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PHYSICAL ACTIVITY, BODY COMPOSITION, INFLAMMATION AND EFFECTS ON ANTIOXIDATIVE CAPACITY IN SEDENTARY ADULTS

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Physical Activity, Body Composition, Inflammation and Effects on Antioxidative Capacity in Sedentary Adults

THESIS FOR LICENTIATE DEGREE

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Don’t sit – get fit

ABSTRACT

Background: For the past fifty years part the population has slowly become sedentary to an extent that it is nowadays considered normal behavior. Parallel with sedentary behavior there is an increase in obesity now reaching epidemic level. Metabolic derangements such as diabetes type 2 and cardiovascular diseases has become an economic burden for the modern society. Implementation of training programs for individuals that have been sedentary for years has often been demonstrated inefficient, with few and short-lasting effects.

Aim: The primary aim of this thesis was to study effects of increased physical activity at different intensities in distinct sedentary populations. The secondary aim was to investigate if there was a gender difference in adaption to short-term endurance training.

Methodology: Study I: Two hundred and twelve (age 45-69 years) sedentary overweight men and women were included. They were classified as normal or impaired glucose tolerance, or diabetes type 2. They were randomized into controls or intervention group. The intervention group was instructed to increase their physical activity by walking with poles (Nordic walking) 5 h per week. Questionnaire, blood samples, anthropometric data and results from an exercise test were collected at baseline and after 4 months.

Study II: Fourteen healthy (22-30 year-old) sedentary normal weight women and men participated in a 3 weeks endurance training program including 2 supervised sessions per day. The training period was followed by 4 weeks of detraining. Blood samples including total antioxidative capacity (TAOC) and oxidative stress (OS), body composition (BC), anthropometric data and evaluation of work capacity were obtained at baseline, after training and after detraining.

Result: Study I: Nordic walking improved waist circumference, body weight and body mass index in the intervention group with normal glucose tolerance. Participants that reported > 80 % adherence to training improved work capacity in terms of work load, $VO_{2\ peak,}$ or both, in all groups. Biochemical markers were unaffected.

Study II: TAOC levels decreased significantly in women after training, while no change was noted in men. After training both gender showed increased work load, $VO_{2\ peak}$ even adjusted for fat-free mass, and ventilation. After detraining, work load and $VO_{2\ peak}$ decreased in women. Adjusted $VO_{2\ peak}$ decreased also in men but remained higher compared to baseline. Fat-free mass (kg) was higher after detraining compared to baseline in women. **Conclusion:** Study I: Nordic walking had positive anthropometric effects among those with normal glucose tolerance. It could be applied in clinical practice, but surveillance of training might be necessary to reach optimal results among sedentary individuals.

Study II: Female participants were more sedentary and their exercise capacity improved to a greater degree. Their decrease in TAOC might reflect an increased consumption of antioxidants to prevent negative effects of OS. A higher level of daily regular physical activity in men could explain their maintenance of improved VO_{2peak} after detraining.

LIST OF SCIENTIFIC PAPERS

I. Fritz T, Caidahl K, Krook A, Lundstrom P, Mashili F, Osler M, Szekeres FL, Ostenson CG, Wandell P and Zierath JR. Effects of Nordic walking on cardiovascular risk factors in overweight individuals with type 2 diabetes, impaired or normal glucose tolerance. Diabetes/ Metabolism Research and Reviews. 2013;29:25-32

II. Petra Lundström, Romain Barrès, Tomas Fritz, Ted Österlund, Anna Krook, Juleen R. Zierath, Kenneth Caidahl, Anette Rickenlund. The Effect of Short-Term Endurance Training on Physical Performance, Total Anti-Oxidative Capacity and Body Composition in Sedentary Young Adults. Manuscript

CONTENTS

LIST OF ABBREVIATIONS

Apo B/A	Apolipoprotein
Bod Pod	A non-invasive dual-chamber plethysmograph
CK	Creatine kinase
CRP	C-reactive protein
DBP	Diastolic blood pressure
DNA	Deoxyribose nucleic acid
FM	Fat mass
FMI	Fat mass index
FFM	Fat-free mass
FFMI	Fat-free mass index
Hb	Haemoglobin
HbA1c	Glycated haemoglobin
HDL	High density lipoprotein
HIT	High-intensity training
IGT	Impaired glucose tolerance
ISAK	International standardization of anthropometry and kinanthropometry
LDL	Low density lipoprotein
MCP-1	Monocyte chemoattractant protein-1
NGT	Normal glucose tolerance
O_2	Oxygen
OS	Oxidative stress
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
RPE	Rating of perceived exertion
RPM	Rate per minute
TAOC	Total antioxidative capacity
T2DM	Diabetes mellitus type II
TNF-α	Tumor necrosis factor alpha
VE	Ventilation
$VO_{2\ peak}$	Peak oxygen uptake
VT	Ventilatory threshold

1 INTRODUCTION

1.1 SEDENTARY LIFE-STYLE, DEFINITION, DEMOGRAPHIC DATA AND HEALTH CONSEQUENCES

The exact mechanisms or causes behind the obesity epidemic are not well defined. However, one of the main contributors is the lack of regular daily physical activity. Over the past five decades there has been a massive decline in calories burned at work (1). Physically active occupations have decreased while there has been a huge increase in sedentary functions at work. Most adults spend many hours every week at work and the possibility to be physically active on a daily basis has therefore decreased substantially. Furthermore, a large proportion of leisure time is spent on sedentary activities, such as watching television, computer and smart phones activities. In addition, the possibility to spontaneous activities also has decreased in the modern society due to environmental factors. In fact, in many places, there is a lack of access to safe and convenient places to walk and bike. Good walkability in the living environment is associated with increased spontaneous physical activity and makes it easier for individuals to meet the recommendations of the degree of physical activity issued by society (2).

Physical inactivity is considered to be one of the leading causes of death in the western countries (2). Actually it jeopardizes the health to the same extent as cigarette smoking and obesity (3). One of the major health consequences of physical inactivity is the risk of developing metabolic disorders, such as type 2 diabetes mellitus (T2DM). If the current trend continues the economic costs will cripple the economy worldwide (4). The costs of physical inactivity are substantial, both in terms of economic and health consequences (5).

Table 1. *List of countries with the highest numbers of estimated cases of diabetes years 2000 and 2030 (6). Reproduced with permission from Diabetes Care.*

	2000		2030	
Ranking	Country	People with diabetes (millions)	Country	People with diabetes (millions)
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Pakistan	13.9
6	Pakistan	5.2	Brazil	11.3
7	Russian Federation	4.6	Bangladesh	11.1
8	Brazil	4.6	Japan	8.9
9	Italy	4.3	Philippines	7.8
10	Bangladesh	3.2	Egypt	6.7

Despite the well-known positive effects of regular physical activity, a large part of the population is physically inactive (7). This causes a number of adverse effects on health conditions including cardiovascular diseases (CVD), obesity, T2DM, sarcopenia (low skeletal muscle mass), osteoporosis (low bone mass), neurological diseases, and inflammatory conditions. (8). In this project we focus on effects related to T2DM.

1.1.1 Diabetes mellitus Type II

Diabetes has been known more than 3500 years and was first described in Papyrus Ebers. The word "diabetes" was established by the Greek physician Aretaios. It refers to the individual´s propensity to drink abundantly, with increased amounts of urine (like a siphon) that smells like "honey", which caused the addition of "mellitus".

The development of T2DM is characterized by impaired glucose tolerance and hyperinsulinemia. Both strength training and aerobic training on a regular basis reduce the incidence of T2DM (9). Low-intensity physical activity lasting at least 40 min per week has been shown to protect against the development of T2DM, with a greater effect on those at high risk. Life-style interventions in randomized control studies have demonstrated that even a modest weight-loss combined with physical activity may prevent the disease in high-risk groups (10).

Physical activity is also effective as a tool to manage established diabetes. Walking two hours per week at low–intensity is associated with a reduced mortality risk (11). Overall training improves the glucose homeostasis, which is crucial for both prevention and management of T2DM. Aerobic training is beneficial for improving cardiovascular function in T2DM (12). It would therefore be interesting to determine if aerobic capacity could be a better predictor of long-term alterations in cardiovascular function than body weight and glycaemic control.

It may seem strange that during the last century physical activity was considered dangerous in situations where today it is advocated (13). In the Physical Activity Guidelines for Americans Advisory Committee (2008) it is stipulated that adults, each week, should be physically active with a minimum of 150 minutes of moderate intensity, 75 minutes of vigorous intensity, or a combination of both (14). In order to prevent obesity and thereby T2DM, increasing the energy expenditure together with restricted dietary intake is considered the most effective tool over time (15). Furthermore, sedentary life-style has been linked to insulin resistance. Only 5 days of bedrest lead to an increase in insulin response to glucose loading in healthy subjects, concomitant with increases in total cholesterol and triglycerides (8). Obesity is also associated with inflammation that per se increases insulin resistance 1 (16), see Figure 1.

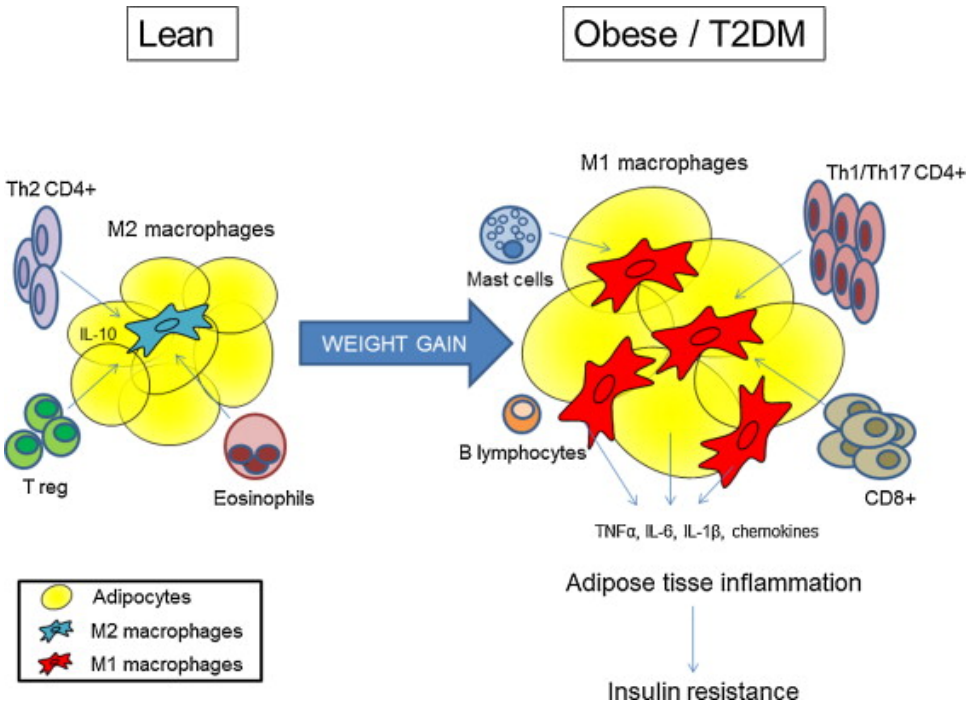

Figure 1. *Effect of fat accumulation on low-grade inflammation and insulin resistance. Permission by the Journal (17).*

The adaptive immune system cells interact with adipose tissue macrophages to modify their activation state. In lean adipose tissue, T lymphocytes promote an M2 macrophage polarization, maintaining an anti-inflammatory state. In obesity and type 2 diabetes, there is a shift to a pro-inflammatory state, promoting systemic inflammation and insulin resistance. Abbreviations: IL, interleukin; TNFα, tumor necrosis factor alpha; T2DM, type 2 diabetes mellitus; Treg, regulatory T lymphocytes.

1.2 PHYSICAL ACTIVITY, DEFINITION AND BENEFICIAL EFFECTS ON HEALTH

Physical activity is defined as bodily movement produced by active skeletal muscle that results in increased energy expenditure (18). In this thesis it refers to walking or jogging. This does not necessarily mean an increased load on the cardiovascular system. Walking, although not necessarily causing physical fitness, may still improve health and well-being. There is a consensus within the field of health and inactivity: regular physical activity is the best approach to avoid a number of health related diseases due to inactivity (19). Both women and men who are physically active on a regular basis have reduced relative risk of death. Daily regular physical activity early in life is, without doubt, far better than treating overweight/obesity (20).

1.2.1 Physical activity versus physical fitness

Physical fitness is defined as a physiological state of well-being that enables demands of a regular living and/or constitute a base for sports performance (20). Physical fitness in terms of cardiovascular function is a stronger predictor of health outcomes than physical activity. People that are endurance trained have a 50 % reduction of mortality compared with low-fit

individuals (21). A low cardiorespiratory fitness (low VO_{2peak}, slow heart rate recovery and failure to achieve target heart rate) are independent predictors of all-cause mortality (21). The greatest health effects are among sedentary subjects. However, in overweight subjects it is more important to increase physical activity rather than focus on physical fitness (19).

Physical activity

- Increases energy expenditure
- Improves metabolic health markers
- Improves carbohydrate metabolism in muscle
- Increases insulin sensitivity
- Is catabolic – break down energy storage
- Activates anabolic cell signaling
- Correlates positively with physical fitness and health

Modified after Caspersen CJ. Public Health Reports, 1985 Vol. 100, No.2

Aerobic training

- Increases energy expenditure
- Energy expenditure range from low to high (kilocalories)
- Is catabolic – break down energy storage
- Planned, structured and repeated over time
- Goes from low to high intensity of $VO_{2\ peak}$
- Improves mitochondrial function, density, and amount
- Increases anabolic cell signals
- Increases aerobic capacity
- Improves relationship between fat and fat-free mass
- Often a clear aim to improve or maintain physical fitness
- Correlates strongly with physical fitness and health

Modified after Caspersen CJ. Public Health Reports, 1985 Vol. 100, No.2

1.2.2 Adaptation in endurance training

The physiological mechanisms that occur during physical activity, as well as the positive effects of chronic endurance training on the body are not fully understood (22). Training changes the whole body homeostasis in which a number of acute and adaptive responses take place; both at cellular and systemic level (23). The system copes with the demand from the contracting skeletal muscle, by increasing the energy and oxygen (O_2) availability. It is the coordinated action from the cardiovascular, respiratory, hormonal, and neural system that supplies the skeletal muscle with O_2 and fuel in order to maintain or increase the level of activity, see Figure 2 (24). These adaptive responses have been essential for human development and crucial for survival.

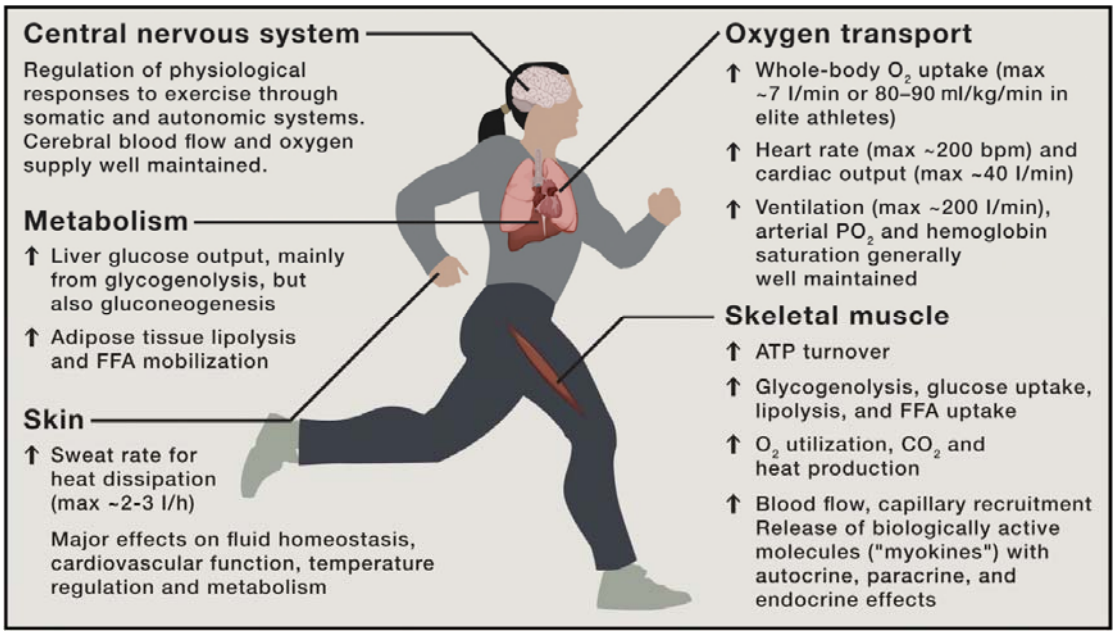

Figure 2. *The Physiological Responses to Voluntary, Dynamic Training*
Endurance training has coordinated multiple systemic effects with main purpose to support working skeletal muscle. Reproduced by permission from Cell (24).

At rest and submaximal level of exercises, endurance trained athletes have a pronounced bradycardia and an increase in stroke volume. These adaptations occur as a result of reduced firing rate of sinoatrial nodal pacemaker tissue (25).

Corrected for body size, the general endurance trained person has larger heart chamber volumes compared to the untrained person. Increased venous return may cause a remodeling of the heart. This increase in volume explains the larger stroke volume and results in the increase of maximum cardiac output commonly seen among endurance trained individuals (26).An endurance athlete has a larger maximal cardiac output mainly through a large stroke volume, but may have similar cardiac output at rest as a sedentary subject, see example in Table 2 (27).

Table 2. *A trained individual has a larger reserve capacity to increase cardiac output by increasing heart rate; an example shown in the table.*

	Stroke volume	Heart rate	Cardiac output
	mL	(beats/min)	(mL/min)
Sedentary	70	71	5000
Trained	100	50	5000

1.2.3 Endurance training and oxidative stress

1.2.3.1 Adaptation to increased free radicals in endurance trainings

Free radicals species (ROS) are compounds that are extremely reactive. Since they oxidize molecules such as DNA, protein or lipids, they were considered harmful. However, later it was discovered that they actually are necessary for other physiological processes e.g. the immune system. The potentially harmful effects of ROS are neutralized by the antioxidant system that acts protective to the organism (28).

However, epidemiological studies have shown that regular training decreases the incidence of OS-associated diseases (29-31). Several studies have also demonstrated lower baseline-levels of OS in athletes compared to untrained subjects (32, 33). The paradox is that acute strenuous training increases ROS even in well-trained athletes, resulting in greater levels of OS (34-37). A significant number of studies suggest that ROS formed during physical activity are involved in beneficial adaptations to training, e.g. in up-regulation of endogenous antioxidative systems and carbohydrate metabolism (38, 39). Concurrently, ROS generated in contracting skeletal muscle plays a crucial role in muscle adaptation to training, in particular stimulation of growth, differentiation, proliferation, apoptosis, and insulin-sensitizing (39-41). This is in contrast to low-grade inflammatory processes which over time increase OS, while antioxidative capacity decreases (42).

1.2.3.2 High intensity training and total antioxidative capacity

Short periods of high intensity training (HIT) are frequently adopted by athletes to improve aerobic/anaerobic capacity (43-47). These periods are characterized by a training load that exceeds an individual's overall capacity, called over-reaching (48). Several studies have shown that the total antioxidative capacity (TAOC) increases after short periods of HIT due to increased activity of antioxidative enzymes (49). ROS also have been identified as key regulators of the intrinsic TAOC via fast and long term up-regulation of the endogenous system enrolled in the antioxidative defense (29, 50, 51). It is established that athletes during

periods of HIT exhibit higher levels of biomarkers of OS, but also have an up-regulated TAOC in comparison with sedentary controls (45, 52, 53). Clearly, training up-regulates TAOC. However, the time course of this response is at present unknown. Moreover, the mode and intensity of training that is required to up-regulate TAOC requires further investigation.

1.2.3.3 Low-intensity versus high-intensity training

Low-intensity physical activity also has a positive effect on health related parameters. It is possible that positive prognostic effects may occur without obvious signs of physiological adaptation. Walking or gardening for more than 60 minutes a week has substantially lowered the risk of T2DM and other diseases related to a sedentary life-style, and e.g. lowered the risk of cardiac arrest (54).

Contrary to low intensity training, there is a clear physiological adaptation in HIT reflected in increasing cardiac output and haemoglobin concentration resulting in increased oxygen transportation, i.e. high levels of aerobic fitness with increased O_2 uptake. This has been investigated in athletes involved in competitive running, cycling, swimming or cross-country skiing, and has been demonstrated to reduce the risk of developing sedentary-related diseases (55). The results are consistent in different studies and independent of age and gender. Other effects are increased glucose uptake and glycogen storage in skeletal muscle, as well as increased release of myokines, blood flow, and capillary recruitment (56).

1.2.4 Work capacity - the prognostic role of a training test

Achieved workload, and oxygen uptake have been identified as strong risk markers for mortality and morbidity, and can therefore be used for prognostic evaluation (57, 58). These variables reflect the state of general health both in a healthy population and in individuals with chronic diseases (60, 61). In heart disease and the majority of the healthy population, decreased workload or oxygen uptake reflects the morbidity risk (21, 59-61).

For most individuals oxygen uptake reflects the cardiac limitation and works as a measure of physical fitness. Work capacity seems to be a robust parameter and often surpassed other traditional markers of increased mortality or morbidity, such as lipoproteins, tobacco use or blood pressure. Although several risk parameters have been identified from cardiopulmonary testing, oxygen uptake and work capacity are the most frequently used methods (21, 62-66). In addition, the amount of exercise, which is an omnipotent risk reducer for morbidity and mortality, may work as a prognostic tool.

1.2.5 Endurance training – an omnipotent reducer of metabolic disease risk

A number of epidemiological studies and controlled experiments have concluded that chronic endurance training overrules any pharmacological treatment of the metabolic syndrome. The idea of the working muscle as an endocrine organ releasing myokines came from Klarlund

Pedersen and her colleagues (67). The plausible explanation of the several fold positive effects in extenuating obesity and the metabolic consequences seems to be the endocrine-like signaling in particular proteins. This signaling together with other nucleic acids, has a “multi systemic” (68) beneficial effects on health due to improved glucose and insulin metabolism as well as reducing low-grade systemic inflammation. Most prominent is the reduction in the incidence of T2DM as well as the direct positive effect on cardiovascular diseases (69).

One of the key factors to maintain an overall health in normal aging is aerobic fitness (70). The capacity depends on a complex interaction between the pulmonary, cardiovascular and skeletal muscle systems as well as the immune function (71). It is a common thought that a regular physical active life-style promotes health and should be used as a powerful tool to prevent diseases related to a sedentary life-style (24). Endurance training in healthy elderly men may improve cardiac function as well as the aerobic capacity to the same extent as in younger adults (72). Several studies have demonstrated an inverse dose-relationship between the amount of physical activity and the risk of premature death, where physical activity is associated with more than 50 % reduced risk of death from cardiovascular disease (73). The benefits of physical activity also comprise patients diagnosed with cardiovascular disease (74). The reduction is similar for hypertension, hypercholesterolemia, and obesity in both women and men (21, 75).

1.3 EFFECTS OF PHYSICAL ACITIVITY ON VARIABLES REFLECTING GENERAL HEALTH

1.3.1 Body Mass Index

Body-mass index (BMI) (the weight in kilograms divided by the square of the height in meters) is frequently used by clinicians and researchers as a simple tool to estimate association with body fat and disease risk. A plethora of diseases have been associated with a high BMI such as: cardiovascular diseases, T2DM, certain cancers, gallstones, sleep apnea, osteoarthritis and Alzheimer's disease, see Figure 3. A large prospective study demonstrated that a high BMI is an independent risk marker for morbidity and mortality (76). Although BMI is well accepted it does not give any information of the relationship between fat mass (FM) and fat–free mass (FFM). It is presumably the excess of body fat and/or its localization, not the body weight per se, that is implicated in the risk of the development of metabolic diseases. Data from NHANES showed that one-quarter of the subjects with normal weight in terms of BMI had cardiovascular and metabolic abnormalities (77). They were characterized as sedentary with a low $VO_{2\,peak}$, and abdominal visceral adiposity (78).

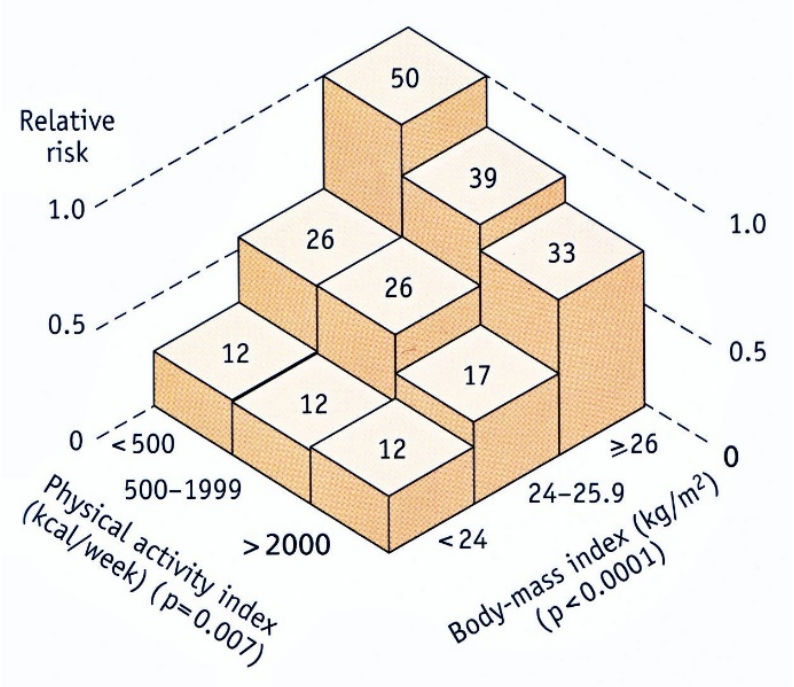

Figure 3. *Age adjusted incidence rates and relative risks of T2DM among 5990 men based on physical activity and BMI. Numbers on each block represent incidence rates during 10,000 man-years, and risk for tallest block is set to 1.0. Adopted from (79).* *Reproduced by permission from the Journal. Copyright Massachusetts Medical Society.*

Another risk for misinterpretation is that obese individuals with sarcopenia have a normal BMI and the associations with morbidity in elderly people might be underestimated due to this factor (80). On the other hand a person could have a BMI in the limits of overweight and at the same time be classified as "underfat". Other factors than excessive fat such as bone, muscle mass, and plasma volume, affects BMI and could lead to an incorrect interpretation. This is not unusual in among athletes. The situation could also be the opposite; an athlete with a clinical eating disorder is overlooked due to a normal BMI (81). BMI could be useful

in well-defined groups in a context with other risk markers for overweight and obesity. The problem with BMI is the assumption that a high BMI correlates with a high amount of body fat. BMI does not distinguish any details in body composition and for comparative purposes should only be used with repeated studies of individuals or matched groups and not as a tool for evaluating fatness.

1.3.2 Body composition

The human body composition reflects net life time accumulation of nutrients and the level and type of physical activity among other variables. Body composition estimations divide the body mass into two major components: FFM and FM. Depending on the method the body mass can be structured in sub components. All methods use different techniques and they cannot be compared with one and another (82).

The FFM consists of skeletal muscle, bone, connective and other tissues. Total body fat consists of subcutaneous, visceral, and intramuscular fat. Parts of the body fat are considered as essential to maintain life and for reproductive functions. In some sports, body composition is an important determinant of performance (e.g. weight class sports, leanness sports). Most athletes have their own thoughts around optimal relationship between FM and FFM, and they are not comparable between sports, see Table 3. To correct for differences in body composition related to height the index of FM and FFM is a simple method (Fat Mass Index (FMI; kg/m2) and Fat-Free Mass Index (FFMI; kg/m^2).

Table 3. *Fat-free mass and body fat in female and male athletes. Adopted from (83), presenting data from (84, 85).*

Body composition in endurance athletes						
	Age (years)	**Height (cm)**	**Body weight (kg)**	**Body fat (%)**	**Body fat (kg)**	**Fat-free mass (kg)**
Females	32	169.4	57.2	5.9	9.1	49.5
Males	-	176.8	62.11	4.3	2.73	59.38

Individuals performing endurance training regularly for many years often have a high FFM and a low FM compared to sedentary individuals, see Table 4. A sedentary life-style over a longer period increases the FM and decreases the FFM. The result is an un-favorable ratio of FM to FFM and the risk of developing sarcopenia and osteoporosis increases. The body mass

is closely related to the variations in peak oxygen uptake ml·kg-1·min-1 (also written as ml/(kg·min) ($VO_{2\ peak}$). This has led to the common practice of expressing oxygen consumption in relation to surface area, body mass, or FFM. The maximal uptake corresponds to the dimension of the body. The size of the contracting muscle mass largely account for the gender difference in aerobic capacity. Adipose tissue in this context is not metabolically active, and could be subtracted from the body weight to get the peak oxygen uptake in FFM. Several studies have come to the conclusion that the average $VO_{2\ peak}$ is independent of gender in elite athletes after correction for FFM (86). In an average population there are other factors that influence the aerobic capacity such as: a sedentary life-style, social and cultural differences.

Table 4 *Ranges of body fat in men and women of different ages. With permission from Human Kinetics Inc. (87).*

Relative body fat in the average Caucasian population			
Age	**Up to 30**	**30-50**	**50+**
Females	14-21%	15-23%	16-25%
Males	9-16%	11-17%	12-19%

1.3.3 Skeletal muscle

The skeletal muscles contribute to whole body energy expenditure and are important for insulin-mediated glucose disposal (88). Reduction in muscle mass lowers total energy expenditure, decreases glucose uptake concomitant with a lower capacity to store muscle glycogen. Thus, glucose will be directed to the adipose tissue and used for lipogenesis rather than being stored as muscular glycogen (89). Physical activity is an anabolic stimulus per se for skeletal muscle mass and therefore a sedentary life-style has been demonstrated to be a risk factor of diseases related to inactivity, notwithstanding that there is not an established causal relationship (90-92). The skeletal muscle is the primary site of fat oxidation during training A low capacity of fat oxidation has been implicated in the development of obesity (93). This concept is termed metabolic fitness and describes a reduced capacity to oxidize fatty acid and affects negatively the total energy expenditure in overweight and obese individuals. In other words skeletal muscle is the greatest contributor to the total energy expenditure in healthy adults and an important predictor of metabolic fitness (94, 95). The human skeletal muscle has a remarkable remodeling capacity even in older subjects. This

remodeling capacity is specific to the type of training, gender and the response to training in any individual (96).

1.3.4 Consequences of reactive oxygen species

An increased production of ROS results in degradation and damage of molecules by oxidative processes causing OS. OS is the result of an imbalance between ROS and TAOC (97). OS is involved in chronic disorders, including cardiovascular, metabolic, neurologic diseases, as well as aging (50, 98). Recently it has been suggested that ROS also is implicated in low estrogen and bone loss (99). Conversely, OS has been demonstrated to elicit muscle damage, muscle fatigue, and impair immune functions, as well as training performance (100, 101). Nevertheless, during the last decade it has been demonstrated that ROS formed during training also have beneficial effects necessary for the immune system as well as other important biological functions such as molecular signaling (102).

1.3.5 Antioxidative capacity

The antioxidant system prevents and limits the effects of free radicals by removing the unpaired electron. When the unpaired electron is eliminated the free radical molecule becomes less reactive, see Figure 4. The antioxidant system consists of an enzymatic endogenous system (catalase, glutathione peroxidase, superoxide dismutase) and a non-enzymatic system that derives from different food components (e.g. vitamin E [tocopherol], vitamin A [retinol], vitamin C [ascorbic acid], glutathione and uric acid). Several minerals (including selenium and copper) are essential components of the endogenous system and both systems regulate the TAOC. Moreover, physical activity per se is considered an antioxidant that up-regulates the endogenous system (50).

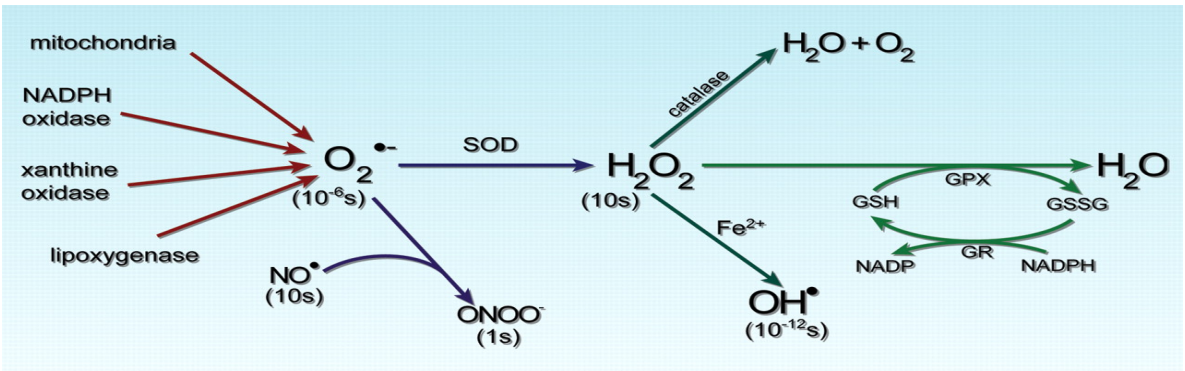

Figure 4. *Major ROS and their elimination in muscle (103).*

GPX, glutathione peroxidase; GSSG, oxidized glutathione; ONOO¯ peroxynitrite; GR, glutathione reductase; NADP, nikotinamid-adenin-dinukleotidfosfat (oxidized), NADPH, nikotinamid-adenin-dinukleotidfosfat (reduced) SOD,superoxid dismutase.

2 AIMS

2.1 STUDY I

The primary aim was to explore the effects of un-supervised low-intensity physical activity in sedentary overweight/obese subjects, with or without T2DM, on cardiovascular risk factors. A secondary aim was to evaluate compliance or adherence to the program. The battery of testing consisted of well recognized health markers such as: work capacity, glucose control, insulin resistance, serum lipids, and blood pressure.

2.2 STUDY II

The primary aim was to investigate the effect of a 3 weeks period of endurance training (ET), in healthy, normal weight, sedentary young adults. The variables were total antioxidative capacity (TAOC), oxidative stress (OS), inflammatory markers, work capacity, anthropometric markers, and body composition. A secondary aim was to investigate if there was a gender response to ET based on the variables.

3 METHODOLOGY

3.1 MATERIAL AND METHODS - STUDY I

3.1.1 Subjects

In study I a total of 213 healthy individuals and patients with T2DM were recruited with a focus in the area of a primary care centre, Gustavsberg outside Stockholm. Invitations were published mainly by advertising in the local newspaper, and by letters of invitation to former participants in the Stockholm Diabetes Prevention Program. Inclusion criteria were age 45-69 years and BMI > 25 kg/m^2. Subjects with insulin treatment, severe physical or cardiovascular impairment, atrial fibrillation determined by ECG, systolic blood pressure (SBP) > 160 or diastolic blood pressure (DBP) > 100 mmHg were excluded. Upon inclusion the subjects were divided in normal glucose tolerance (NGT, n=128), impaired glucose tolerance (IGT, n=35), and T2DM (n=50) based on an oral glucose tolerance test (OGTT). On the same occasion body weight, BMI, waist circumference, blood chemistry, blood pressure, plasma lipids, as well as medication for diabetes, hypertension, and dyslipidaemia were registered. The training test was performed at a different occasion at the department of Clinical Physiology.

The study protocols were approved by the Regional Ethical Review Board, Stockholm, Sweden (Dnr. 2007/717-31/1-4).

3.1.2 Study design

Blood sampling, work capacity tests and anthropometric measurements were performed at baseline and after 4 months. The subjects were randomized in a control group, and a training group walking 5h per week with poles (Nordic walking) for a 4-month period. The study continued May to September 2006, 2007 and 2008. All participants in the intervention group were informed of the effects of physical activity on glucose and insulin metabolism. They also received instructions on how to walk with poles to avoid injuries. Finally, all participants were instructed not to change their dietary intake and to maintain their regular life-style. The physical activity was un-supervised, but the participants kept a diary over the hours they walked. At baseline and after 4 month the participants estimated the level of their regular physical activity as: low, medium, or high (see Paper I).

3.1.3 Accelerometer

To compare the participant subjective assessment of their regular physical activity with an objective estimation 25 consecutive participants (11 controls and 14 in the intervention group) agreed to wear an accelerometer (Actigraph model GT1M Pensacola Florida, USA) during daytime for 7 days. The activity was estimated as counts per minute per day, divided into low, medium, and high intensity based on the frequency and estimated as Metabolic Equivalent of Task (MET).

3.2 MATERIAL AND METHODS - STUDY II

3.2.1 Subjects

The subjects (14 subjects, 8 women and 6 men, 22-30 years) were recruited by advertising at local universities in the Stockholm area. The individuals were classified as sedentary if they were not involved in any regular moderate or vigorous intensity training for more than 2 h per week for two years prior to the study. Low intensity physical activity was permitted, which allowed the students to bike or walk between home and universities on a regular basis, the normal transport pattern for students in Stockholm (see Paper II, Table 1). The participants were screened by physical examination, ECG, echocardiography and blood tests for general health to exclude participants who had any sign of cardiovascular disease or other major acute or chronic diseases.

Written informed consent was obtained from all participants and the study was approved by the Regional Ethical Review Board, Stockholm, Sweden (Dnr. 2007/717-31/1-4).

3.2.2 Study Protocol and Training Program

Aerobe capacity, body composition, anthropometric measurements, and blood samples were measured at baseline, after 3 weeks of training, and after 4 weeks of detraining, i.e. returning to regular sedentary life-style, see Figure 5.

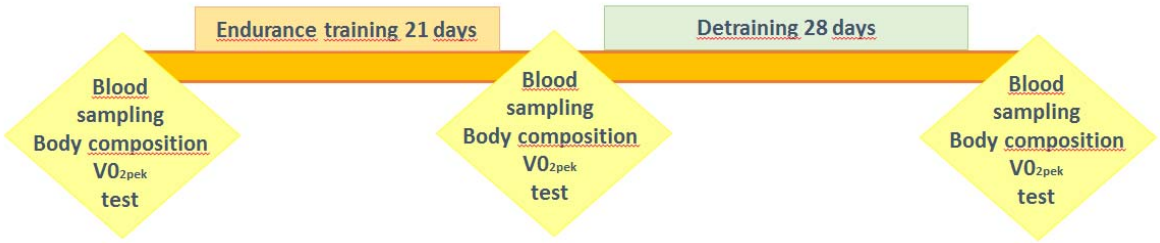

Figure 5. *Protocol Study II.*

The training program included 2 sessions per day, 6 days per week for 3 weeks. The training sessions were all supervised and all participants attended all sessions. All sessions consisted of treadmill running and indoors or outdoors cycling. Within the individual there is a relationship between the heart rate and percentage of maximal work capacity and adaptation to training was continuously monitored by increasing aerobe intensity at the target heart frequency. After one week, cycling and running sessions lasted 50 and 40 minutes (min), respectively. The morning sessions were performed on an ergometer cycle that followed a strict protocol: The participants started with a 10 min warm-up at low intensity followed by

30 min cycling at a heart rate defined at VO_2 test to approximate the lactate threshold. After finishing the aerobe training the subjects recovered at a low intensity for 10 min. In the afternoon the sessions consisted of running indoors/outdoors. The sessions always began with a 10 min warm-up followed by 25 min running at an intensity corresponding to the cycling session. Every running session was always followed by a 5 min recovery at low intensity. After 3 weeks of training, the same samples and the measurements at baseline were repeated. After 3 weeks of training the measurement protocol that was performed at baseline was repeated. The participants were informed not perform any activity except for the regular walking and biking. All participants had a detraining period for 4 weeks. At the final day of the detraining, all the baseline measurements were repeated.

3.2.3 Oxidative stress and total antioxidative capacity

Total antioxidative capacity (TAOC) and oxidative stress (OS) were determined by a system that is based on the Lambert-Beer law and the Fenton reaction respectively.

Antioxidative capacity: By adding a stable colored radical (FeCl3) to a 50 µl of blood sample in an acidic solution (pH = 5.2), a decolored solution detectable at 505 nm is produced. The radical decreases in absorbance in proportion to the blood antioxidant concentration of the sample. A permeable cell derivative of vitamin E, used as an established antioxidant, the absorbance values in the samples is compared with a standard curve. The results correspond to the overall antioxidant activity that is present in the solution (104).

Oxidative stress: OS was established by adding prepared reagents to each blood sample. Free radicals are produced in the solution and through the interaction with phenylenediamine [2CrNH2]), a colored, averagely long-lived radical cation is formed. The quantity is directly proportional to the quantity of peroxides present in the sample. The intensity of the red-colored solution correlates with the hydroperoxides concentration and the quantity of free radical compounds. This is a measure of the oxidative status of the sample according to the Lambert-Beer law.

Blood was obtained from finger and analyses were performed according to the manufactures instructions using a dedicated spectrophotometer (Form CR2000, Callegari, Parma, Italy). The participants were instructed not to carry out any strenuous physical activity the 24 hours prior to test, and be fasted for 4 hours prior to the blood test.

3.2.4 Body composition and anthropometry measurements

Body composition in terms of FM and FFM was determined with the Bod Pod (Life Measurements Incorporated Concord, software version 1.68, CA USA), which is a whole body plethysmograph, measuring body composition. The Bod Pod is considered as an easy non-invasive method with high reproducibility with test scores ($r>0.90$) within and across days. Body density computes as total body mass, is converted to density, using the Siri

equation to percentage of body fat (105). The day to day variation was controlled one week before the study, in one male and two female subjects during five consecutive days.

To ensure the reliability of the measurements subjects were wearing tights and a swimming cap provided by the manufacturer. The system was calibrated daily when in use, according to the guidelines. The participant's waistline and hip were measured according to International Standardization of Anthropometry and Kinanthropometry (ISAK), and their body weight was determined on a calibrated scale connected to the Bod Pod, Participants were instructed not to take part in any strenuous physical activity the last 24 hours, and be fasted for 4 hours prior to body composition measurements. Possible differences in FM and FFM associated with height, was eliminated by calculating the Fat Mass Index (FMI; kg/m2) and Fat-Free Mass Index (FFMI; kg/m2).

3.3 TESTS FOR BOTH STUDY I AND STUDY II

3.3.1 Work capacity test

In both studies the participants performed a continuous incremental cycle ergometer test (Rodby, RE990, Rodby innovation AB, Uppsala, Sweden) with a 12-lead ECG (CASE/ Carestream, GE Healthcare, Freiburg, Germany). In study 1 the starting load was 30W in women and 50W in men followed by 10W increase per minute. In study 2, the starting load for all participants was 70 W, followed by 20 W increased per minute. Oxygen uptake was measured breath-by-breath and averaged in 20-s intervals using indirect calorimetry (Vmax ENCORE, VIASYS/CareFusion, Germany). Prior to each test, the system for O_2 and CO_2 was calibrated with standardized gases. The work rate was continuously increased until the participant volitionally terminated due to exhaustion. In study 2, participants were considered to have reached volitional exhaustion when the they could no longer maintain a pedal frequency of 60 Rate Per Minute (RPM) along with at least two of the following criteria: a respiratory exchange ratio (RER) >1.10, rating of perceived exertion exceeded 16 out of 20 according to the Borg's RPE scale and a maximal heart rate exceeding 85 % of the estimated age-predicted maximal heart rate according to commonly used equations. Peak workload was determined from the moment the subject could not maintain the targeted RPM or when participants terminated due to exhaustion. VO_{2peak} and peak ventilation were determined from the highest 20-s-period during the training before the test was interrupted.

3.3.2 Biochemical markers

Haemoglobin, and erythrocyte concentration, C- reactive protein, glycated haemoglobin (HbA1c), total cholesterol, high and low density lipoproteins (HDL and LDL), apolipoprotein (Apo) B and A1 were analyzed by standardized methods at the University Laboratory, Karolinska University Hospital. Tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6)

Monocyte Chemoattractant Protein -1(MCP-1), Hepatocyte growth factor (HGF), leptin and insulin were analyzed by Luminex technology (106).

3.4 STATISTICS

3.4.1 Study I

Pearson's paired t-test was used to analyze the within-group difference. This was followed up by a two-sample t-test for between-group differences. Differences of sex distribution, medication and physical activity levels were analyzed by $\chi 2$ or Fisher's exact test. A p-value of <0.05 was considered statistically significant. For statistical analyses, Stata statistical software (Stata Corporation, College Station, Texas, USA) was used. Follow-up data were missing for ten participants, and the principle of last-observation-carried-forward was applied in those cases. Analyses were performed as intention-to-treat.

3.4.2 Study II

Values are reported as arithmetic mean and SD. Women and men were evaluated on 3 occasions. Comparisons between the 3 time-points were made for the whole group by repeated-measures ANOVA. Sphericity was tested and when there was a significant difference in variance between time-points we applied the Greenhouse–Geisser test. When ANOVA indicated significance, we performed paired t tests between time-points and $p < 0.05$ was considered significant. All analysis was performed using SPSS/IBM v.23 (IBM, Chicago, IL, USA).

4 RESULTS

4.1 STUDY I

The subjects differed only marginally between intervention and control groups at baseline. In the NGT intervention group, the lipid-lowering statin medication was more frequent, the total cholesterol level lower and high physical activity reported less frequent compared to the NGT control group. The triglyceride level was higher in the T2DM intervention group compared to the control group. We found no other significant differences between the control and intervention groups.

The change in serum lipids, Hba1c, HOMA IR, and blood pressure during 4 months did not differ between the intervention and control groups.

Work capacity was slightly increased in the T2DM, in the other groups there was no difference between baseline and after 4 months of intervention.

Four individuals, all allocated to the intervention group (1 NGT, 2 IGT, 1 T2DM) altered their medications. Two altered their anti-hypertension treatment, and two stopped the treatment. For those participant's, blood pressure levels were not included at the end of the study. According to intention-to-treat practice, baseline SBP and DPB were carried forward.

In the NGT group weight, waist and body mass index were statistically improved after the intervention. The subjects in NGT intervention group also experienced a higher level of self-reported physical activity in terms of medium and high-intensity ($p<0.001$).

The adherence to Nordic walking was aberrant within the intervention groups. Hence, a separate analysis for the 55 subjects that reported ≥ 80 % of prescribed time in their log-books. In this group (all interventions group included) VO_{2peak} and/or power output significantly improved compared with their respective control group.

4.1.1 Withdrawal rate

Ten participants decided to leave the study before the final examination after 4 month. The reasons to withdraw were: personal /lack of time (n=4), medical (n=4), and unknown (n=2). Their baseline data were included in the intention – to- treat analysis. They were 7 female (5 NGT, 1 IGT, 1 T2DM - 5 interventions and 2 controls) and 3 male subjects (1 IGT, 2 T2DM - 1 intervention and 2 controls).

4.2 STUDY II

4.2.1 Effects of endurance training and detraining on work capacity

The training effects differ between women and men. In women workload, $VO_{2\ peak}$ and VE increased significantly after 3 weeks of endurance training ($p<0.001$, $p<0.05$ and $p<0.01$

respectively). After 4 weeks of detraining workload and $VO_{2\ peak}$ decreased ($p<0.01$, $p<0.05$) but VE did not change. Compared to baseline workload and VE but not $VO_{2\ peak}$ were still increased (both $p<0.01$). After 3 weeks of training VT increased ($p<0.05$) and the improvement remained after 4 weeks of detraining.

Also in men there was a significant increase in workload, $VO_{2\ peak}$ and VE after training (all $p<0.05$), and for all variables the effect remained after detraining (p=ns). Compared to baseline workload and $VO_{2\ peak}$ were still increased after 4 weeks of detraining ($p<0.001$, $p<0.01$). In men VT and VE were not affected.

When adjusted $VO_{2\ peak}$ for FFM the average results equals for both groups: women (57.8±6.6, 64.5±5.3, 61.2±3.9); men (62.1±12.3, 67.2±11.2, 65.5±13.6). However, the results within the groups remain: both groups ameliorate from training but men preserved their improvement after detraining compared to baseline.

There were no significant differences in RPE according to the Borg scale, RER and the maximal heart rate for the participants before and after endurance training and after detraining, indicating that the subjects performed and interrupted at the same level of exhaustion at all three occasion.

One participant in the female group continued some physical exercise during the detraing period. She maintained her aerobic capacity to the same extent as after ET. Correcting for her results the aerobic capacity in women declined even more. Notwithstanding, we decided not to exclude her from the study.

4.2.2 Effects of endurance training and detraining on biochemical markers

The effects of training on blood analyses differ between men and women. After 3 weeks of endurance training the levels of TAOC decreased but only in women ($p<0.05$). In men there was just a trend to decreased TAOC (p=0.082). OS decreased significantly in women after 4 weeks of detraining compared to baseline ($p<0.05$), in men there were no significant changes.

Other inflammatory markers display differences between men and women. MCP-1 decreased after training compared to baseline ($p<0.05$). In men MCP-1 decreased detraining compared to baseline ($p<0.05$). TNF-α decreased significantly in women after 3 weeks of training, and after detraining compared to baseline (both $p<0.05$). In men TNF-α decreased significantly after detraining compared to baseline ($p<0.01$). HbA1c increased significantly in women after detraining compared to training ($p<0.05$). In men there was a tendency (p=0.058) to increased HbA1c. IL-6 decreased significantly in women after training compared to baseline ($P<.01$), there were no significant changes in men.

CK increased significantly only in women after training and remained elevated after detraining compared to training ($p<0.05$, $p=<0.001$). Haemoglobin decreased in women after training ($p<0.5$) but returned to baseline levels after detraining. In men there were no significant changes.

4.2.3 Effects of endurance training and detraining on body composition and anthropometric measurements

Except for a slight increase in FFM (kg) and FFMI after detraining compared to baseline ($p<0.05$) seen in women, there were no significant changes in body composition.

5 DISCUSSION

Physical activity has been associated with so many positive health effects that it should be considered as an effective multi-pill (107). We observed positive effects of training in two completely different populations during relatively short periods. Despite of low adherence to walk with poles 5 h per week, the results were positive.

The secondary aim of study was to investigate the compliance to unsupervised Nordic walking as a useful tool in a clinical setting. Already in 1989 the importance of counseling patients to increase physical activity was considered meaningful in clinical settings (108). We demonstrated that it is possible to increase the weekly physical activity, beneficial for health, even among sedentary overweight as long as the goal is interpreted as achievable. This is of clinical relevance and could therefore be used as a tool to reduce the negative impact of overweight in sedentary patients with reduced glucose tolerance.

5.1.1 The effects of low-intense activity on body weight, BMI and waist circumference in NGT subjects

At baseline the body weight, BMI, and waist were lowest in the NGT and highest in the T2DM. After 4 months the NGT group self-reported an enhanced physical activity, and they also achieved a loss of body weight, BMI and waist although modest. This indicates that low-intensity physical activity is more effective in subjects with normal glucose metabolism. Another theory is that the participants in NGT have had a higher level of both physical activity and/or intensity at baseline. They were more active, less overweight and therefore had a better ability to perform the walking sessions.

One of the major effects in our study was the decrease in waist circumference in the NGT group. We did not measure the visceral fat; however it is reasonable to assume that the modest decrease in body weight was due to a loss of visceral fat.

The excess of visceral fat is proposed to be one of the main risk factors in the development of the metabolic syndrome (109). It is well accepted that chronic training even at low-intensities, prevents an increase of FM and induces a loss of FM, although the dose response relationship is poorly understood. The fact that low-intensity activities preserves and sometimes even increases FFM is often underestimated (110). Thus, positive effects may be reached without change in body weight (111). Although overweight is a risk factor in itself it is important to investigate what the weight loss consists of; a reduction of FM, FFM or both (112, 113).

5.1.2 The effect of low-intensity physical activity on body weight, BMI and waist circumference in IGT and T2DM

In the IGT and T2DM groups we did not find the same positive results. Numerical change does not indicate group size as an obvious reason. One possible explanation is that subjects with T2DM are resistant to training in some way, or/and perform less intensive training. One reason is that the walking speed is reduced in subjects with T2DM (114). They often demonstrated a reduced metabolic and mitochondrial function that decreases ATP-

production. This reduces skeletal muscle function, aerobic capacity, and ultimately walking speed (115-118). It has been demonstrated in women with T2DM, that low-intensity activity did not alter the metabolic profiles. However in postmenopausal women without T2DM, both visceral fat and aerobic fitness improved after a low-intensity activity (119). Aerobic fitness and metabolic fitness do resemble each other; nevertheless improvements can be obtained independently in just one of them (93). The DREW study reported that participants with low level of fitness might need training at higher intensity/and/or dose of training to improve fitness (120).

5.1.3 Beyond the recommendations of physical activity in sedentary population

Although, it is more than well established that physical activity protects from the development of obesity it is extremely difficult to motivate sedentary individuals to increase their physical activity on a regular basis. Both overweight and/or sedentary individuals with normal weight have a number of reasons to remain sedentary such as: low motivation, negative previous experiences, lack of know-how and high costs of access to training facilities (121, 122). Desharnais et al 1986 demonstrated that in overweight women too high expectations for weight loss, and a failure to meet them, was actually a prediction of low adherence to a structured training program (123). Being sedentary for a decade or more makes it is difficult to implement daily physical activity. We observed that unsupervised Nordic walking was achievable and provided a sensation of actually training.

5.1.4 Effect of short-term endurance training on work capacity in young healthy sedentary subjects

The larger effect of training in women measured by VT is likely a consequence of the fact that female participants were more sedentary. Women in an average population are less physically active than men and therefore they have a lower aerobic capacity (83).The training response depends on the initial fitness level meaning that someone who starts at a low level has more beneficial effects with respect to central circulatory capacity, in our study reflected in the increase of VT (124). Other crucial factors are: training intensity, training frequency, and training duration. A physically fit person requires higher level of stimuli to achieve a training response. All men except for one had a regular daily physical activity pattern of bicycling or walking to school and home again. This is a probable why the ET did not provide enough stimuli in the male group. The decline in women after detraining has been demonstrated in other studies with a similar design (125). Even in endurance-trained subjects the aerobic as well as the metabolic capacity decline already after 1-2 weeks of detraining (126). From the age of 25 people intend to accumulate the amount of body fat parallel with a slow decrease in aerobic capacity. Our results indicate that ordinary bicycling and/or walking every day is sufficient to maintain an increased aerobic capacity.

5.1.5 Body composition and anthropometry after endurance training and detraining

The relationship between body fat and FFM is important for health status in the average population. Men normally have more FFM and women considerably more FM due to different hormonal status after puberty. There is little doubt that being leaner is an advantage since FM is not involved in transportation of oxygen (127). A high amount of body fat expressed in percentage could be interpreted in two ways: a high amount of total body fat in kg and/or in combination with a low amount of FFM. In our study women had a considerable relative and absolute high amount of body fat. The situation was similar in men although their values were lower. The body fat index and the FFM index in both groups were within the normal range (128).

A loss of FFM occur in relation to a too low energy intake or so called semi starvation (129). There were no changes in FFM after training in both groups, indicating that they were in energy balance during ET. It is well accepted that athletes when increasing their training regime, not always follow up with a matching energy intake particularly in carbohydrates. After detraining, women had an increase in FFM (kg), FFMI, and Hba1C, indicating a compensating increase in energy intake particularly from carbohydrates. This is confirmed by the theory that individuals with reduced glycogen store gain more weight than those with larger glycogen stores (93, 130). A too low intake of carbohydrates will negatively affect fat and glucose oxidation followed by difficulties to lose body fat (131). This could be one explanation of the lack of loss in FM. Increasing the amount of physical activity by 2 hours per day, 6 times per week should theoretically increase energy expenditure by 2-300 kilo calories per day, which has been demonstrated, be enough to lose 0.5 kg/week of body weight (132). An important finding is that despite of a normal BMI, the relative and absolute amount of body fat was in the upper range for their age. If this continues it could have future implication on the overall health, as well as it increases the risk of metabolic complications.

5.1.6 Effect of short-term endurance training on total antioxidative capacity, oxidative stress, and other biochemical markers in young healthy sedentary subjects

Endurance athletes at elite level train about 1500 h per year. There is substantial evidence that prolonged ET increases the production of free radicals causing OS. Regular physical activity reduces OS and inflammation by increasing the endogenous antioxidative capacity. (133). It is not clear to what extent OS is harmful to the athlete. Contrary to what we expected we found a decrease in TAOC in women, while in men there was a tendency. This could be explained by the fact that women were more sedentary and thus the training was more strenuous for them. This was also confirmed by higher levels of CK in women. An interesting finding was that the levels of OS in women decreased significantly after detraining compared to baseline, indicating a positive effect of ET. The antioxidant reserve capacity in most tissues is rather small, and continued and/or strenuous endurance training could theoretically deplete the antioxidant system (134).

Ultra-endurance events attract recreational as well as elite athletes. A number of studies have investigated the effect on OS and antioxidative capacity in association with these events. It has been found that different markers of OS remain elevated for several days in athletes after the ironman triathlon. These athletes have higher concentration of antioxidants in erythrocytes at rest but after a race they are reduced (47). An imbalance between ROS and TAOC favors an excessive accumulation of ROS following an increase in OS. It has been suggested that an increase of TAOC counter balance the increase of ROS. Lower TAOC precedes higher levels of OS and thus TAOC could be a predictor than OS (42).

The normal range for OS with our method is between 210-310 Fort Units. The levels of OS in women were higher than normal at all occasions. Inflammatory markers were within the normal range; however they values decreased significantly after training in both men and women.

5.1.7 Methodological considerations

5.1.7.1 Limitations study I

Several limitations of this thesis should be addressed. The subjects were a mixture of patients in the primary health care system in Sweden. Therefore it was difficult to have the same number of participants in each group. Especially subjects with IGT are difficult to recruit since IGT is not a common diagnosis in primary health care. With a higher number of participants the statistical power could have been stronger. The walking sessions were not supervised or performed in groups and the information regarding compliance is scarce. Except for diaries there is no other information. The self-reported amount of physical activity could have been over/underestimated. Neither do we have no knowledge if they the intervention group increased their weekly activity with 5 h per week. We have no information of dietary intake or alcohol habits. We did not measure the body composition and resting metabolic rate. Neither did we establish the metabolic flexibility test when performing VO_{2peak} test.

5.1.7.2 Strength study I

The study was executed in a clinical setting in the participants neighborhood based on the advices that would normally be given to patients. This was motivating and encouraging enough to actually have a reasonable adherence. The low-intensity activity with poles was also designed to prevent the patients from injuries or inconveniences also in subjects with T2DM. Physical performance tests were performed by a well-established evaluated method.

5.1.7.3 Limitations study II

It was difficult to recruit sedentary participants in the Stockholm area willing and capable of training two times per day. The total number of subjects was thus limited. The training protocol did not contain any resistance training or HIT. It is difficult to follow a training

schedule for 3 weeks without variation. This will probably influence the willingness to train that could affect the training quality and ultimately the results. The level of physical activity at baseline and during detraining was self-reported and not controlled. We have used an evaluated method to measure body composition adapted from a "golden standard" method. However, it should be recognized that with this method we only have information on FM and FFM. We have no knowledge of the amount or distribution of skeletal muscle, localization of FM, bone mineral content or hydration status.

Although the FORT/FORD method is well-established it does not give any information about the OS or antioxidative capacity locally e.g. in skeletal muscle or mitochondria. However, together with other inflammatory markers in plasma this method could reflect the overall balance between TAOC and OS.

We did not register the dietary intake and the participants did not receive any nutrition support during the training period. We could only speculate if the outcome would have been different with nutrition support and controlled energy intake. Dietary intake is also closely related to the TAOC and this lack of information limits our interpretation of the results. Furthermore we did not measure the resting metabolic rate or estimated the metabolic flexibility.

5.1.7.4 Strength study II

The strength of this study is that all training sessions were supervised and the participant did not fail any session. They were always training together with the same instructor. The tests and blood sampling were standardized and performed by the same persons. Physical performance test was performed by a well-established evaluated method.

5.1.8 General conclusions

- Low-intensity nordic walking 5 h per week improved body weight, BMI, and waist in NGT. Adherence > 80 % improved the results in all groups in terms of power output and VO_{2peak} – compliance is important for results.
- Young women and men increased their work capacity by short-term endurance training.
- Young men preserved the improved aerobic fitness to a greater extent after detraining possibly due to a higher level of daily physical activity.
- Women had a significant decrease of TAOC after training.
- Women had a higher range of body fat than normal for their age despite a normal BMI.
- Future studies on work capacity and effect of detraining should include daily activity, such as active transportation to and from work.

5.1.9 Future perspectives

This thesis contributes with information of clinical relevance of the importance of daily regular physical activity in different sedentary groups. In order to evaluate further mechanism of a sedentary life-style, it is intended to study resting metabolic rate, metabolic flexibility, and body composition in accordance with nutritional information. Furthermore mechanistic studies are needed to get a better understanding of cell-signaling events when going from active to sedentary life-style.

An understanding of the need of a regular daily physical activity has to be put into a multidisciplinary perspective. This is important to develop better counseling in clinical settings to patients

The design in study II could be used as a new way to understand the physiological effects useful in preventing and/or maintaining a healthy body composition and aerobic fitness (135).

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