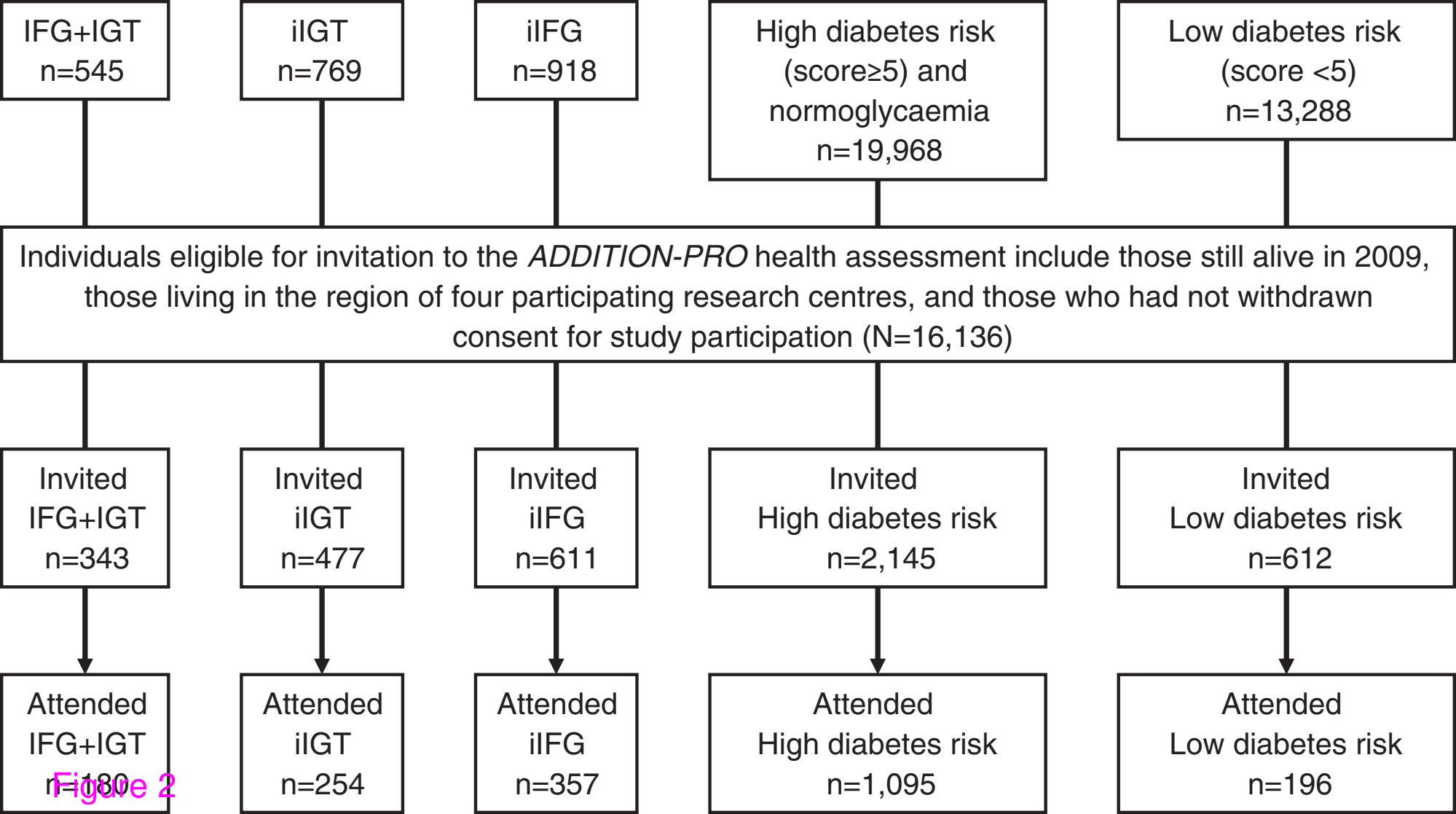
IFG+IGT
n=545
iIGT
n=769
iIFG
n=918
High diabetes risk
(score≥5) and
normoglycaemia
n=19,968
Low diabetes risk
(score <5)
n=13,288
Individuals eligible for invitation to the *ADDITION-PRO* health assessment include those still alive in 2009, those living in the region of four participating research centres, and those who had not withdrawn consent for study participation (N=16,136)
Invited
IFG+IGT
n=343
Invited
iIGT
n=477
Invited
iIFG
n=611
Invited
High diabetes risk
n=2,145
Invited
Low diabetes risk
n=612
Attended
IFG+IGT
n=180
Attended
iIGT
n=254
Attended
iIFG
n=357
Attended
High diabetes risk
n=1,095
Attended
Low diabetes risk
n=196

Figure 2

PAPER I

Physical activity patterns and glucose metabolism in an adult Danish population: the Health2008 study

(*submitted to Journal of Epidemiology and Community Health*)

Title page

Title: 'Physical activity patterns and glucose metabolism in an adult Danish population: the Health2008 study'

Authors: Anne-Louise S Hansen[1], Dorte Vistisen[1], Bendix Carstensen[1], Jørn W Helge[2], Allan Linneberg[3], Daniel R Witte[1], Mette Aadahl[3]

[1]Steno Diabetes Center A/S, Gentofte, Denmark; [2]Centre for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; [3]Research Centre for Prevention and Health, The Capital Region of Denmark, Glostrup, Denmark

Correspondence: Anne-Louise Smidt Hansen, Niels Steensens vej 1, building NLD 2.07, DK-2820 Gentofte. Fax: (+45) 4443 0706; Tel: (+45) 4442 0111; E-mail: asih@steno.dk *or* al.smidthansen@gmail.com

Word Count: 2,998 (without title page, abstract [250 words], tables, figures, references, and thumbnail box)

Tables/Figures: 1 table, 2 figures, 1 Web only file (supplemental figure)

Keywords: Heart rate, accelerometer, latent class analysis, physical activity, sedentary behaviour

Abstract

Background Physical activity (PA) patterns vary between groups of individuals and the influence of different dimensions of PA on health-outcomes is not fully understood.

Objective Using cross-sectional data, we aimed to identify subgroups with different PA patterns in a healthy Danish sample using latent class analysis. Subsequently, we aimed to describe characteristics including the glucose metabolic profile of potential groups.

Methods Direct assessment of PA was obtained using the ActiHeart activity monitor and self-report questionnaire in 360 participants (30–60 years) from the Danish 'Health2008' study. Five PA indicators: 1) overall PA energy expenditure ≥35.2 kJ/kg/day (PAEE); 2) active transportation ≥0.25 hrs/day; 3) occupational sitting ≥4 hrs/day; 4) leisure time sitting >3hrs/day; and, 5) time spent in moderate-to-vigorous PA ≥150 min/week, were used to derive latent classes of PA patterns. Glucose homeostasis markers were derived based on an oral glucose tolerance test.

Results Two latent classes were identified: 'overall active exercisers' (n=311) and 'inactive occupational sitters' (n=49). Physical activity levels and physiological characteristics differed between the groups (PAEE: 37.4 vs. 27.3 kJ/kg/day, *P*<0,001). Compared to 'inactive occupational sitters', the 'overall active exercisers' had lower levels of fasting insulin (32.1 vs. 40.1 pmol/l, *P*=0,017) and insulin resistance (0.6 vs. 0.7, *P*=0.030).

Conclusions In healthy Danish men and women, we identified two subgroups with different PA patterns. 'Overall active exercisers' had significantly lower levels of fasting insulin and insulin resistance than 'inactive occupational sitters', suggesting an unhealthier glucose metabolic profile when being less physically active and having higher levels of prolonged sitting.

INTRODUCTION

Regular physical activity (PA) has several beneficial health effects including protection against diabetes and cardiovascular disease(1;2). In addition to PA-related energy expenditure, several dimensions of PA have been suggested to be important for achieving beneficial effects on health related outcomes; total volume of PA, frequency, intensity and duration of physical activity bouts(3;4). The mix of PA dimensions forms a person's PA pattern and the multidimensional concept of PA suggests that it is possible to accumulate PA in many ways. However, how different PA patterns are associated with glucose metabolism is not fully understood. In diabetes research, adults' PA levels have been suggested to be influenced by other people, such as peers(5). Thus, within a population, subgroups of persons with internally similar PA patterns are likely to exist. Recent studies on exercise intervention strategies suggest that people dislike exercising in groups of people markedly dissimilar to themselves(6;7). Thus, exploring whether subgroups of persons with different PA patterns exist, and, describing group-specific characteristics, could provide important knowledge contributing to the design of successful PA interventions in diabetes prevention and treatment or environmental and policy actions for promoting PA in public health.

A previous study used latent class analysis to identify five different classes of PA patterns among a nationally representative sample of Americans(8). For example, a 'Weekend Warrior' group was identified (persons who accumulate a large quantity of PA during a short period of time – mostly during weekends). Additionally a subgroup of persons who were highly active during the week but less active in the weekends was identified(8). These results were found in a large, heterogeneous US sample, and evidence that similar subgroups of persons with different PA patterns are present in other (more homogenous) populations are scarce.

We examined if subgroups of persons with different PA patterns could be found in a generally healthy and relatively homogenous (in terms of age, health status and physical capacity) Danish sample, and subsequently aimed to describe the demographic, clinical and behavioural characteristics of any subgroups.

METHODS

Study participants

A random sample of 2,218 men and women, aged 30–60 years, living in the Western part of Copenhagen, was extracted from the Danish Civil Registration System and invited for a general health examination(9). The invitation letter stated that persons could participate only if they were able to cycle or climb stairs and were free of the following conditions: cardiovascular disease, diabetes, chronic obstructive pulmonary disease, hypertension, and history of blood clots. Pregnant women were not eligible. A total of 795 eligible participants (36%) accepted the invitation and underwent health examination between September 2008 and December 2009. The study was approved by the Ethics Committee of the Copenhagen Region (KA-20060011) and all participants provided written informed consent. More women than men agreed to participate (56% women among participants vs. 49% women among non-participants) and participants were in average 46.8 years old (non-participants: 46.3). Participants were asked to wear a direct PA monitor (ActiHeart) for 7 days. In total, 463 participants (58% of attending participants) agreed to wear the monitor. Only participants with a minimum ActiHeart wear-time of 24 hours throughout the measurement period (n=392), with valid data from the PA questionnaire (n=366), and, who had been fasting prior to examination were included in the present study (n=360).

Measurement methods

Demographic variables

Information on employment status (yes/no), school education (≤7 years, 8-9 years, 10 years, high school, or other), marital status (unmarried, married, divorced, widowed), smoking status (current, former, or never smoker), and alcohol consumption (in units per week, one unit ~12grams of pure alcohol) was obtained from a self-report questionnaire.

Physical activity

When estimating PA patterns, methodological issues are of great concern. Objective monitors provide a valid and reliable measure of the PA energy expenditure, whereas self-report methods provide information on the context and self-perception of the PA performed(10). As such, by combining these measurement methods, a more detailed picture of the PA patterns can be obtained. Direct measures of PA were obtained using the combined accelerometer and heart-rate (HR) monitor, 'ActiHeart' (CamNtech Ltd., Cambridge, UK)(11), measuring acceleration and HR every 60 seconds. The monitor was placed horizontally on the participants' chest on two standard electrocardiogram electrodes (3M™, Minnesota, US), one at the lower part of the sternum and the other one placed to the left as laterally as possible on the same horizontal level. The monitor was worn continuously for up to seven days, and participants were asked to maintain their normal activity patterns. Participants also completed a PA questionnaire: the 'Physical Activity Scale II' (PAS2), which inquires about usual time spent in various daily and weekly physical activities, including occupational and leisure time sitting, active commuting and moderate-to-vigorous PA(12).

Clinical measures

Weight was measured to the nearest 0.1 kg using a Tanita scale (Model TBF-300, Tokyo, Japan), with participants in light clothing and without shoes. Clothes were estimated to weigh 0.5 kg and this weight was deducted from the total weight. Measures of height were obtained using a fixed rigid stadiometer (Seca, Medical Scales and Measuring Systems, Hamburg, Germany). Waist circumference was measured midway between the lower rib margin and the

iliac crest to the nearest 0.5cm using a non-stretchable measuring tape (Xenical, US). Fitness level and maximal oxygen consumption were measured using the 'Watt max test'(13).

Glucose homeostasis markers

Venous blood samples were drawn after an overnight fast (≥8 hours), and 120 minutes after ingestion of a standard oral glucose tolerance test (75g glucose dissolved in 2.5dl water). Plasma insulin was analysed using a fluoro-immunoassay (Auto-DELFIA, Perkin Elmer, Massachusetts, US), and plasma glucose levels were analysed enzymatically by the hexokinase/G6PDH method (Hitachi 912, Roche Diagnostics, Basel, Switzerland). Glycated haemoglobin A_1, HbA_{1C}, was assessed using high pressure liquid chromatography (Tosoh G7, Roche Diagnostics, Basel, Switzerland). Homeostasis model assessment (HOMA2) insulin resistance and HOMA2-beta-cell function were calculated based on model-derived estimates using the HOMA2-calculator, version 2.2(14).

Data processing

Direct measures of PA obtained by the ActiHeart were downloaded using the manufacturer's software (www.camntech.com), cleaned and pre-processed using a Gaussian Regression method as described by Stegle et al(15). The combination of minute-by-minute data registered from accelerometry and heart rate to derive daily PA energy expenditure under normal life conditions was based on the 'branched equation model' as described by Brage et al(11). Heart rate data were converted to energy expenditure using a group calibration heart rate equation modified from the equation published by Brage et al (2007)(16). The group calibration was based on data from the INTERACT study(17). Accelerometry data were converted to energy expenditure using group calibrated accelerometry equations corresponding to walking or running acceleration(16). Basal resting metabolic rates were estimated based on the 2005 Oxford model by Henry et al(18). Branched equation modelling from raw time-series

measures was performed in STATA® version 10.0 (StataCorp, College Station, TX, USA)(19).

For each minute of ActiHeart wear-time, we derived: 1) PA energy expenditure, PAEE (kJ/kg); 2) heart rate above sleep, HRaS (beat per minute, bpm); 3) fraction of time spent in PA intensities expressed as multiples of predicted resting metabolic rate (METs): <1MET, 1-3MET and >3MET; and, 4) average sleeping heart rate, SHR (bpm). These measures were then summarized to hourly (PAEE) and daily measures of PAEE, HRaS, METs and SHR. From the self-report Physical Activity Scale(12), time spent in different PA domains (hours per day) and time spent in moderate-to-vigorous PA (minutes per week) were computed.

We defined 5 binary variables: 1) PA energy expenditure, PAEE, equal to or above the median value for the study population (≥ 35.23 kJ/kg/day) (yes/no); 2) Active transportation equal to or above the median value (0.25 hours/day) (yes/no); 3) Occupational sitting equal to or above four hours per day (self-reported) (yes/no); 4) Leisure time sitting equal to or above three hours per day (self-reported) (yes/no); and, 5) Moderate-to-vigorous PA equal or above 150 minutes per week (self-reported) (yes/no). These 5 variables were used as input for a latent class analysis.

Statistical analyses

Latent Class Analysis

A latent class model was used to classify the study population in groups based on the 5 defined binary variables(20); The 32 groups defined from the 5 binary variables are collapsed to a few groups, where a group is characterized by 5 posterior probabilities of a positive score for each binary variable. These posterior probabilities were reported as indicators of the group characteristics. Moreover, each person is assigned a posterior probability of belonging to each of the classes. We assigned each person to the class with the largest posterior probability. Since the number of classes must be chosen a priori for a particular analysis, we fitted models

with 1, 2, 3 and 4 classes, comparing the fit of the models using the Akaike Information Criterion(21) . When comparing different models with the same set of data, models with lower values are preferred(22). In addition, choice of the optimal number of classes was based on the distinctiveness and interpretability of the classes. In this study sample, the model with two classes was found to be most appropriate. The model was fitted by PROC LCA(23) in SAS version 9.2 (SAS Institute, Cary, NC).

Characteristics of the study population by class-membership

We compared demographic, behavioural and physiological characteristics between the latent classes identified, using linear models for normally distributed variables, the Kruskal-Wallis test for highly skewed variables, and Chi-square tests for testing equal distribution of proportions.

RESULTS

Of the 360 participants with valid ActiHeart (median ActiHeart wear-time: 6.8 [5.8; 6.9] days) and PAS2 data, 43 % were men. Mean age (SD) for the entire population was 46.9 (8.1) years and median PAEE was 35 kJ/kg/day. The participants included in the present study (n=360) did not differ from the eligible participants (n=795) in terms of gender distribution, age, time spent with moderate-to-vigorous PA, occupational sitting time, leisure time sitting, or active transportation (results not shown).

Latent class profiles

The LCA revealed two latent classes with different PA patterns: Class 1 (n=49, [14%]) was characterized by the highest probability of low PAEE and high level of occupational sitting. Class 2 (n=311, [86%]) was characterized by a high probability of high PAEE, more than four hours of occupational sitting per day, and more than 150 self-reported minutes per week of moderate-to-vigorous PA. The probability of active transportation and leisure time sitting was not different between the two groups (Figure 1).

Therefore, we named the two classes ‘inactive occupational sitters’ and ‘overall active exercisers’.

INSERT FIGURE 1 ABOUT HERE

When plotting the PAEE (J/kg/min) as a function of time of day, for each class, the same diurnal shape of PAEE emerged, albeit specific levels of PAEE were different (Figure 2 and Web only files Figure S1).

INSERT FIGURE 2 ABOUT HERE

Class-specific characteristics

The demographic, socioeconomic, behavioural and physiological characteristics of the two classes are shown in Table 1. The physiological characteristics, PA behaviour and measures of fasting serum insulin and insulin resistance were different between the groups. In contrast, we found no differences between the groups in socio-demographic characteristics or other behavioural characteristics (Table 1).

INSERT TABLE 1 ABOUT HERE

DISCUSSION

Class-specific physical activity patterns

In a generally healthy Danish population, we identified two subgroups with different PA patterns: 'inactive occupational sitters' and 'overall active exercisers'. Despite differences in PA accumulation between the two subgroups, their objectively measured PAEE showed similar diurnal patterns: lowest PAEE during the night and early mornings, and peaks around 8-9am, at 12-1pm, 5-7pm, and levelling off during the evening. Only a few studies have used the latent class analysis approach when exploring the PA patterns in adults(8;24;25). However, these and other previous studies utilizing the LCA approach in PA research performed in children or adolescents, have found LCA to be a valid method with regards to grouping individuals into different groups based on response-patterns(8;24-28).

Class-specific characteristics

We found the 'overall active exercisers' group to be younger than the 'inactive occupational sitters', which is in accordance with an earlier study showing PA levels to decrease with age in adults(29). The level for occupational sitting was consistent with findings from an Australian study of workers, suggesting occupational sitting time contributes to more than half of the overall sitting time during a day(30). However, in our study, the 'inactive occupational sitters' were dominated by women, whereas other studies have found men to have the highest amount of time spent in occupational sitting(30). This inconsistency may be due to the relatively well-educated study population and the fact that in Denmark a relatively high proportion of women have a higher education and are on the labour market, compared to other countries(31).

The lower levels of fasting insulin levels and insulin resistance among the 'overall active exercisers' in the present study is consistent with previous findings, suggesting that membership of the more active PA classes are associated with lower odds of all risk factors

for the metabolic syndrome(24). It is generally accepted that higher PA levels are associated with a lower risk of developing diabetes(2), and that time spent in sedentary behaviours (e.g. sitting and watching TV) is adversely associated with glucose metabolism(32;33). Recent interest has focused on the potential role of breaking up sedentary time on metabolic functions and has suggested breaks in sedentary time to be associated with a healthier cardio-metabolic profile(34;35). As such, despite the relatively high volume of sitting time in the 'overall active exercisers', they could potentially have more breaks in sedentary time, leading to a healthier metabolic profile. This hypothesis was, however, not explored in our study as there is currently no valid algorithm to define 'breaks' when using the ActiHeart monitor. However, as seen in Table 1, 'overall active exercisers' reported to spend more time with walking or standing at work than the 'inactive occupational sitters', suggesting more breaks in occupational sitting time.

Strengths and weaknesses of this study

The current study has a number of limitations that should be noted. First, the study population consisted of persons who were free of chronic illnesses and in generally good physical condition. As such, persons who are more physically active than the Danish background population might be overrepresented in this study. Though, the identification of 14% of the population with a lifestyle characterized by large amounts of prolonged sitting is surprising within a population assumed to be sufficiently physically active. It is possible, that this tendency might be even more pronounced in a heterogonous sample. Additionally, the clinical significance of the differences between the two subgroups may be more distinct in a more heterogeneous population. The modest number of participants may have influenced the number of the latent classes, although the number of classes was chosen based on model-fit using an established information criterion to guide the decision. Additionally, dichotomizing the variables by using other cut-points could have led to a somewhat different latent class

structure. However, the cut-points were based on either the observed median values for the entire population (similar to the median values of the eligible participants, n=795) or based on values from existing literature. Despite these limitations, the use of LCA in this study has several benefits: The classification of the participants into groups was based only on the individual's responses, hence exclusively data-driven. The combination of both objective and subjective measures of PA increased the dimensions of information on PA. While the subjective data gave insight into the context of the performed PA, the objective measurements of PAEE provided a valid and precise measure known to have low measurement error(10;36). This study provides an important descriptive insight into the PA patterns in a generally healthy and homogenous Danish population. This information can be used for the development and targeting of interventions and policies to promote: 1) overall PA, and, 2) reduction in sitting time. Based on these and other previous findings(34;35), strategies to avoid prolonged sitting should be encouraged. Additionally, as there is a dose-response relationship between PA and metabolic outcomes(37) and since recently published data suggests that even a minimum amount of low-volume PA seems to be associated with longevity(38); persons already engaging in some sort of PA should be encouraged to maintain a healthy lifestyle. Studies of PA promotion suggest that an individual's adherence to an exercise program is influenced by how similar other participants in the program are with respect to determinants such as age, gender, and PA level(6;7;39). Identifying characteristics of persons with similar PA patterns may provide useful knowledge for the design of future health promotion strategies aiming to increase PA through co-active exercise settings. Additionally, the information of hour-by-hour variation in PAEE could be used to identify periods during the day with particularly low PA levels.

We identified two subgroups in a generally healthy Danish population, characterized by different PA patterns: 'overall active exercisers' and 'inactive occupational sitters'. The

persons in the two subgroups had different physiological characteristics. In contrast, there were no differences in socio-economic characteristics between the groups. Compared to the 'inactive occupational sitters', the 'overall active exercisers' had significantly lower fasting insulin levels and insulin resistance, suggesting a more beneficial glucose metabolic profile in men and women who perform regular PA and avoid prolonged occupational sitting. When planning future public health strategies, the knowledge of potential population-specific subgroups with different PA patterns may be beneficial for tailoring and targeting interventions aimed at increasing PA level.

Acknowledgements

The authors are most grateful to the support staff and participants for their contribution to the study. The Health2008 study was funded by Timber Merchant Vilhelm Bang's Foundation, The Danish Heart Foundation and The Health Insurance Foundation (Helsefonden). Additionally, this work was supported by grants to A.L.S.H, awarded by Carpenter Sophus Jacobsen and wife Astrid Jacobsen's Foundation, and the Danish Medical Laboratory Technicians' Development and Research Foundation. A.L.S.H., D.V., and B.C. are employed by Steno Diabetes Center A/S, a research hospital working in the Danish National Health Service and owned by Novo Nordisk A/S. Steno Diabetes Center receives part of its core funding from unrestricted grants from the Novo Foundation and Novo Nordisk A/S. A.L.S.H., D.V., B.C., and D.R.W. own shares in Novo Nordisk A/S. J.W.H., A.L. and M.A. have no potential conflicts of interest.

Figure legends

Figure 1. Class-specific physical activity patterns. Item-response probabilities for the different classes. Black ='inactive occupational sitters' (class 1); White='overall active exercisers' (class 2). PAEE = physical activity energy expenditure. MVPA = moderate-to-vigorous physical activity

Figure 2. Class-specific patterns of median daily physical activity energy expenditure (PAEE J/kg/min) per time of day. Symbols represent classes: black triangles = 'inactive occupational sitters' (class 1); white circles= 'overall active exercisers' (class 2).

Web only files (supplemental material)

Figure S1. Class-specific median daily physical activity energy expenditure (J/kg/min) per hour of day. Boxes represent inter-quartile ranges. Whiskers indicate 1.5 x inter-quartile ranges.

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Tables

Table 1. Demographic-, socioeconomic-, and physiologic characteristics of the study population stratified by latent class membership

	Class		
	Inactive occupational sitters (1) (*n=49*)	**Overall active exercisers (2)** (*n=311*)	*P***
Characteristics			
Men (n, %)	17 (34.7)	136 (43.7)	0.234
Age (years)*	49.3 (6.8)	46.5 (8.3)	0.024
Employment status (n, % yes)	49 (100.0)	306 (98.4)	0.671
Education level (n, % high school or higher)	26 (54.2)	132 (42.7)	0.445
Marital status (n, % married)	33 (67.4)	215 (69.4)	0.465
Smoking (n, % current smoker)	8 (16.3)	60 (19.3)	0.315
Alcohol consumption (units per week, 1 unit=12g alcohol)§	7.0 (2.0; 15.0)	5.0 (2.0; 9.0)	0.084
Sleeping heart rate (bpm)*	58.9 (7.3)	54.8 (7.1)	<0.001
Heart rate above sleep (bpm)*	14.0 (2.6)	16.5 (4.1)	<0.001
Fitness level (VO_2max, mlO_2/kg/min)*	28.7 (6.5)	32.7 (7.5)	<0.001
Maximal oxygen consumption (lO_2/min)*	2.5 (0.7)	2.6 (0.7)	0.165
Physical activity energy expenditure (kJ/kg/day)§	27.3 (21.5; 30.2)	37.4 (28.3; 47.1)	<0.001
Time spent in physical activity intensity categories (hours/day) §			
<1 MET	10.4 (9.5; 11.7)	9.9 (8.8; 11.2)	0.107
1-3 MET	13.3 (11.9; 14.0)	12.9 (11.7; 14.0)	0.739
>3 MET	0.6 (0.3; 0.8)	1.0 (0.5; 1.4)	<0.001
Time spent in physical activity domains (hours/day)§			
Occupational sitting	6.5 (6.0; 7.5)	5.0 (2.0; 6.5)	<0.001
Standing/walking at work	1.3 (0.5; 2.0)	2.0 (1.0; 4.3)	<0.001
Strenuous work	0.0 (0.0; 0.0)	0.0 (0.0; 0.5)	<0.001
Active transportation (walking/cycling)	0.3 (0.0; 0.5)	0.3 (0.0; 0.6)	0.616
Height (cm)*	174.2 (11.4)	172.7 (8.9)	0.273
Weight (kg)*	79.2 (17.1)	76.1 (15.2)	0.191
Waist circumference (cm)*	90.3 (12.9)	87.7 (11.4)	0.160
Body Mass Index, BMI (kg/m^2)*	25.9 (4.1)	25.4 (4.1)	0.410
Glycated Haemoglobin, HbA_{1c} (mmol/mol)*	35.5 (3)	35.5 (3)	0.670
Glycated Haemoglobin, HbA_{1c} (%)*	5.4 (0.3)	5.4 (0.3)	0.670
Fasting serum insulin (pmol/l)*	40.1 (26.6)	32.1 (20.8)	0.017
Fasting plasma glucose (mmol/l)*	5.7 (0.7)	5.5 (0.5)	0.121
2-hours plasma glucose (mmol/l)*	5.5 (1.4)	5.4 (1.4)	0.584
Insulin resistance (HOMA2-IR)§	0.7 (0.5; 1.1)	0.6 (0.4; 0.9)	0.030
Insulin secretion (HOMA2-%B)§	62.2 (48.8; 74.8)	54.0 (44.2; 68.5)	0.069

* Mean (SD), §Median (1^{st}-3^{rd} quartile), ** P-value: ANOVA test for normally distributed variables, Kruskal-Wallis test for highly skewed variables, Chi-square test for proportions

Figures

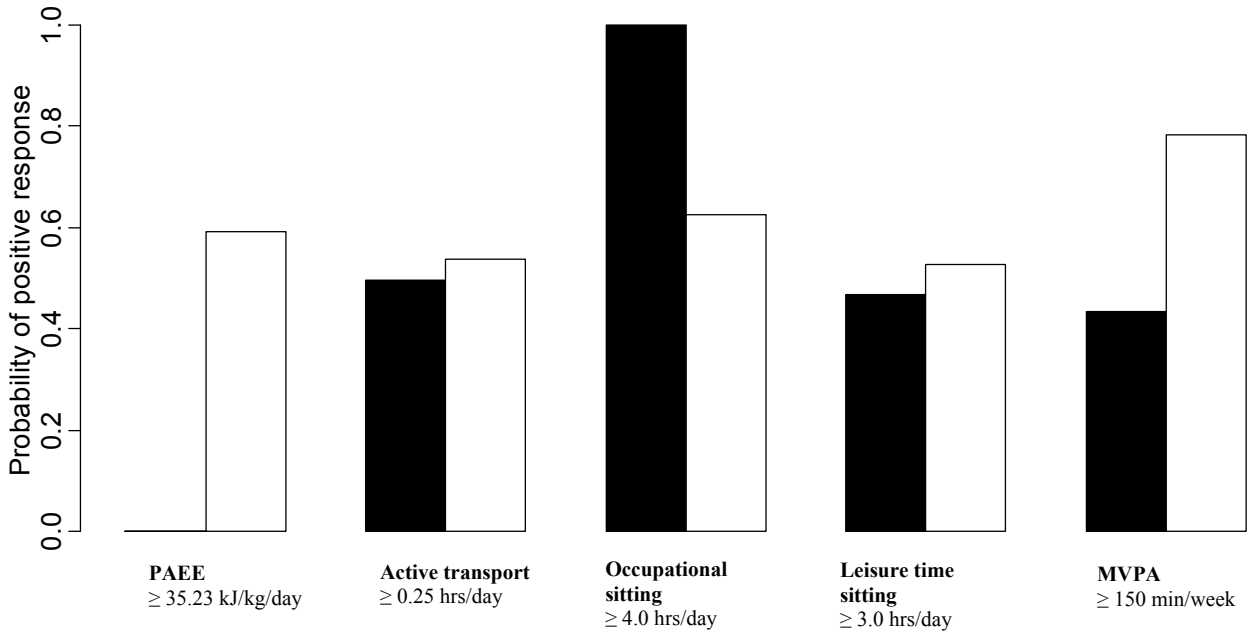

Figure 1. Class-specific physical activity patterns. Item-response probabilities for the different classes. Black ='inactive occupational sitters' (class 1); White='overall active exercisers' (class 2). PAEE = physical activity energy expenditure. MVPA = moderate-to-vigorous physical activity

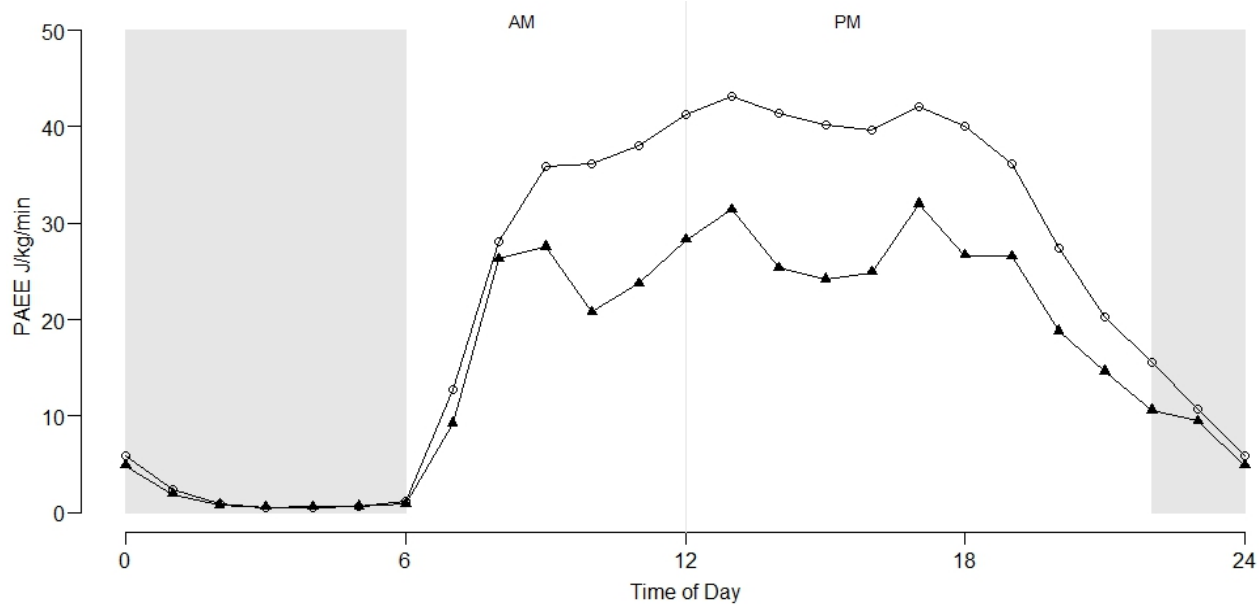

Figure 2. Class-specific patterns of median daily physical activity energy expenditure (PAEE J/kg/min) per time of day. Symbols represent classes: black triangles = 'inactive occupational sitters' (class 1); white circles= 'overall active exercisers' (class 2).

Supplemental Material

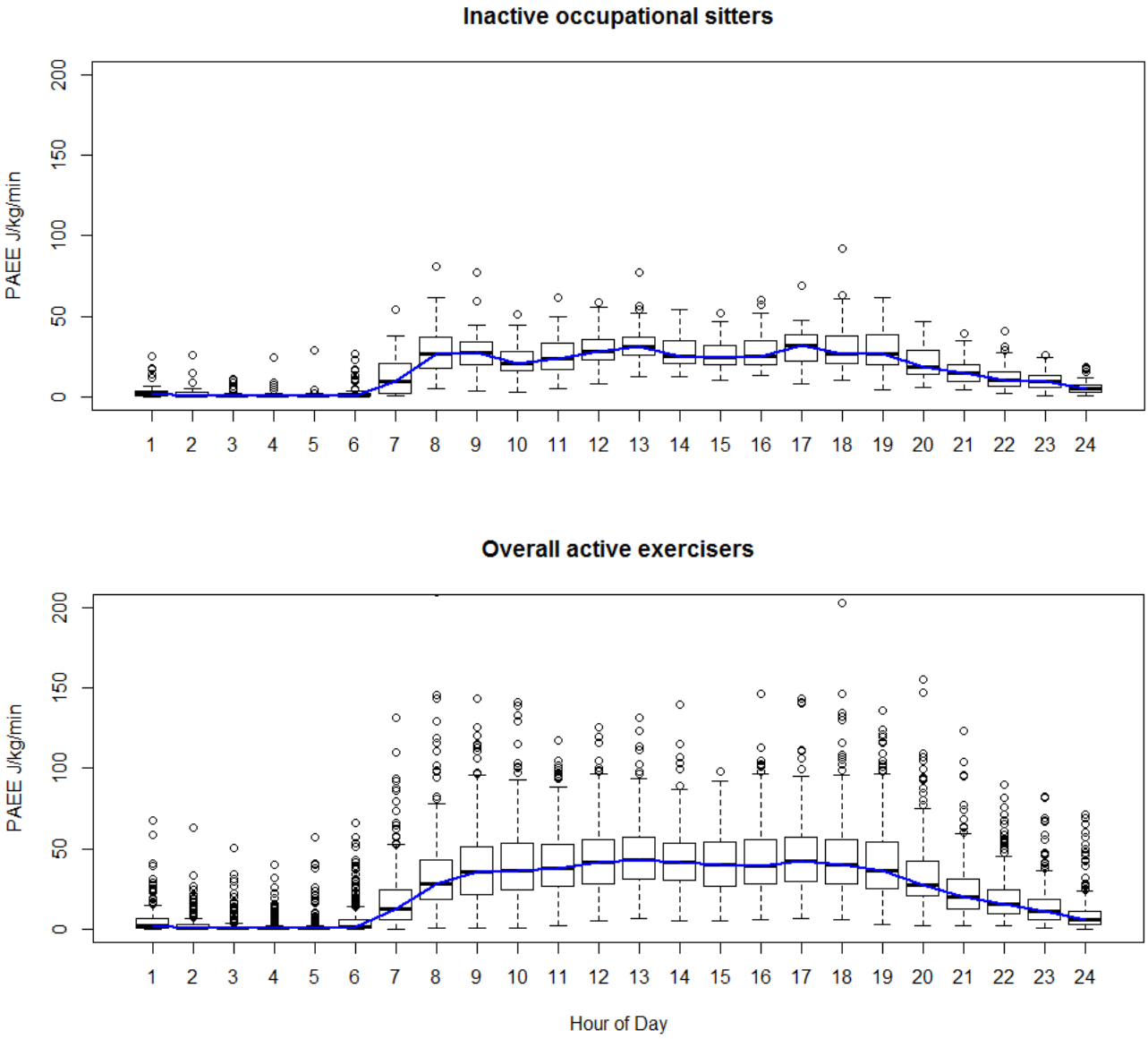

Figure S1. Class-specific median daily physical activity energy expenditure (J/kg/min) per hour of day. Boxes represent inter-quartile ranges. Whiskers indicate 1.5 x inter-quartile ranges.

PAPER II

Combined heart rate- and accelerometer- assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes. The ADDITION-PRO study

(*submitted to Diabetes Care*)

Title page

Title: Combined heart rate- and accelerometer- assessed physical activity energy expenditure and associations with glucose homeostasis markers in a population at high risk of developing diabetes. The ADDITION-PRO study

Authors: Anne-Louise S Hansen, MSc.[1]; Bendix Carstensen, MSc.[1]; Jørn W Helge, MSc., PhD[2]; Nanna B Johansen, MD[1]; Bibi V Gram, MSc., PhD[3]; Jens S Christiansen, MD, DMSc.[4]; Torsten Lauritzen, MD, DMSc[5]; Marit E Jørgensen, MD, PhD[1]; Mette Aadahl, MPH, PhD[6]; Daniel R Witte, MD, PhD[1,7], ADDITION-Denmark steering committee[*]

[1]Steno Diabetes Center A/S, Gentofte, Denmark; [2]Centre for Healthy Aging, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; [3]Department of Endocrinology, Hospital of South West Jutland, Esbjerg, Denmark; [4]Department of Endocrinology, MEA Aarhus University Hospital, Aarhus, Denmark; [5]Department of Public Health, Section of General Practice, Faculty of Health Sciences, Aarhus University, Aarhus, Denmark; [6]Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; [7]Centre de Recherche Public de la Santé, Strassen, Luxembourg

*ADDITION-Denmark Steering Committee: Marit E. Jørgensen, Steno Diabetes Center A/S, Gentofte, Denmark; Daniel R. Witte, Centre de Recherche Public de la Santé, Strassen, Luxembourg, Annelli Sandbæk, Department of Public Health, Section of General Practice, Faculty of Health Sciences, Aarhus University, Aarhus, Denmark; Torsten Lauritzen, Department of Public Health, Section of General Practice, Faculty of Health Sciences, Aarhus University, Aarhus, Denmark; Knut Borch-Johnsen, Institute of Public Health, University of Southern Denmark, Odense, Denmark.

Correspondence: Anne-Louise Smidt Hansen, Niels Steensensvej 1, building NLD 2.07, DK-2820 Gentofte. Fax: (+45) 4443 0706; tel: (+45) 4442 0111; E-mail: asih@steno.dk *or* al.smidthansen@gmail.com

Journal: Diabetes Care

Running title: Physical activity and glucose homeostasis markers in diabetes high risk individuals

Word Count: 3,999 (excl. abstract, references, and figure legends), abstract: 250 words

Tables/Figures: 3 tables, 1 figure, 1 online-only supplemental figure

Abstract

Objective: Regular physical activity (PA) reduces the risk of developing type-2-diabetes and different subtypes of dysglycemia have shown different associations with PA. To better understand the associations of PA and glucose homeostasis, we examined the association of objectively measured PA energy expenditure (PAEE) with detailed measures of glucose homeostasis.

Research Design and Methods: In 1,531 Danish men and women, with low-to-high risk of developing type 2 diabetes, we measured 7-days of PAEE using a combined accelerometry- and heart rate-monitor (ActiHeart). Measures and indices of glucose homeostasis were derived from a 3-point oral glucose tolerance test in addition to measures of long-term glycemia (glycated hemoglobin A_{1c} and Advanced Glycation Endproducts). Associations of PAEE with glucose homeostasis markers were examined using linear regression models.

Results: Median (IQR) age was 66.6 (62.1; 71.6) years (54% men) with a median ActiHeart wear-time of 6.9 (6.0; 7.1) days and PAEE level of 33.0 (23.5; 46.1) kJ/kg/day. In fully adjusted models, we found higher levels of PAEE to be positively associated with insulin sensitivity and negatively with insulin two hours after glucose load ($p<0.05$).

Conclusions: Even in an elderly population with relatively low levels of PA, we found higher objectively measured PAEE-levels to be associated with a more beneficial glucose metabolic profile. This indicates that even without high intensity exercise, increasing the overall level of PAEE slightly in an entire population at risk of developing type-2-diabetes may be a realistic and worthwhile goal to reach, in order to achieve beneficial effect in terms of glucose metabolism.

Regular physical activity (PA) reduces the risk of developing type-2-diabetes by 15-60%. In cross-sectional and prospective studies, PA of light intensity as well as moderate-to vigorous intensity have been related to a better glucose homeostasis (1-3), whereas other studies have found overall PA to be the main determinant of insulin sensitivity (1,4).Although some of the current evidence linking PA with glucose homeostasis has been established in large studies with prospective designs (2,5), epidemiological studies have traditionally used PA measures obtained by self-report methods, which are subject to bias. Even though heart rate monitors and accelerometers have increased in popularity as PA measurement methods (6), both have disadvantages in the assessment of PA (7). In contrast, PA monitors combining heart rate monitoring and accelerometry have shown to give more precise estimates of PA than the subjective methods and the separately measured accelerometry and heart rate (8). Even though the use of the combined PA monitors is increasing, no studies have yet studied the association between PA as measured with these monitors and detailed glucose homeostasis measures in larger population based studies. Previous epidemiological studies have investigated the association of PA measures with glucose homeostasis based on fasting and 2-hour samples of glucose and insulin (1,2,4,9). The use of more detailed indices of insulin resistance and beta cell function, based on a 3-point rather than a 2-point oral glucose tolerance test, may lead to deeper insight into the pathophysiological derangements which precede and lead to diabetes (10). Since subtypes of dysglycemia may show different associations with PA (5), including specific indices of peripheral insulin sensitivity, hepatic insulin resistance, beta cell function and the absolute insulin response to a glucose load, may provide a more detailed picture of the association of PA and glucose homeostasis. Furthermore, glycated hemoglobin A_{1c} (HbA_{1c}), which reflects the average glucose level over a longer period of time, has been suggested to be modifiable by exercise of moderate to vigorous intensity (11). Another measure of the even longer term load of protein glycation is

the skin accumulation of 'Advanced Glycation Endproducts' (AGE_{skin}) which is in part affected by hyperglycemia (12). AGE_{skin} has, in type-2-diabetes patients, been postulated to be associated with HbA_{1c} in cross-sectional studies and with cardiovascular disease and cardiovascular mortality in prospective studies (13,14). It is not known whether PA affects levels of AGE_{skin}, or whether associations of PA with HbA_{1c} and AGE_{skin} points in the same direction.

The current study tests the hypothesis that higher PA levels, as measured by a combined accelerometer- and heart rate- monitor, are associated with a better glucose metabolic profile. In addition to delineating the PA levels in a population at high risk of developing diabetes, we examined the association of accelerometer- and heart rate- assessed physical activity energy expenditure (PAEE) with various glucose homeostasis markers and long term glycemia in a cross-sectional design. Furthermore, we studied the course of glucose and insulin during a 3-point oral glucose tolerance test (OGTT) by different levels of PAEE, in order to better understand the pathophysiological pathways linking PA and glucose homeostasis.

Research Design and Methods

Study Design

We carried out a cross-sectional analysis based on PAEE and glucose homeostasis measurements at the 6-year follow-up health examination of a population at low-to-high risk of developing diabetes, recruited based on a stepwise screening procedure in 2001-2006 (15). Persons with different elevated diabetes risk profiles at the time of screening, but without diabetes, were invited to participate in the follow-up health examination (the ADDITION-PRO study) (16). Health examinations took place from 2009 to 2011 at four centers in Denmark. The study was approved by the ethical committee of the Central Denmark Region (journal no. 20080229) and was conducted in accordance with the 1996 Helsinki Declaration. All participants provided written informed consent.

Study Population

In total, 4,188 persons were invited to the ADDITION-PRO study. Of these, 2,082 persons (50% of invited) participated and underwent a health examination. A full description of the invitation procedure and the baseline diabetes risk groups: combined impaired fasting glycemia and impaired glucose tolerance (IFG+IGT); isolated impaired glucose tolerance (iIGT); isolated impaired fasting glycemia (iIFG); high diabetes risk but normal glucose tolerance (NGT); elevated-; moderate-; and, low- diabetes risk have been defined elsewhere (17). For the present analysis, participants with incident diabetes since screening (n=329) and participants fasting <8 hours prior to the health examination (n=11) were excluded. Of the 1,742 remaining participants, only participants with valid data in the outcome variables (markers of glucose homeostasis and long term glycemia) were included in the present analysis (n=1,531) (Supplemental Figure S1).

Measurement Methods

Information on age (years) and sex was derived from the unique Danish civil registration number. Employment status (yes/no), alcohol consumption (units per week, 1 unit equals 12grams of pure alcohol), and smoking status (current smoker/never smoker/ex-smoker) were obtained from a self-report questionnaire completed at the ADDITION-PRO examination day. Height in meters was measured without shoes to the nearest millimeter using a stadiometer (Seca, Hamburg, Germany), weight in kilograms was measured and rounded to the nearest 0.1 kg, using a Tanita scale with the participants wearing light indoor clothes but without shoes (Tanita Corporation Inc., Tokyo, Japan). Clothes were estimated to weigh 0.5 kg and thus this weight was subtracted from the total weight. Waist circumference, in centimeters, was measured to the nearest millimeter, at the mid-point between the lower rib margin and the iliac crest using an un-stretchable tape measure without any pressure on the skin.

Physical activity

PA was measured using a combined accelerometer- and heart rate- monitor (ActiHeart®, CamNTech Ltd., Cambridge, United Kingdom) (18).To ensure individual calibration of the heart rate to PA intensity, a sub-maximal step test was performed on the day of the health examination. The eight-minute step test was administered from the ActiHeart software to indicate the cycles of stepping up and down a 20.5cm step bench (Rucanor Europe B.V., Nieuwerkerk, The Netherlands). The stepping frequency ranged from 15 to 33 step cycles per minute over the duration of the test, followed by a two-minute recovery period (sitting). After participants had completed the step test, the monitor was set up to record long-term PA, registering movement and heart rate every 60 seconds. The monitor was placed horizontally on the participants' chest with two standard electrocardiogram electrodes (Maxensor, Alton, United Kingdom), one at the lower part of the sternum and the other one to the left on the same horizontal level, as laterally as possible. Participants were asked to wear the monitor for seven days and nights and to maintain their usual PA pattern during the period. Participants additionally completed a PA questionnaire, a modified (Danish) version of the Recent Physical Activity Questionnaire (RPAQ) (19), asking about type, frequency, intensity, and context of PA performed in the last four weeks prior to the health examination. Additionally, as part of the general questionnaire, participants categorized their typical leisure time PA (modified from Saltin & Grimby (20)) as: '1. Mainly sedentary' (e.g., reading, watching television or movies); '2. Low physical activity level': Engaging in light physical activities for more than 4 hours per week (e.g. leisurely walking, leisurely cycling, light do-it-yourself tasks, light house chores, table tennis and bowling); '3. Moderate physical activity level': engaging in sports or exercises minimum three times per week or vigorous leisure time activities (e.g. heavy gardening); or, '4. High physical activity level': Engaging in competitive sports or long distance running several times per week.

Glucose homeostasis markers

At the health examination, venous blood samples were drawn after a verified overnight fast (≥8.0 hours). Following this, participants drank a glucose drink (75g glucose dissolved in 250ml water) as part of a standardized OGTT (21), with blood samples drawn 30 and 120 minutes after the glucose intake. Plasma glucose (0, 30, 120 min) was determined using the Hitachi 912 system (Roche Diagnostics, Mannheim, Germany) from 2009 to April 2010. From April 2010, glucose was assessed using the Vitros 5600 system (Ortho Clinical Diagnostics, IllkirchCedex, France). Since the agreement between the methods was modest, all 'Vitros'-values were converted to the 'Hitachi'-values, using the regression equation from a method comparison (adjusted glucose value = original glucose value + 0.2637/0.983). Serum insulin (0, 30, 120 min) was determined by an immunoassay method (AutoDELFIA, Perkin Elmer,Massachusetts, UnitedStates). HbA_{1c} was determined by high performance liquid chromatography (TOSOH G7, Tokyo, Japan). AGE_{skin} was assessed using skin autofluorescence (AGE Reader SU, Diagnoptics Technologies B.V., Groningen, The Netherlands). Glycemic indices were derived from glucose and insulin measures from the OGTT. Measures of insulin resistance were derived from homeostasis model assessment (HOMA-IR (mmol/l xmU/L) = fasting plasma glucose (mmol/l) x (fasting plasma insulin (pmol/l)/6.945)/22.5 (22). The insulin sensitivity index ($ISI_{0,120}$) was calculated according to Gutt et al. (23) to give an estimate of insulin sensitivity in the peripheral tissues. Beta-cell function was determined by calculating the disposition index (24). To do this, first phase insulin release was calculated as described by Stumvoll et al (25). Disposition index (DI) was then calculated using the following formula: DI = first phase insulin release$_{Stumvoll}$ x $ISI_{0,120}$. Absolute insulin response to the glucose load was determined by calculating the insulinogenic index$_{t30min}$ (26).

Data processing

Physical activity measures

Heart rate and accelerometry measures from the ActiHeart monitor were downloaded to the manufacturer's software (www.camntech.com). Noisy heart rate measures were reduced and periods of non-wear were inferred from the combination of non-physiological heart rate and prolonged periods of inactivity (to minimize diurnal information bias when summarizing the intensity time-series into PAEE measures), using the procedure published by Stegle et al (27). PA measures were derived by combining minute-to-minute heart rate and accelerometry measures using a 'branched equation model' (18). The relation between heart rate and PAEE was calibrated using data from the individually performed sub-maximal step test. Based on 1,046 ADDITION-PRO participants with a valid step test, a 'group calibration' was derived based on regression coefficients from the heart rate to PAEE relationship. This group calibration was then used to calibrate the relation between heart rate and PAEE for participants who did not perform the sub-maximal step test, including information on sex, age, and sleeping heart rate of the individual (28). Accelerometry data were converted to energy expenditure using equations corresponding to walking or running (28). The 2005 Oxford Model (29) was used to estimate basal metabolic rate. ActiHeart provided minute-by-minute measurements of PAEE in kJ/kg. From these, fraction of time (per hour) spent in PA intensity groups, expressed as multiples of predicted resting metabolic rate (METs), were derived. All measures were summarized to daily measures. Only measures from participants with a minimum of 24 hours of ActiHeart wear-time were considered valid for the present analysis. From the RPAQ, time (hours per week) spent in different activities was computed.

Statistical analyses

For all explanatory variables, we performed multivariate imputation by chained equations (MICE) procedure (30), using 50 imputations. We examined the association of daily PAEE

(kJ/kg/day) with the different glucose homeostasis markers and long term glycemia using multiple linear regression analyses. We adjusted for baseline diabetes risk group based on the results of the ADDITION-Denmark stepwise screening procedure performed in 2001 to 2006 (15) to control for different invitation procedure and as an indicator of participants' clinical history. Further adjustments included age, sex, employment status, smoking, and alcohol consumption. Furthermore, due to the potential confounding role of obesity on the link between PA and glucose homeostasis (31), adjustments for waist circumference were included in the full model.

Repeated measures of glucose and insulin (at 0, 30, and 120- minutes during the OGTT), were analyzed by a random effects mixed model with random slope of time since glucose intake. The plasma glucose and serum insulin trajectories for different levels of PAEE were plotted over time since glucose load accounting for age, sex and diabetes risk group. All statistical analysis were performed in R statistical software program version 2.15.0.

Results

Demographical-, clinical-, and behavioral- characteristics for the study sample are presented in Table 1. The median (25^{th}; 75^{th} percentile) ActiHeart wear-time was 6.9 (6.0; 7.1) days. Median PAEE was 33 (23.5; 46.1)kJ/kg/day, amounting to 2591 kJ/day for a person weighing 78.5 kg (median weight of the study population). Over 24-hours, 72% of the time was spent ≤1.5 METs (including sleeping), 23% of the time was spent with light intensity activities (>1.5 to 3.0 METs), and 5% with PA of moderate-to-vigorous intensity (≥3METs) (Table 2). The majority of the population (66%) reported a leisure time characterized by 'low PA level' with e.g. walking, cycling, and light home activities (Table 2), whereas one quarter of the population reported having a 'moderate PA level' during leisure time participating in sports or exercising more than three times per week. Only 9% of the population reported to have leisure

time characterized as ‘mainly sedentary’. Median values of glucose homeostasis markers revealed a population with incipient deterioration in glucose metabolism (Table 1).

INSERT TABLE 1 & 2 ABOUT HERE

In models adjusting for age, sex, diabetes risk group (at baseline screening), occupation, alcohol intake, and smoking status, PAEE was positively associated with insulin sensitivity $index_{0,120}$ and negatively associated with 2-hours plasma glucose, plasma serum insulin (0-, 30-, 120-minutes), and with HOMA-insulin resistance (Table 3, model 2). After additionally adjusting for waist circumference these associations remained significant for 120-minutes plasma insulin and insulin sensitivity $index_{0,120}$ only (Table 3, model 3).

INSERT TABLE 3 ABOUT HERE

Figure 1 illustrates the effects shown in the previous models for an example of a 66 year-old man in the baseline diabetes risk group ‘high diabetes risk but normal glucose tolerance’. The modeled values indicate a more rapid glucose uptake with higher PAEE level (Figure 1a), while the log-insulin response to the glucose load is lower the higher PAEE levels (Figure 1b).

INSERT FIGURE 1 ABOUT HERE

Conclusions

We found that in a population at low-to-high diabetes risk, objectively assessed PA levels were generally of light intensity, but nonetheless positively associated with insulin sensitivity and negatively associated with insulin concentration two hours after glucose load.

Physical activity patterns

We found the median PAEE level to be 33 kJ/kg/day, equaling 2,590 kJ/day for a person weighing 78.5 kg. In comparison, Vaughan et al. (1991) found slightly lower mean PAEE (~2,190kJ/day) in a slightly older (mean age: 71 years) American population as measured by a respiratory chamber method (32). In different European populations, PAEE levels seem to differ markedly according to the assessment method. Adult populations where PAEE has been assessed by heart rate monitors alone (9) show higher PAEE levels than adult populations where PAEE have been assessed by accelerometry alone (33). This is consistent with the known disadvantages of the heart rate and accelerometer monitors, where heart rate monitors tend to over-estimate PAEE and accelerometers tend to under-estimate PAEE as compared to gold standard methods. However, in a healthy Danish sample (mean age: 58 years old), the median ActiHeart-assessed PAEE level was found to be 40 kJ/kg/day or approximately 3,140 kJ/day for a person weighing 78.5 kg (34). In UK, the PAEE levels were somewhat lower (35 kJ/kg/day) in a slightly older population (34). Thus, when taking the population specific characteristics into account (age and anthropometric measures), our results are comparable with those of other studies using combined heart rate and accelerometry to estimate PAEE level.

In accordance with other findings in aging populations (35), the most prevalent leisure time PAs included walking, gardening, and cycling. Time spent in different PA intensity categories was consistent with reports from other western populations (3,4). We saw a tendency towards more time spent in lower PA intensities, possibly due to the slightly older population and the

fact that the majority of the population consisted of individuals with a higher diabetes risk score (performed at screening), which included physical inactivity. The majority of the population reported to have low PA levels in leisure time, consisting of mainly household activities and some physical movement (≥4 hours per week). This reveals a population where PAEE is mainly composed of 'daily activities' rather than by regular exercise sessions.

Physical activity energy expenditure and glucose homeostasis

Heart rate and accelerometer measured PAEE in daily life was positively associated with peripheral insulin sensitivity ($ISI_{0,120}$) and negatively associated with 2-hours insulin response after a glucose load. Our findings are consistent with those of other studies using accelerometer (4) and questionnaire (36) based estimates of PAEE. Others have found higher PA levels (as measured by questionnaire as well as by heart rate monitors and accelerometry) to be associated with lower HOMA-insulin resistance (37), fasting serum insulin (1), and 2-hours plasma glucose levels (38) even when adjusting for body composition measures. In our study, when additionally adjusting analyses for waist circumference, the associations with fasting-, and 30-minutes plasma insulin levels, HOMA-insulin resistance, and 2-hours plasma glucose levels were attenuated and lost statistical significance, probably due to the small effect size in this elderly population performing mainly sedentary activities and light intensity PA. The reported 10kJ/kg/day increment in PAEE level (which would approximate one hour of walking with a pace of 3.2 km per hour for a person weighing 73kg) would result in a 1% increment in peripheral insulin sensitivity; a small, but clinically relevant increment if seen in conjunction with other metabolic improvements and non-metabolic benefits of being physically active. We did not find any association of PAEE with indices of the beta cell function (disposition index and insulinogenic index) or with measures of long term glycemia (HbA_{1c} and $AGEs_{skin}$), indicating peripheral glucose uptake to be most important with regards to explaining the association of PAEE with glucose homeostasis in persons performing

mainly light intensity PA. This is in line with other studies showing high volume but not low volume exercise to be associated with a decrease in HbA_{1c} (11).

Glucose uptake and insulin response during the OGTT

Our models, although cross-sectional, indicate that an increment in PAEE level would result in a more rapid decline in glucose concentrations from 30 to 120 minutes after the OGTT (Figure 1a) rather than in differences in fasting or 30-minute glucose levels. This finding is in line with laboratory studies showing that persons with high PA have better glucose uptake than persons with lower PA level (39), due to the increased glucose transport activity in skeletal muscles as a response to muscle contraction. Furthermore, the effects of PA on peripheral insulin sensitivity have, in laboratory studies and exercise interventions, been suggested to be mainly due to an increased oxidative capacity and mitochondrial function in muscles (40). As such, despite starting at almost the same fasting plasma glucose levels and ending with slightly different 2-hours plasma glucose levels by different PAEE levels, our results suggest that persons with a higher PAEE level spend less time at the highest levels concentrations of circulating glucose. The initial insulin response to glucose load was almost the same (equally steep slope) for all PAEE levels (Figure 1b). However, 2-hours insulin seemed to be lower for higher PAEE levels and with a slightly steeper slope from 30 minutes to 120 minutes, probably due to a higher insulin action in persons with a high PAEE level since they are more likely to have higher peripheral insulin sensitivity (table 3).

We found that only insulin sensitivity $index_{0,120}$ and 2-hours insulin levels were associated with PAEE, after examining several detailed measures of glucose homeostasis including derived indices. Although the associations found were modest in magnitude, it is encouraging that these differences are observable even within a population with only modest PA levels. This indicates that even without high intensity exercise, aiming to increase the overall level of

PAEE by small but reasonable amounts in an entire population at risk of developing type 2 diabetes, may be a realistic and worthwhile goal to aim for from a public health perspective.

Strengths and limitations

A major strength of this study is the large number of participants with objectively measured PA during daily living. Although, the population approach hindered the use of gold standard methods of glucose regulation (e.g. the use of a euglycemic clamp test to determine insulin sensitivity), we did obtain the most precise measures of glucose homeostasis achievable in an epidemiological setting.

We examined the association of objectively measured PAEE with glucose homeostasis markers in a population with different risk profiles for developing type 2 diabetes. Since participants were invited and included in the study according to their diabetes risk, their PA level could potentially be affected by their risk status, as participants with different risk status could have been approached differently by their general practitioners regarding prevention strategies for type 2 diabetes (e.g. advice on enhancing PA level). To account for this, we adjusted for baseline diabetes risk group as a surrogate measure of the participants' clinical history. Even though this could potentially have resulted in over-adjustments of the analysis, our results are more likely to be generalizable to other populations when including the adjustments. Although based on objective measurements, the obtained PAEE levels are results of estimations by modeling of heart rate and accelerometry data. Hence, PAEE is not measured directly. Lastly, because of the cross-sectional design of the current study, we cannot be sure about the directions of causality of the associations found.

In spite of the above listed limitations, our study documents and confirms the associations of PAEE with peripheral insulin sensitivity by using robust measures of objectively measured PAEE and insulin sensitivity in an epidemiological setting.

Even in an elderly population with relatively low levels of PA, performed mainly during daily activities, we found a positive association of heart rate and accelerometer assessed PAEE with insulin sensitivity and a negative association with insulin concentration two hours after a glucose load. This indicates that even without high intensity exercise, increasing the overall level of PAEE sligthly in an entire population at risk of developing type 2 diabetes, may be a realistic and worthwhile goal, when aiming to achieve beneficial effects in terms of glucose metabolism. When looking into the activities of the elderly population, suggestions to increase overall PAEE level could include brisk walking, cycling, and in general, increased activity levels during home-based activities.

Acknowledgements

A.L.S.H collected and researched data, contributed to discussion and wrote the manuscript; B.C. researched the data, contributed to discussion, and, reviewed and edited the manuscript; D.R.W. , T. L. and N.B.J. designed the study, contributed to discussion, and, reviewed and edited the manuscript; M.A. , J.W.H. , M.E.J., J.S.C., and B.V.G. contributed to discussion and reviewed and edited the manuscript. All authors approved the final version of the manuscript. A.L.S.H is the paper guarantor.

A.L.S.H., B.C., N.B.J., M.E.J. and D.R.W. are employed by Steno Diabetes Center A/S, a research hospital working in the Danish National Health Service and owned by Novo Nordisk A/S. Steno Diabetes Center receives part of its core funding from unrestricted grants from the Novo Foundation and Novo Nordisk A/S. A.L.S.H., B.C., N.B.J., M.E.J., T.L., and D.R.W. own shares in Novo Nordisk A/S. J.W.H., B.G., J.S.C., and M.A. have no potential conflicts of interest.

The ADDITION-Denmark study was supported by the National Health Services in the counties of Copenhagen, Aarhus, Ringkøbing, Ribe, and Southern Jutland in Denmark; the Danish Council for Strategic Research; the Danish Research Foundation for General Practice;

Novo Nordisk Foundation; the Danish Centre for Evaluation and Health Technology Assessment; the Diabetes Fund of the national Board of Health; the Danish Medical Research Council; and, the Aarhus University Research Foundation. Additionally, the ADDITION-PRO study was funded by an unrestricted grant from the European Foundation for the Study of Diabetes/Pfizer for Research into Cardiovascular Disease Risk Reduction in Patients with Diabetes (74550801), by the Danish Council for Strategic Research, and by internal research and equipment funds from Steno Diabetes Center. A.L.S.H. additionally received scholarship funding from the Capital Region of Denmark. We acknowledge the ADDITION-PRO study centers and are most grateful to the support staff and the participants for their contribution to the study. We furthermore acknowledge Søren Brage and Kate Westgate, MRC Epidemiology Unit, Cambridge, UK, for guidance in processing the PA data from the ActiHeart monitor.

Figure legends

Figure 1. Glucose concentration (mmol/l) (A) and log-insulin concentration (B) per time since glucose load for a 66 year-old man with baseline high diabetes risk but normal glucose tolerance by different PA levels (10-70 kJ/kg/day)(black=10 kJ/kg/day, light grey=70kJ/kg/day).

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Tables

Table 1. Demographical- , behavioral-, and clinical-, characteristics of the ADDITION-PRO population (n=1,531)

Characteristic	*Number of participants with available data*	*Median (IQR) or n (%)*
Demographical		
Men, n (%)	1531	825 (53.9)
Age (years)	1531	66.6 (62.1; 71.6)
Occupation, n (% working)	1528	589 (38.5)
Behavioral		
Alcohol consumption (units per week)	1285	7.0 (3.0; 14.0)
Smoking status, n (% yes)	1527	254 (16.6)
Clinical		
Height (m)	1531	1.71 (1.64; 1.78)
Weight (kg)	1531	78.5 (68.2; 88.4)
BMI (kg/m2)	1531	26.7 (24.1; 29.6)
Waist circumference (cm)	1531	94.9 (86.0; 103.8)
Glucose homeostasis markers		
Fasting plasma glucose (mmol/l)	1531	5.9 (5.6; 6.3)
30-minutes plasma glucose (mmol/l)	1531	9.1 (8.1; 10.1)
120-minutes plasma glucose (mmol/l)	1531	6.3 (5.3; 7.7)
Fasting plasma insulin (pmol/l)	1531	37.0 (25.0; 56.0)
30-minutes plasma insulin (pmol/l)	1531	221.0 (156.0; 321.5)
120-minutes plasma insulin (pmol/l)	1531	191.0 (112.0; 320.0)
Glycated hemoglobin A1c, HbA_{1c} (%)	1531	5.7 (5.5; 5.9)
Glycated hemoglobin A1c, HbA1c (mmol/mol)	1531	39 (37; 41)
Advanced glycation endproducts in skin (AU)	1531	2.3 (2.0; 2.6)

Median (1st quartile; 3rd quartile) if not stated else.

Table 2. ActiHeart-assessed and self-reported physical activity characteristics of the ADDITION-PRO population (n=1,531)

	Number of participants with available data	
Heart rate and accelerometry assessed physiological measures average for days with ActiHeart monitoring		*Median (IQR) or n (%)*
Physical activity energy expenditure (kJ/kg/day)	1184	33.0 (23.5; 46.1)
Sedentary activities incl. sleeping, ≤ 1,5 METs (hours/day)	1184	17.2 (15.5; 19.0)
Light intensity physical activity, 1.5-3.0 METs (hours/day)	1184	5.4 (4.1; 6.8)
Moderate intensity physical activity, ≥3.0-6.0 METs (hours/day)	1184	1.1 (0.5; 1.9)
Vigorous intensity physical activity, ≥6.0 METs (hours/day)	1184	0.0 (0.0; 0.0)
Self-report leisure time category, n (%)	*1520*	
Mainly sedentary (mainly sitting activities)		136 (8.9)
Low PA level (physical movement ≥ 4 hours per week)		1008 (66.0)
Moderate PA level (sports and exercise ≥ 3 times per week)		370 (24.2)
High PA level (elite sports several times per week)		1 (0.1)
Watching TV (hours/day)	1481	2.8 (2.0; 3.6)

Median (1st quartile; 3rd quartile) if not stated else. PA= physical activity

Table 3. Associations of physical activity energy expenditure with glucose homeostasis markers (n=1531)

Determinant	*Glucose homeostasis markers*						
	Difference per 10kJ/kg/day PAEE increment (ß-estimates and 95% CI)						
PAEE (kJ/kg/day)	**Fasting plasma glucose (mmol/l)**	**30-minutes plasma glucose (mmol/l)**	**120-minutes plasma glucose (mmol/l)**	**HbA_{1c} (%)**	**HbA_{1c} (mmol/mol)**	**AGEs (AU)**	
Model 1	-0.01 (-0.03; 0.00)	-0.04 (-0.07; -0.01)*	-0.08 (-0.13; -0.03)*	-0.01 (-0.01; 0.00)	-0.03 (-0.03; 0.08)	-0.01 (-0.02; 0.01)	
Model 2	-0.01 (-0.02; 0.01)	-0.03 (-0.06; 0.00)	-0.05 (-0.09; -0.01)*	-0.00 (-0.01; 0.01)	0.08 (-0.03; 0.19)	-0.00 (-0.01; 0.01)	
Model 3	-0.00 (-0.02; 0.01)	-0.02 (-0.04; 0.01)	-0.03 (-0.07; 0.01)	0.00 (-0.01; 0.01)	0.08 (-0.03; 0.19)	-0.00 (-0.01; 0.01)	
	*% Decrement per 10kJ/kg/day PAEE increment (95% CI)***						
	Fasting plasma insulin	**30-minutes plasma insulin**	**120-minutes plasma insulin**	**Insulin sensitivity index (0-120)**	**HOMA-insulin resistance**	**Disposition index**	**Insulinogenic index ($\Delta I_{30}/\Delta G_{30}$)**
Model 1	2.5 (1.1; 3.8)§	2.1 (0.8; 3.3) §	4.1 (2.1; 6.1) §	-2.0 (-3.2; -1.0) §	2.8 (1.0; 4.4) §	-0.8 (-2.4; 0.8)	0.50 (-1.0; 1.9)
Model 2	2.1 (0.7; 3.4)*	1.9 (0.7; 3.2)*	3.4 (1.5; 5.5) §	-1.6 (-2.6; -0.6)*	2.2 (0.5; 3.9)*	-0.2 (-1.7; 1.3)	0.80 (-0.7; 2.2)
Model 3	1.0 (-0.1; 2.0)	1.1 (0.0; 2.2)	2.5 (0.8; 4.1)*	-1.0 (-1.9; -0.2)*	1.0 (-0.2; 2.2)	-0.3 (-1.8; 1.3)	0.20 (-1.2; 1.7)

* $p<0.05$ § $p<0.001$
**Estimates from plasma insulin and derived indices are back-transformed from naturally log transformed values
Model 1: adjusting for age, sex
Model 2: adjusting for age, sex, diabetes risk group at baseline screening, occupation, alcohol intake, smoking status
Model 3: adjusting for age, sex, diabetes risk group at baseline screening, occupation, alcohol intake, smoking status, and waist circumference
PAEE=physical activity energy expenditure

Figures

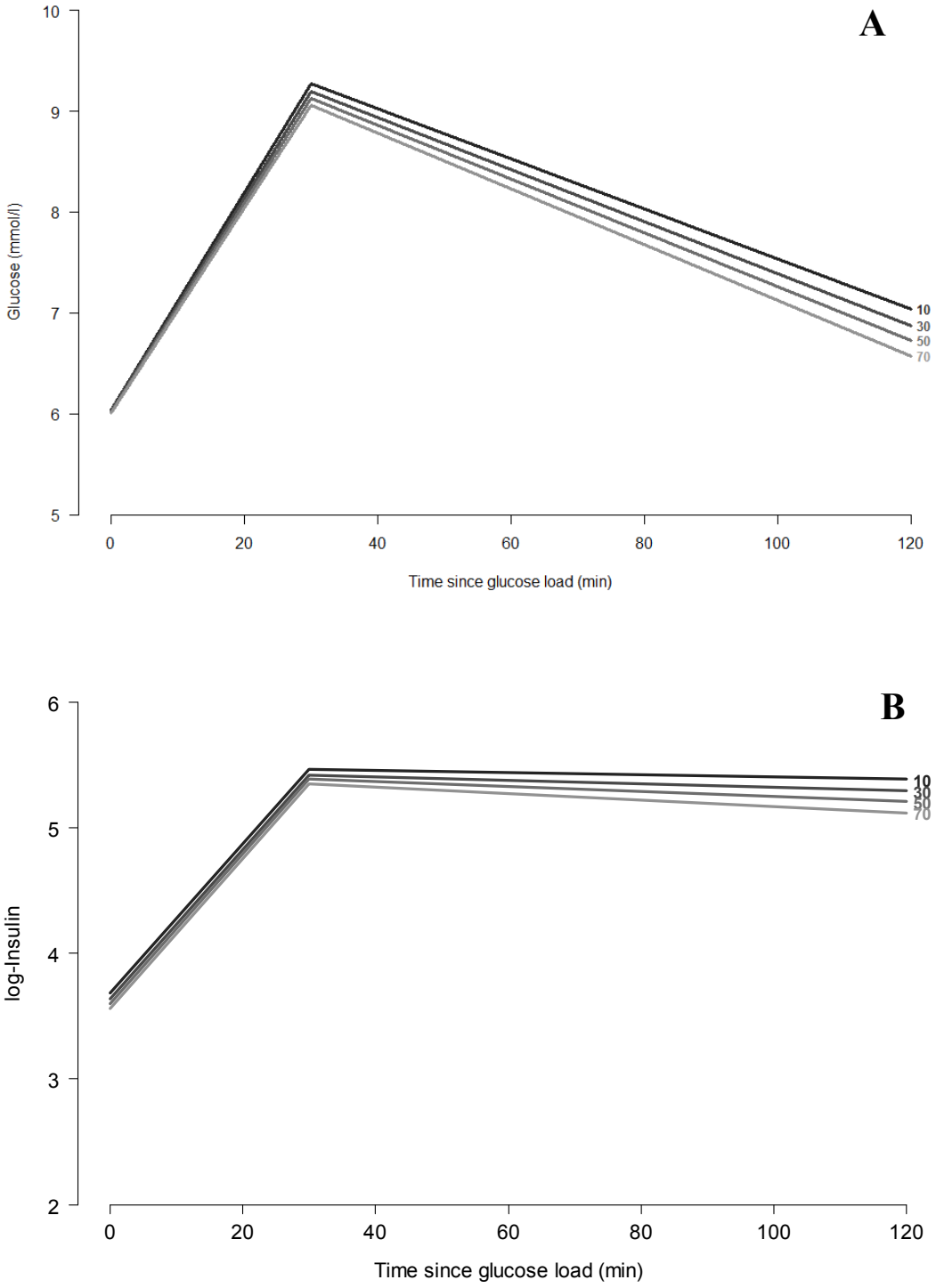
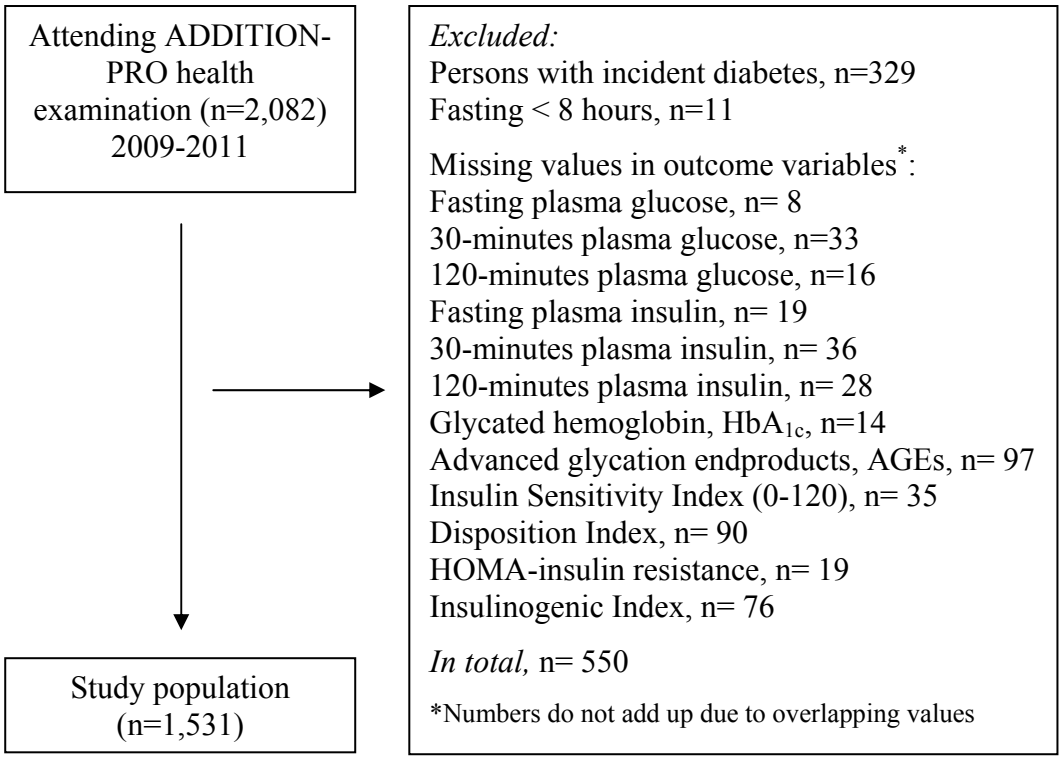


Figure 1. Glucose concentration (mmol/l) (A) and log-insulin concentration (B) per time since glucose load for a 66 year-old man, with baseline high diabetes risk but normal glucose tolerance, by different physical activity levels (10-70 kJ/kg/day)(black=10 kJ/kg/day, light grey=70kJ/kg/day).

Online-only supplemental material



Supplemental Figure S1. Flow chart of the ADDITION-PRO study population

PAPER III

Adverse associations of increases in television viewing time with 5-year changes in glucose homoeostasis markers: the AusDiab study

(*Diabetic Medicine 2012;29:918-925*)

DOI: 10.1111/j.1464-5491.2012.03656.x

Article: Epidemiology

Adverse associations of increases in television viewing time with 5-year changes in glucose homoeostasis markers: the AusDiab study

A. L. S. Hansen[1,2], K. Wijndaele[3,4,5], N. Owen[2,6], D. J. Magliano[2,7], A. A. Thorp[2], J. E. Shaw[2,7] and D. W. Dunstan[2,6,7,8,9]

[1]Steno Diabetes Center A/S, Epidemiology Group, Gentofte, Denmark, [2]Baker IDI Heart and Diabetes Institute, Melbourne, Vic., Australia, [3]Research Foundation Flanders (FWO), Brussels, [4]Department of Movement and Sports Sciences, Ghent, Belgium, [5]MRC Epidemiology Unit, Cambridge, UK, [6]The University of Queensland, School of Population Health, Brisbane, Qld, [7]School of Public Health and Preventive Medicine, Monash University, Melbourne, [8]School of Exercise and Nutrition Sciences, Deakin University, Melbourne, Vic. and [9]ECU Health and Wellness Institute, Edith Cowan University, Perth, WA, Australia

Accepted 16 March 2012

Abstract

Background Television viewing time is associated cross-sectionally with abnormal glucose tolerance and diabetes risk; however, the impact of changes in television viewing time on glycaemic measures is less understood. We examined relationships of 5-year change in television viewing time with 5-year change in glucose homeostasis markers.

Methods Participants in the Australian Diabetes, Obesity and Lifestyle study with data available at the 1999–2000 baseline and the 2004–2005 follow-up were included (4870; 45% men). Television viewing time (h/week) was assessed by questionnaire. Fasting plasma glucose, serum insulin and 2-h plasma glucose were obtained from an oral glucose tolerance test. Beta-cell function and insulin resistance were ascertained using the homeostasis model assessment 2-calculator. Associations of change in television viewing time with changes in glucose homeostasis markers were examined using linear regression models [β-coefficients (95% CI)]. Adjustments included baseline measures of age, television viewing time and glycaemic marker, and baseline and 5-year change in diet quality, energy intake, physical activity and waist circumference.

Results For every 5-h per week increase in television viewing time from baseline to 5-year follow-up, changes in glucose homeostasis markers were observed: among women there was a significant increase in fasting plasma glucose [0.01 (0.00–0.02) mmol/l] insulin resistance [0.03 (0.01–0.05)] and insulin secretion [1.07 (0.02–2.12) %]; insulin levels increased [men: 1.20 (0.30–2.09); women: 1.06 (0.32–1.80) pmol/l]; in men, 2-h plasma glucose levels increased [0.06 (0.01–0.1) mmol/l].

Conclusion Five-year increases in television viewing time were associated adversely with changes in glucose homeostasis markers. These findings add to earlier cross-sectional evidence that television viewing time can be associated with biomarkers of diabetes risk.

Diabet. Med. 29, 918–925 (2012)

Keywords glucose homeostasis, prospective studies, sedentary behaviour, television viewing

Abbreviations AusDiab, Australian Diabetes, Obesity and Lifestyle; HOMA2, homeostasis model assessment; TV, television

Introduction

Television (TV) viewing time, a highly prevalent sedentary behaviour [1], is associated cross-sectionally with abnormal glucose homeostasis [2,3] and increased cardio-metabolic risk [4,5]; prospective studies also show associations with premature mortality risk [6]. While post-exercise mechanisms leading to improved glucose homeostasis have been well elucidated [7], mechanisms responsible for deleterious associations of sedentary behaviour with glycaemic measures are less clear. It has been postulated that reduced insulin action associated with prolonged sitting could be an early indication of an adverse metabolic response [8], possibly the consequence of changes in the muscle glucose transporter protein content [9].

Correspondence to: Anne-Louise S. Hansen, Epidemiology Group, Steno Diabetes Center A/S, Niels Steensens Vej 1, Building NLD 2.07, DK-2820 Gentofte, Denmark. E-mail: asih@steno.dk

Alterations in hepatic insulin resistance may also have a role as insulin resistance in the liver is strongly correlated with insulin resistance in skeletal muscle [10]. This may be attributable to the molecular defects from genetic predisposition for insulin resistance that affects both liver and skeletal muscle, as well to excessive fat deposition in the liver [10]. However, the impact of sedentary behaviour on hepatic insulin sensitivity is not well understood.

Disturbances in glucose homeostasis are observed before the onset of pre-diabetes and diabetes [11], highlighting the need to identify lifestyle factors that may adversely influence such metabolic alterations. Findings from prevention studies suggest that lifestyle modifications such as increased physical activity can ameliorate the pathophysiology of abnormal glucose tolerance and have a favourable impact on the progression to diabetes [12]. However, it is unclear whether changes in sedentary behaviours such as TV viewing can be related to changes in glucose homeostasis. In a large, population-based sample of Australian adults, we examined the associations of 5-year changes in TV viewing time with 5-year changes in continuous glucose homeostasis markers.

Subjects and methods

Study population

The Australian Diabetes, Obesity and Lifestyle (AusDiab) study included adults ≥ 25 years of age from six Australian states and the Northern Territory, examined in 1999/2000 (baseline) and in 2004/2005 (follow-up). The overall aim of the AusDiab study was to determine the national prevalence of diabetes and other non-communicable diseases and their risk factors. Details of the study design, including the stratified cluster sampling strategy, are described elsewhere [13]. At the baseline (1999/2000) survey, 11 247 participants completed a household interview and attended a biomedical examination. Of these, 6538 participants attended the 2-year follow-up examination in 2004/2005. For the present analyses, participants with less than 8 h of fasting prior to the respective examinations (n = 60), pregnant women (n = 43) at baseline or follow-up and those with known diabetes (n = 229) at baseline were excluded. Those without data on any of the outcome variables of interest (n = 1363) were also excluded (Fig. 1). The Ethics Committee of the Baker IDI Heart and Diabetes Institute (formerly known as the International Diabetes Institute) approved the study and all participants provided written informed consent.

Measurement methods

TV viewing time

Time (h and min) spent watching TV or DVDs or playing games on the TV was reported separately for workdays and non-workdays during the last week as part of an interviewer-administered general health and well-being questionnaire. Total TV viewing time (h/week) was calculated as the sum of workdays TV viewing time and non-workdays TV viewing time. This measure has been shown to have reasonable reliability and validity for estimating TV viewing time in adults [14].

Glucose homeostasis markers

Venous blood samples were collected after an overnight fast (≥ 8 h fast) and 2 h after intake of a standardized glucose solution (75 g glucose dissolved in 2.5 dl water) according to the World Health Organization (WHO) protocol. The

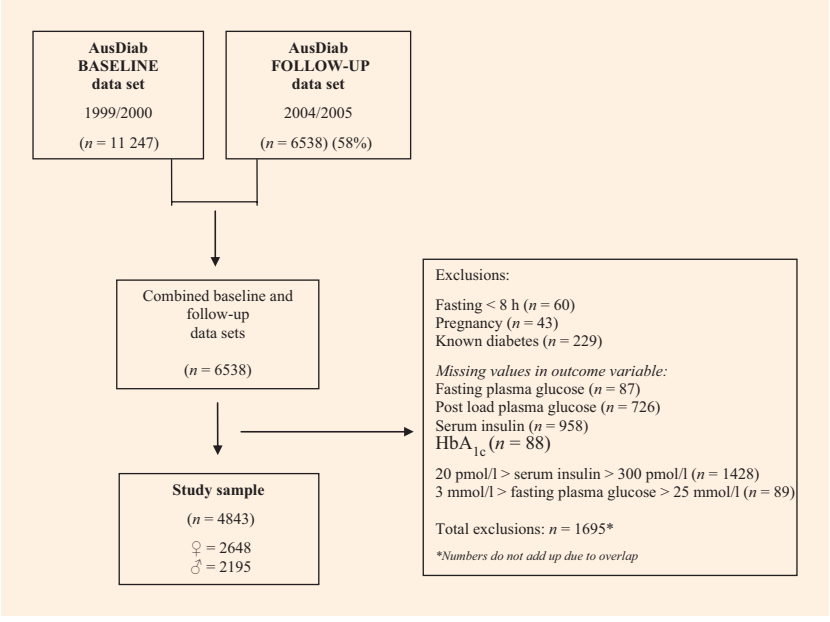


FIGURE 1 Flow chart showing participant recruitment and exclusion criteria.

measurement methods of glucose and HbA_{1c} have been described in detail elsewhere [2]. Baseline serum insulin (1999/2000) was measured (in participants ≥ 35 years old) using a human insulin-specific radioimmunoassay (RIA) kit (Linco Research Inc., St Charles, MO, USA). At follow-up (2004/2005). serum insulin was measured by chemiluminiscence (Bayer Advia Centaur; Bayer HealthCare AG, Leverkusen, Germany). A published relationship from a method comparison between insulin measures determined by the Linco RIA method and the Bayer chemiluminescence method [15] was used to corrugate the baseline serum insulin values using the following equation: Adjusted baseline serum insulin (pmol/l) = –24.4 + 0.99 × baseline insulin values (Linco RIA measures) (R^2 = 0.98). Adjusted baseline serum insulin values were used in all subsequent calculations and statistical analyses.

Homeostasis model assessment (HOMA2) of insulin resistance and HOMA2–β-cell function was calculated based on model-derived estimates using the HOMA2-calculator, version 2.2 [16]. Before importing data to the calculator, observations with extreme values of fasting plasma glucose ≤ 3 or ≥ 25 mmol/l (n = 1) and/or serum insulin < 20 or > 300 pmol/l (n = 405) were excluded as this is the validity range of the HOMA2-calculated values based on specific insulin measures.

Potential confounding factors

Potential confounding factors on the association of TV viewing time with glucose metabolism were identified from the literature [6]. Time spent in moderate-to-vigorous intensity physical activity (min/week) was measured using the Active Australia questionnaire [17]. This questionnaire has previously been found to provide reliable and valid estimates of leisure-time physical activity among adults [18]. Sociodemographic and behavioural information on employment status (employed in full-time or part-time job; yes/no), income level (≥ $A1500/week; yes/no), educational attainment (never attended school, primary school, some high school, completed high school, completed university/technical and further education), smoking status (current heavy smoker ≥ 20 cigarettes per day, current light smoker < 20 cigarettes per day, non-smoker and ex-smoker), alcohol consumption (non-drinker, light-drinker, moderate to heavy drinker) and parental history of diabetes (yes/no) was obtained using an interviewer-administered questionnaire. A self-administered, validated food frequency questionnaire developed by the Anti-Cancer Council of Victoria was used to calculate total energy intake (KJ/day) and to derive a measure of diet quality based on recommended daily macronutrient intakes (Diet Quality Index – Revised, scale 1–100 with 100 being high diet quality) [19].

Potential mediating factor

Waist circumference was included in the analysis as a potential mediator rather than a confounder because of the role of adiposity in causal pathways leading to insulin resistance [20]. Measures of waist circumference (cm) were obtained in duplicate using a steel measuring tape [13].

Statistical analyses

Five-year changes for all parameters were derived by subtracting the baseline values from the follow-up values.

Missing data on baseline and follow-up determinants (n = 924) for all participants included in the study (n = 4843) were imputed using the Multivariate Imputation by Chained Equations (MICE) method in R software [21] with missing-at-random assumptions. Fifty copies of the data, each with missing values suitably imputed, were independently assessed in the analyses described below. Fifty imputations were chosen to be sufficient to obtain valid inference. Estimates of parameters of interest were averaged across the copies to give a single mean estimate. Standard errors and P-values were adjusted according to Rubin's rules [22]. Parameter estimates and 95% confidence intervals from the analyses using imputed data are presented as main results (Table 2). Parameter estimates and 95% confidence intervals from analyses using non-imputed data (thus excluding missing values) are presented in the Supporting Information (Table S1).

Multiple linear regression analyses with 5-year change in TV viewing time (exposure) and 5-year change in glucose homeostasis markers (outcome) were applied. Covariates included in the regression models were: model A—baseline age, baseline TV viewing time, baseline glucose homeostasis marker under study; model B—additionally adjusted for baseline and 5-year change in educational attainment, employment status, income level, smoking status, alcohol consumption, diet quality, energy intake and parental history of diabetes at follow-up; model C—model B covariates and adjustments for baseline and 5-year change in time spent in moderate-to-vigorous intensity physical activity; model D—model C covariates and adjustments for baseline and 5-year change in waist circumference.

Parameter estimates from the regression analyses are reported per 5-h units (Table 2) as a reasonable proportion of the population (25%) had an increased TV viewing time by 5 h per week over the 5 years.

Analyses were stratified by gender given previous AusDiab study findings showing differences between men and women in TV time/biomarker associations [2;3]. Level of significance was set at $P < 0.05$. All analyses were performed using the statistical software program 'R' version 2.13.0.

Results

Demographic, behavioural, anthropometric and glucose homeostasis measures at baseline and at follow-up are presented in Table 1 for the study sample.

On average, TV viewing time increased by 0.9 (± 8.8) h/week for men and by 1.2 (± 9.3) h/week for women over the 5 years from baseline to follow-up. Mean moderate-to-vigorous intensity physical activity levels increased over the

Table 1 Characteristics of the AusDiab study population with complete data at baseline (1999–2000) and follow-up (2004–2005)

	Men (n = 1812)			Women (n = 2133)		
Characteristics	Baseline	Follow-up	P	Baseline	Follow-up	P
Demographical						
Gender (%)	45.9			54.1		
Age (years)	53.6 (11.1)	58.6 (11.1)	< 0.001	53.0 (10.9)	58.0 (10.9)	< 0.001
University/technical and further education, n (%)†	785 (43.3)	827 (45.6)	< 0.001	769 (36.1)	783 (43.2)	< 0.001
Employed, n (%)†	1,309 (72.2)	1,176 (64.9)	< 0.001	1,233 (57.8)	1,160 (64.0)	< 0.001
Income ≥ $A1500/week, n (%)†	402 (22.5)	574 (31.6)	< 0.001	362 (17.5)	486 (26.8)	< 0.001
Parental history of diabetes, n (%)†	321 (17.7)	437 (24.1)	< 0.001	401 (18.8)	579 (27.1)	< 0.001
Behavioural						
Current heavy smoker, n (%)†	240 (13.3)	185 (10.2)	< 0.001	200 (9.4)	156 (7.3)	< 0.001
Moderate to heavy drinker, n (%)†	683 (37.7)	723 (39.9)	< 0.001	335 (15.7)	437 (24.1)	< 0.001
Diet quality, 1–100	61.2 (12.5)	62.0 (12.4)	0.002	67.3 (12.7)	67.4 (12.0)	0.620
Total energy intake (kJ/day)	10036 (3271)	9337 (3167)	< 0.001	7451 (2767)	7055 (2768)	< 0.001
TV viewing time (h/week)*	12.0 (7.0–18.0)	13.0 (7.0–19.0)	< 0.001	10.5 (5.0–16.0)	12.0 (6.0–19.0)	< 0.001
Physical activity (min/week)*	200.0 (60.0–450.0)	210.0 (60.0–436.0)	0.972	135 (30.0–330.0)	180.0 (60.0–390.0)	< 0.001
Markers of glucose homeostasis						
Fasting plasma glucose (mmol/l)	5.6 (0.6)	5.6 (0.7)	< 0.001	5.3 (0.5)	5.3 (0.6)	0.025
Post-load plasma glucose (mmol/l)	6.0 (2.0)	6.1 (2.4)	0.062	6.2 (1.8)	6.0 (2.0)	< 0.001
Fasting serum insulin (pmol/l)*	66.3 (45.7–93.5)	48.6 (34.7–83.3)	< 0.001	58.8 (42.3–84.9)	48.6 (34.7–76.4)	< 0.001
HbA_{1c} (mmol/mol)	36 (4)	37 (4)	< 0.001	32 (3)	36 (3)	< 0.001
HbA_{1c} (%)	5.4 (0.4)	5.5 (0.4)	< 0.001	5.1 (0.3)	5.4 (0.3)	< 0.001
HOMA2–insulin resistance (100/S%)	1.7 (0.9)	1.4 (0.9)	< 0.001	1.5 (0.9)	1.3 (0.9)	< 0.001
HOMA2–β-cell function (%)	104.0 (37.5)	94.5 (38.2)	< 0.001	108.1 (36.5)	96.6 (36.6)	< 0.001
Anthropometrical						
Waist (cm)	97.8 (10.3)	99.4 (10.8)	< 0.001	85.2 (12.5)	87.6 (12.8)	< 0.001
Height (cm)	175.8 (7.0)	174.9 (7.1)	< 0.001	162.6 (6.5)	161.6 (6.6)	< 0.001
Weight (kg)	84.3 (13.0)	85.5 (13.7)	< 0.001	70.7 (14.2)	71.1 (15.0)	< 0.001

Means (SD) if not stated else and paired t-test for differences in baseline and follow-up values.
*Median (interquartile ranges) and signed rank test for differences in baseline and follow-up values.
†Proportions and χ^2-test for differences in baseline and follow-up proportions.
HOMA, homeostasis model assessment.

Table 2 Five-year change in markers of glucose homeostasis per 5-year change in TV viewing time*

Outcome variable	Model	Men (n = 2195)		Women (n = 2648)	
		β (95% CI)	*P*	β (95% CI)	*P*
Fasting plasma glucose (mmol/l)†	A	0.009 (−0.007 to 0.025)	0.245	**0.013 (0.001–0.025)**	**0.031**
	B	0.009 (−0.007 to 0.025)	0.282	**0.013 (0.001–0.025)**	**0.039**
	C	0.009 (−0.007 to 0.025)	0.290	**0.012 (0.001–0.024)**	**0.048**
	D	0.006 (−0.010 to 0.022)	0.468	0.009 (−0.003 to 0.021)	0.141
Post-load plasma glucose (mmol/l)	A	**0.076 (0.026–0.127)**	**0.003**	**0.041 (0.001–0.081)**	**0.047**
	B	**0.062 (0.011–0.114)**	**0.018**	0.033 (−0.009 to 0.074)	0.121
	C	**0.063 (0.011–0.114)**	**0.017**	0.032 (−0.010 to 0.074)	0.131
	D	**0.052 (0.000–0.103)**	**0.048**	0.022 (−0.019 to 0.063)	0.303
Fasting serum insulin (pmol/l)	A	**1.325 (0.444–2.206)**	**0.003**	**1.342 (0.620–2.063)**	**0.000**
	B	**1.164 (0.268–2.060)**	**0.011**	**1.146 (0.405–1.867)**	**0.002**
	C	**1.195 (0.302–2.088)**	**0.009**	**1.060 (0.320–1.800)**	**0.005**
	D	0.780 (−0.031 to 1.591)	0.060	**0.712 (0.027–1.398)**	**0.042**
HbA_{1c} (mmol/mol)	A	0.022 (−0.055 to 0.098)	0.581	0.055 (0.000–0.109)	0.060
	B	0.044 (−0.033 to 0.120)	0.301	0.044 (−0.022 to 0.098)	0.200
	C	0.044 (−0.033 to 0.120)	0.246	0.033 (−0.022 to 0.098)	0.216
	D	0.022 (−0.055 to 0.098)	0.556	0.022 (−0.044 to 0.077)	0.508
HbA_{1c} (%)	A	0.002 (−0.005 to 0.009)	0.581	0.005 (0.000–0.010)	0.060
	B	0.004 (−0.003 to 0.011)	0.301	0.004 (−0.002 to 0.009)	0.200
	C	0.004 (−0.003 to 0.011)	0.246	0.003 (−0.002 to 0.009)	0.216
	D	0.002 (−0.005 to 0.009)	0.556	0.002 (−0.004 to 0.007)	0.508
HOMA2–β-cell function (%)	A	**1.208 (0.158–2.258)**	**0.024**	**1.264 (0.259–2.270)**	**0.014**
	B	**1.075 (0.003–2.146)**	**0.049**	**1.140 (0.098–2.182)**	**0.032**
	C	**1.109 (0.038–2.179)**	**0.042**	**1.073 (0.023–2.122)**	**0.045**
	D	0.792 (−0.262 to 1.845)	0.141	0.850 (−0.185 to 1.886)	0.108
HOMA2–insulin resistance	A	**0.027 (0.005–0.050)**	**0.019**	**0.034 (0.012–0.055)**	**0.002**
	B	**0.024 (0.000–0.047)**	**0.046**	**0.029 (0.007–0.050)**	**0.009**
	C	**0.024 (0.001–0.047)**	**0.041**	**0.027 (0.005–0.049)**	**0.015**
	D	0.015 (−0.006 to 0.037)	0.160	0.018 (−0.002 to 0.038)	0.078

*Regression coefficients (95% CI). Unit for TV viewing time is 5 h/week. Analyses on imputed data (19% missing data).
Change in TV viewing: model A—baseline age, baseline TV-viewing time, baseline glycaemic marker under study; model B—baseline age, baseline TV-viewing time, baseline glycaemic marker under study and baseline and change in education level, employment status, income, smoking status, alcohol consumption, diet quality, energy intake and parental history of diabetes at follow-up; model C—baseline age, baseline TV-viewing time, baseline glycaemic marker under study and baseline and change in education level, employment status, income, smoking status, alcohol consumption, diet quality, energy intake, physical activity and parental history of diabetes at follow-up; model D—baseline age, baseline TV-viewing time, baseline glycaemic marker under study and baseline and change in education level, employment status, income, smoking status, alcohol consumption, diet quality, energy intake, physical activity and waist circumference (as mediating factor), parental history of diabetes at follow-up. †Results on fasting plasma glucose previously presented by Wijndaele *et al.* [5].

5 years by 28.8 (± 323.4) min/week among women and decreased by 8.0 (± 378.4) min/week among men. Mean differences (± SD) in markers of glucose homeostasis among men and women (respectively) from baseline to follow-up were: fasting plasma glucose (mmol/l): –0.1 (± 0.6) and –0.0 (± 0.5); 2-h plasma glucose (mmol/l): 0.1 (± 2.0) and –0.2 (± 1.8); fasting serum insulin (pmol/l): –10.8 (± 36.3) and –10.6 (± 33.7); HOMA2–insulin resistance: –0.2 (± 0.8) and –0.2 (± 0.7); HOMA2–β-cell function (%): –8.8 (± 33.7) and –11.2 (± 33.8); HbA_{1c} (mmol/mol): 4 (± 3) (men and women); HbA_{1c} (%): 0.3 (± 0.3) (men and women).

Five-year change in TV viewing time was positively associated with the 5-year change in 2-h plasma glucose and insulin levels, HOMA2–insulin resistance and HOMA2–β-cell function in men (Table 2). In women, for every 5 h/week increase in TV viewing time from baseline to follow-up, there was an increase in the 5-year change in fasting plasma glucose, HOMA2–insulin resistance, HOMA2–β-cell function and fasting serum insulin (model C). These associations remained statistically significant in models adjusted for baseline age, baseline TV viewing, baseline glycaemic marker under study, and baseline and change in education levels, employment status, income, smoking status, alcohol consumption, diet quality, energy intake, moderate-to-vigorous intensity physical activity and parental history of diabetes at follow-up (model C). However, after additionally including baseline and change in waist circumference (model D) as a potential mediator, only the association with fasting serum insulin in women and 2-h plasma glucose in men remained significant (Table 2).

Analyses on complete cases (see also Supporting Information, Table S1) showed similar, albeit slightly weaker, associations with changes in 2-h plasma glucose levels, fasting serum

insulin, HOMA2–insulin resistance and HOMA2–β-cell function as that reported in Table 2 using imputed data. In women only, the associations of change in TV viewing time with fasting plasma glucose was markedly different in complete case analyses (non-significant associations) compared with the associations from analyses on imputed data (significant associations). Scatter plots showing 5-year changes in TV viewing time vs. 5-year changes in fasting serum insulin and 2-h plasma glucose are included in the Supporting Information (Fig. S1a and b).

Discussion

In this cohort of Australian adults, TV viewing time increased on average by approximately 1 h/week from 1999/2000 to 2004/2005. This is consistent with findings from the 2006 Australian Time Use Survey, which reported a 1-h/week increase in TV viewing time for adults from 1997 to 2006 [23]. Change in TV viewing time over the 5-year period was associated with significant increases in continuous measures of insulin resistance, β-cell function, fasting serum insulin and plasma glucose levels in women and, with increased levels of 2-h plasma glucose and fasting serum insulin, insulin resistance and β-cell function in men. Importantly, the associations were shown to be independent of moderate-to-vigorous intensity physical activity time and other confounders, including dietary quality and energy intake. These findings are consistent with previous studies that have shown cross-sectional associations of TV viewing time with glucose homeostasis markers, impaired glucose tolerance and diabetes [6].

It is widely acknowledged that the development of Type 2 diabetes involves specific pathophysiological changes that lead to derangements in glucose homeostasis and that these metabolic changes occur in different stages before the clinical onset of diabetes [24]. Our findings suggest that increased TV viewing could be another influential lifestyle factor in this process and some corroborative evidence exists. In a study that followed participants of the European Prospective Investigation into Cancer and Nutrition—Potsdam Study for 7.8 years [25], it was found that time spent watching TV predicted future incident diabetes. An elevation in 2-h plasma glucose levels (such as the one observed in men in the present study) is consistent with the hypothesized later stages of the pathophysiological changes leading to diabetes [24]. Although the associations identified in the present study are modest in magnitude, they nevertheless may be important from a public-health perspective [26]. In isolation, a 5-year increase in serum insulin of 1.0 pmol/l per 5-h increase in TV viewing time could be viewed as a small increase, but in conjunction with other metabolic changes this could be significant for cardiovascular risk. Furthermore, if a person has a 2-h-fasting plasma glucose level near the cut-off point for the diagnostic criteria for impaired glucose tolerance or diabetes, an additional increase of 0.05 mmol/l would be of potential significance. The found associations of 5-year increases in TV viewing time with increased insulin levels is supported by recent physiological insights in the acute response of 1 day of prolonged sitting on insulin action in healthy men and women [27]. In a counterbalanced, crossover trial, Stephens and colleagues found that, compared with a condition in which sitting was minimized, prolonged sitting was associated with a substantial reduction (18%) in insulin action over 24-h, even when taking into account the lower energy expenditure associated with prolonged sitting [27].

As expected, the inclusion of waist circumference, which is considered to be a better marker of central adiposity than BMI [28], as a mediating factor in the analyses attenuated the associations of 5-year change in TV viewing time with 5-year change in fasting plasma glucose, insulin resistance and insulin secretion among women and with insulin resistance, insulin secretion and fasting serum insulin among men. It is well known that central adiposity and overweight/obesity are strongly associated with insulin resistance [20], and it has been proposed that sedentary behaviour and obesity should be considered as separate entities in relation to health outcomes [29]. Thus, including waist circumference as a potential mediator might represent a statistical overcorrection [30].

The major strengths of this study are the prospective design with measures of TV viewing time, continuous glycaemic measures and potential confounding factors at two time-points over 5 years. Additionally, the large population-based sample includes an equal distribution of men and women of a varying age range. TV viewing time was measured as total hours spent watching TV on both workdays and non-work days during the week as opposed to a measure of frequency of TV viewing. To minimize misclassification of the exposure variable, participants were asked to report the time they spent watching TV when it was their main activity, and not when the TV was switched on whilst other tasks were being undertaken, such as preparing a meal.

Most published studies examining the associations of TV viewing time with markers of glucose homeostasis have focused on one of the following outcomes: classifications of glucose tolerance or incident diabetes; limited to one marker of glucose homeostasis (fasting plasma glucose only) [2,25]; or had examined the exposure of TV viewing according to categories [3]. The use of 5-year changes in continuous outcome measures enabled a more detailed picture of the associations of change in TV viewing time with the initially observed derangements of glucose homeostasis. Thus, we were able to identify small changes, if any, rather than missing these changes because of categorization of data using arbitrary cut-points.

A limitation of utilizing a self-report measure of sedentary behaviour, such as TV viewing time, is the propensity for measurement bias as a result of participants' over- or under-reporting of the behaviour. In the case of TV viewing, this is likely to be under-reporting [1]. Another study limitation is that the measurement methods for analysing plasma glucose and serum insulin levels changed over the duration of study. The findings from the assessment of plasma glucose levels did not differ between the two methods. For serum insulin levels,

the two measurement methods gave different results, with the baseline measurement method determining systematically higher results than the measurement method at follow-up. To account for this, we used the equation derived from a recent method comparison study of the two assessment methods [15] to adjust the baseline insulin levels. The equation from the method-comparison study was based on participants with different glucose tolerance status to ensure the derived regression equation would be valid for the wide spectrum of insulin levels. However, as the validation was not performed with participants from the present population, the baseline insulin levels might be slightly biased. Changes in TV viewing and glucose homeostasis markers occurred over the same 5-year period. Thus, we cannot speculate on causality of any associations found. However, our findings provide unique evidence that change in TV viewing time is associated differently with changes in glucose homeostasis markers for men and for women. These differences are mainly in the associations of change in TV viewing time with 2-h plasma glucose in men and with fasting insulin in women. Although we adjusted our models for a substantial number of potential confounding factors, it is plausible we did not include all relevant covariates, which might have resulted in residual confounding of the associations found. The associations that we have identified may be influenced by the decrease in other non-measured activities (particularly light-intensity ambulatory activities that are difficult to measure by self-report) rather than by an increase in TV viewing time. When examining the associations of changes in TV viewing time with changes in homeostasis markers, we performed multiple regression analyses using seven different models. Multiple testing has the potential to lead to false positive findings. It could be that the associations found of the present study are spurious. However, we utilized a sizeable number of parameter estimates when including covariates in different models. Also, our findings are consistent with those of previous cross-sectional studies [3–5].

Conclusions

In this prospective study of Australian adults without known diabetes, an increase in TV viewing time over 5 years was shown to be adversely related to 5-year changes in insulin resistance, β-cell function and fasting plasma glucose and insulin levels in women and with 2-h plasma glucose and insulin levels, insulin resistance and β-cell function in men. However, after additional adjustment for waist circumference as a potential mediating factor, only the associations with insulin remained significant in women and with 2-h plasma glucose in men. The findings of the present study suggest that initial pathophysiological changes that contribute to the development of diabetes may be adversely associated with increased TV viewing time. This is an important finding, given that TV viewing time is increasing in the Australian population [23] and in many European countries [6].

Competing interests

ALSH is employed by the Steno Diabetes Center A/S, a research hospital working in the Danish National Health Service and owned by Novo Nordisk A/S. The Steno Diabetes Center receives part of its core funding from unrestricted grants from the Novo Foundation and Novo Nordisk A/S. ALSH own shares in Novo Nordisk A/S. KW, NO, DJM, AAT, JES and DWD have nothing to declare.

Acknowledgements

We acknowledge the AusDiab project manager Shirley Murray, administrative associate Sue Fournel and the field coordinators Marita Dalton (1999–2000), Theresa Whalen (2004–2005) and Annaliese Bonney (2004–2005). We are most grateful to the support staff and participants for their contribution to the study. This work was supported by: a National Health and Medical Research project grant (no. 233200); the Victorian Government's Operational Infrastructure Support Program; a scholarship awarded to ALSH by the University of Copenhagen, Denmark; the Danish Cardiovascular Research Academy; and Steno Diabetes Center A/S; an Australian Research Council Future Fellowship to DWD; a National Heart Foundation Postdoctoral Fellowship to AAT; a National Health and Medical Research Council (NHMRC no. 586623) Senior Research Fellowship to JES; and a NHMRC Program Grant (no. 569940) and a Senior Principal Fellowship (no. 1003960) to NO. For further acknowledgements please refer to Thorp *et al.* [4].

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Scatter plots of 5-year changes in TV viewing time (h/week) vs. 5-year changes in (a) fasting serum insulin and (b) 2-h plasma glucose for women and men.

Table S1. Five-year change in markers of glucose homeostasis per 5-year change in TV viewing time.

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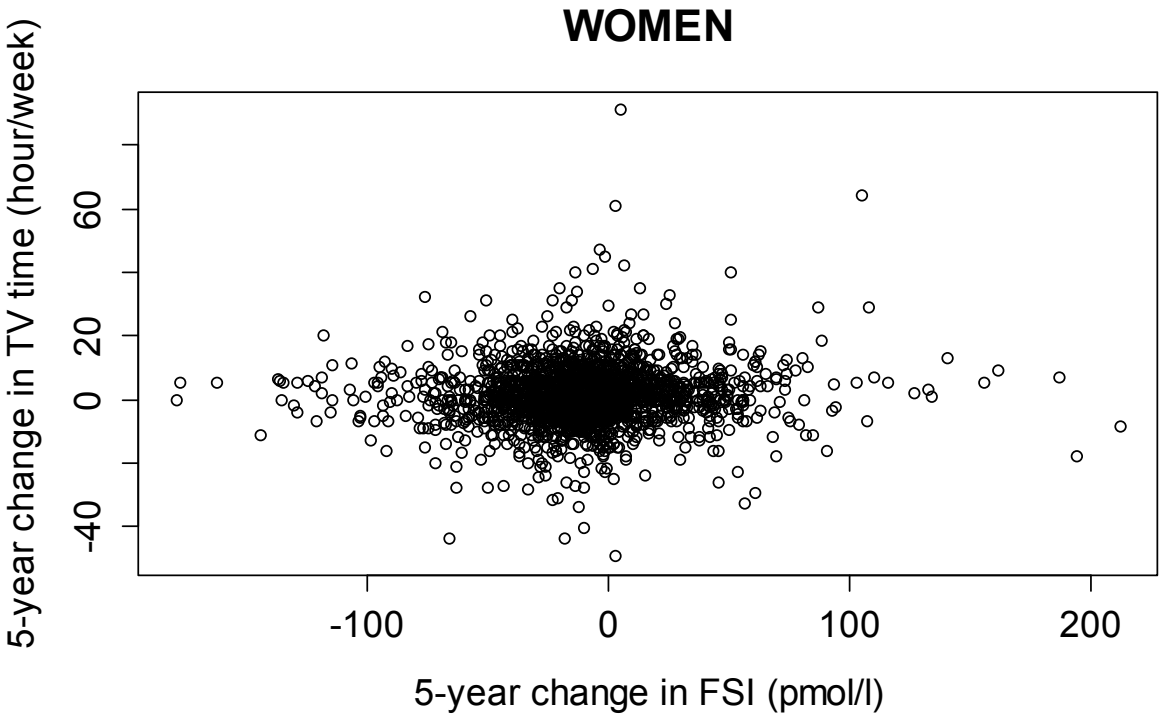
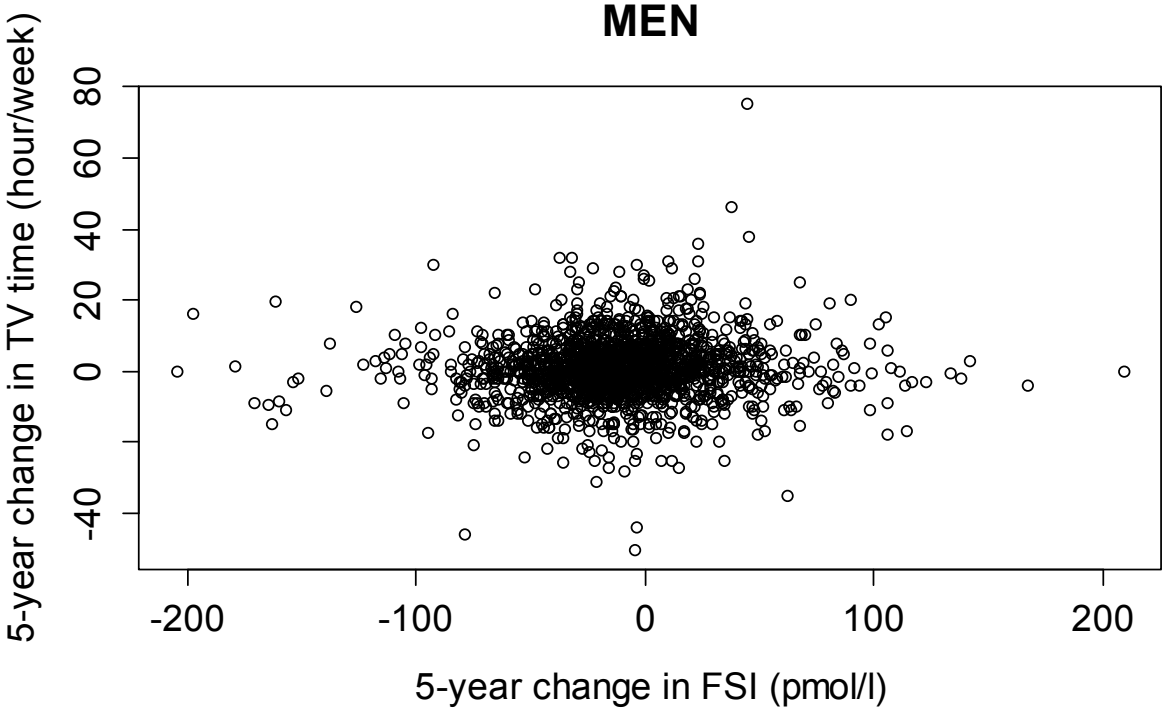
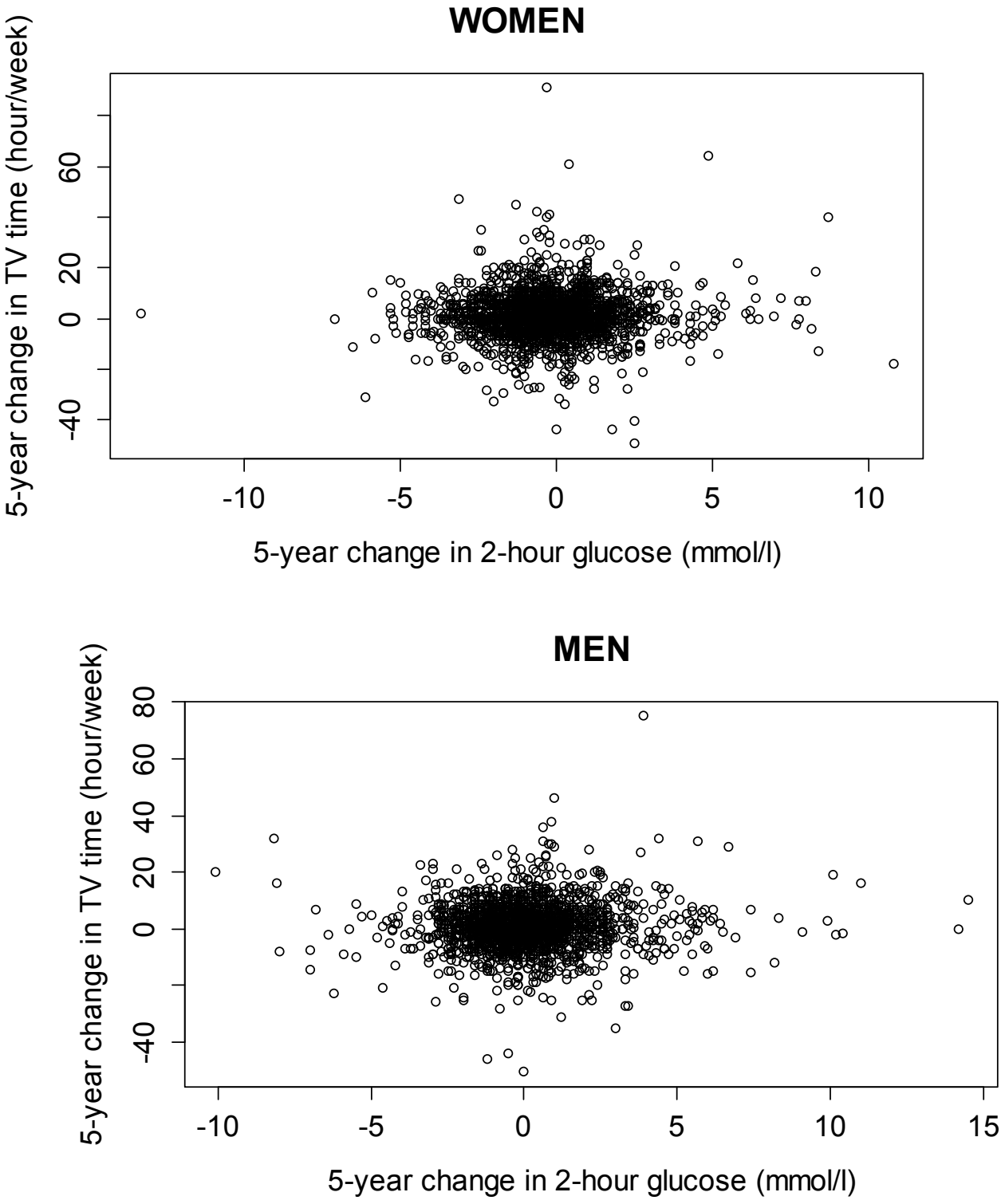




Supplemental figure 1a. Scatterplots of five-year changes in TV viewing time (hour/week) versus five-year changes in fasting serum insulin (FSI, pmol/l) for women and men.



Supplemental figure 1b. Scatterplots of five-year changes in TV viewing time (hour/week) versus five-year changes in 2-hour plasma glucose (mmol/l) for women and men.

Supplemental online material

TABLE 2a. . *Five-year change in markers of glucose homeostasis per five-year change in TV viewing time. Analysis on complete cases (n=3,945). Regression coefficients (95% CI). Unit for TV viewing time is five hrs/week*

		Men (n=1,812)		***Women (n=2,133)***	
Outcome variable	***Model***	***β (95% CI)***	***P***	***β (95% CI)***	***P***
*Fasting plasma glucose (mmol/l)**	*A*	*0.006 (-0.012; 0.024)*	*0.516*	*0.011 (-0.002; 0.023)*	*0.102*
	B	*0.005 (-0.013; 0.023)*	*0.587*	*0.011 (-0.002; 0.024)*	*0.109*
	C	*0.005 (-0.013; 0.023)*	*0.587*	*0.010 (-0.003; 0.024)*	*0.124*
	D	*0.002 (-0.016; 0.020)*	*0.831*	*0.008 (-0.005; 0.021)*	*0.227*
Post load plasma glucose (mmol/l)	*A*	***0.080 (0.022; 0.137)***	***0.007***	*0.036 (-0.007; 0.078)*	*0.101*
	B	***0.061 (0.003; 0.120)***	***0.040***	*0.028 (-0.015; 0.072)*	*0.203*
	C	***0.062 (0.003; 0.120)***	***0.040***	*0.029 (-0.016; 0.071)*	*0.218*
	D	*0.049 (-0.009; 0.107)*	*0.099*	*0.018 (-0.025; 0.061)*	*0.406*
Fasting serum insulin (pmol/l)	*A*	***1.409 (0.423; 2.395)***	***0.005***	***1.203 (0.418; 1.988)***	***0.003***
	B	***1.083 (0.084; 2.082)***	***0.034***	***1.055 (0.251; 1.859)***	***0.010***
	C	***1.124 (0.130; 2.119)***	***0.027***	***0.979 (0.176; 1.782)***	***0.017***
	D	*0.705 (-0.202; 1.612)*	*0.128*	*0.660 (-0.083; 1.403)*	*0.082*
Glycated hemoglobin, HbA_{1C} (%)	*A*	*0.000 (-0.007; 0.008)*	*0.938*	*0.003 (-0.003; 0.009)*	*0.310*
	B	*0.001 (-0.007; 0.009)*	*0.792*	*0.001 (-0.004; 0.007)*	*0.651*
	C	*0.002 (-0.006; 0.009)*	*0.706*	*0.001 (-0.005; 0.007)*	*0.706*
	D	*-0.001 (-0.009; 0.007)*	*0.806*	*0.000 (-0.006; 0.005)*	*0.896*
Glycated hemoglobin, HbA_{1C} (mmol/mol)	*A*	*0.000 (-0.077;0.087)*	*0.938*	*0.033 (-0.033;0.098)*	*0.310*
	B	*0.011 (-0.077;0.098)*	*0.792*	*0.011 (-0.044;0.077)*	*0.651*
	C	*0.022 (-0.066;0.098)*	*0.706*	*0.011 (-0.055;0.077)*	*0.706*
	D	*-0.011 (-0.098;0.077)*	*0.806*	*0.000 (-0.066;0.055)*	*0.896*
HOMA2-Beta cell function (%)	*A*	***1.478 (0.555; 2.401)***	***0.002***	***1.234 (0.446; 2.021)***	***0.003***
	B	***1.206 (0.272; 2.139)***	***0.011***	***1.076 (0.270; 1.882)***	***0.011***
	C	***1.249 (0.319; 2.178)***	***0.008***	***0.996 (0.192; 1.801)***	***0.018***
	D	***0.928 (0.050; 1.806)***	***0.038***	*0.713 (-0.049; 1.475)*	*0.084*
HOMA2-Insulin resistance	*A*	***0.029 (0.008; 0.051)***	***0.008***	***0.026 (0.009; 0.043)***	***0.002***
	B	***0.022 (0.000; 0.044)***	***0.049***	***0.023 (0.005; 0.040)***	***0.009***
	C	***0.023 (0.001; 0.045)***	***0.039***	***0.021 (0.004; 0.039)***	***0.015***
	D	*0.014 (-0.006;0.033)*	*0.174*	*0.014 (-0.002; 0.030)*	*0.067*

Change in TV-viewing*: Model A: Baseline age, baseline TV-viewing time, baseline glycaemic marker under study; Model B: Baseline age, baseline TV-viewing time, baseline glycaemic marker under study and baseline and change in education level, employment status, income, smoking status, alcohol consumption, diet quality, energy intake and parental history of diabetes at follow-up; Model C: Baseline age, baseline TV-viewing time, baseline glycaemic marker under study and baseline and change in education level, employment status, income, smoking status, alcohol consumption, diet quality, energy intake, physical activity and parental history of diabetes at follow-up; Model D: Baseline age, baseline TV-viewing time, baseline glycaemic marker under study and baseline and change in education level, employment status, income, smoking status, alcohol consumption, diet quality, energy intake, physical activity, and waist circumference (as mediating factor), parental history of diabetes at follow-up. * Results on fasting plasma glucose previously presented by Wijndaele*