Henna Cederberg

RELATIONSHIP OF PHYSICAL ACTIVITY, UNACYLATED GHRELIN AND GENE VARIATION WITH CHANGES IN CARDIOVASCULAR RISK FACTORS DURING MILITARY SERVICE
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RELATIONSHIP OF PHYSICAL ACTIVITY, UNACYLATED GHRELIN AND GENE VARIATION WITH CHANGES IN CARDIOVASCULAR RISK FACTORS DURING MILITARY SERVICE

Academic dissertation to be presented with the assent of the Faculty of Medicine of the University of Oulu for public defence in the Auditorium of Kastelli Research Centre (Aapistie 1), on 25 November 2011, at 12 noon

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Abstract

The increase in the prevalence of overweight and obesity parallels the increase in physical inactivity and sedentary lifestyle, and leads to the worsening of cardiorespiratory fitness. Both overweight and physical inactivity are recognised risk factors for the development of cardiovascular disease, insulin resistance and type 2 diabetes, but the independent effects of cardiorespiratory fitness and obesity on cardiovascular risk factors remain debated.

Lifestyle interventions are the key treatment for overweight and obesity. There are however, limited data from large population-based studies on the efficacy of exercise in modifying cardiovascular risk factors in young adults. Many of the mechanisms underlying the changes in body composition and metabolism achieved by exercise interventions are not well understood. The role of adipokines, and particularly unacylated ghrelin has been proposed in relation to changes in glucose metabolism. Individuals also vary in their response to exercise, which is, at least in part, explained by genetic factors. Improved understanding of the gene-exercise interaction is needed for the development of more targeted intervention strategies.

In Finland, military service is compulsory for men. Military service includes large amounts of physical exercise but no dietary restriction. The current study evaluated the health benefits of exercise in young men attending military service in the Sodankylä Jaeger Brigade from 2005 to 2006 (N=1,112, mean age 19.2 years). Changes in endurance and strength performance, body composition, cardiometabolic risk factors and unacylated ghrelin levels were recorded at the beginning and end of the military service (6 to 12 months follow-up).

Improvement in cardiometabolic risk factors was observed with improved exercise performance, an association which was attributable to changes in weight and waist circumference. Increase in unacylated ghrelin level was associated with beneficial changes in body composition and fat distribution, as well as in lipid and glucose metabolism. Significant gene-exercise interactions were observed for variants in PPARG, IRS1 and TCF7L2 on changes in weight and/or body composition.

This study shows the efficacy of physical activity for the improvement of cardiometabolic health among young men. It shows that unacylated ghrelin plays an important role in the improvement of body composition, and glucose and lipid metabolism achieved by exercise. Finally, the harmful effects of common genetic variants on body composition can be counteracted by improvement in exercise performance.

Keywords: body composition, cardiovascular risk factors, genes, ghrelin, military, obesity, physical activity
Viimeaikaiset tutkimukset ovat osoittaneet, että väestötasolla ylipaino ja lihavuus lisääntyvät ja liikunta vähenee. Sekä ylipaino että vähäinen liikunta ovat tunnettuja sydän- ja verisuonitautien, insuliniresistenssin ja tyyppi 2 diabeteksen vaaratekijöitä.


Parantuneen fyysisen vaikutuksen painon ja/tai kehonkoostumukseen vaikuttajat olivat muutokset painonlaskuun ja keskivartalolihavuuden vähentämiseen. Liikunnan ansiosta asyloimattoman greliinin plasmataso lisääntyi ja se oli yhteydessä edullisiin muutoksiin kehonkoostumukseessa ja rasvanjakautumisessa sekä glukoosi- ja lipidaineenvaihdunnassa. Tärkeitä geneen-liikunta interaktioita todettiin insuliniherkkyystä säätellevien geneen (PPARG, IRS1 ja TCF7L2) vaikutuksissa painon ja/tai kehonkoostumukseen muutoksiin.


Asiasanat: geenit, greliini, kehonkoostumus, liikunta, sydän- ja verisuonitautien riskitekijät, varumiespalvelus, ylipaino

Cederberg, Henna, Liikunnan, asyloimattoman greliinin ja geenimuutosten vaikutus sydän- ja verisuonitautien vaaratekijöiden muutoksiin varusmiespalvelusaikana.

Oulun yliopisto, Lääketieteellinen tiedekunta, Terveystieteiden laitos, PL 5000, 90014 Oulun yliopisto; Itä-Suomen yliopisto, Lääketieteiden tiedekunta, Laakkotieteen laitos, Kliinisen laakkotieteen yksikkö, Sisäaudit, PL 1627, 70211 Kuopio; Oulun yliopiston sairaala, Yleislääketieteen yksikkö, PL 10, 90029 OYS

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Oulu
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This doctoral thesis research has been carried out at the University of Oulu, Institute of Health Sciences, and University of Eastern Finland, Department of Medicine, and is based on the data which was collected at the Sodankylä Jaeger Brigade in the years 2005-2006 in collaboration with the Finnish Defence Forces. I wish to express my most sincere gratitude to all of those who contributed to this project.

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particularly Jari Jokelainen, MSc for the statistical expertise and Alena Stančáková, MD PhD, for the expertise on genetical statistics.

The data collection for this study was a unique undertaking, and would not have been possible without the incredible effort of all the team involved. I wish to offer my sincere thanks to Mauri Laakso, MD, Pirjo Härkönen, MNSc, research nurse Eero Saastamoinen, all the staff in the Sodankylä Jaeger Brigade and Sodankylä Health Center, as well as all the staff and volunteers from the University of Oulu who took part in the data collection. Medical Colonel Ari Peitso, MD PhD is acknowledged for his help with the study. Warm thanks are also due to all the young men who participated in this study.

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Kuopio, 3rd November, 2011

Henna Cederberg
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AG</td>
<td>acylated ghrelin</td>
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<tr>
<td>BIA</td>
<td>bioimpedance analysis</td>
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<tr>
<td>BP</td>
<td>blood pressure</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>dBP</td>
<td>diastolic blood pressure</td>
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<tr>
<td>DPP</td>
<td>Diabetes Prevention Program</td>
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<tr>
<td>DPS</td>
<td>Diabetes Prevention Study</td>
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<tr>
<td>fat%</td>
<td>body fat percentage</td>
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<tr>
<td>FFM</td>
<td>fat free mass</td>
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<tr>
<td>FM</td>
<td>fat mass</td>
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<tr>
<td>GWAS</td>
<td>genome-wide association study</td>
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<td>GOAT</td>
<td>ghrelin O-acyltransferase</td>
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<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
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<tr>
<td>IRS1</td>
<td>insulin receptor substrate 1</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
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<tr>
<td>LBM</td>
<td>lean body mass</td>
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<tr>
<td>MetS</td>
<td>metabolic syndrome</td>
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<tr>
<td>MFI</td>
<td>muscle fitness index</td>
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<tr>
<td>MRI</td>
<td>magnetic resolution images</td>
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<tr>
<td>PA</td>
<td>physical activity</td>
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<tr>
<td>PPARG</td>
<td>Peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>sBP</td>
<td>systolic blood pressure</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>T2D</td>
<td>type 2 diabetes</td>
</tr>
<tr>
<td>TBW</td>
<td>total body water</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>transcription factor 7-like 2</td>
</tr>
<tr>
<td>TG</td>
<td>triglyceride</td>
</tr>
<tr>
<td>UAG</td>
<td>unacylated ghrelin</td>
</tr>
<tr>
<td>VFA</td>
<td>visceral fat area</td>
</tr>
<tr>
<td>VO$_2$max</td>
<td>maximal oxygen uptake</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHR</td>
<td>waist-to-hip-ratio</td>
</tr>
</tbody>
</table>
List of original articles

This thesis is based on following articles, which are referred to in the text by their Roman numerals.


Some unpublished results will also be presented.
Contents

Abstract
Tiivistelmä
Acknowledgements 7
Abbreviations 9
List of original articles 11
Contents 13
1 Introduction 17
2 Review of the literature 19
  2.1 Overweight and obesity ................................................................. 19
    2.1.1 Overweight among adolescents and young adults .............. 19
    2.1.2 Abdominal obesity ................................................................. 19
  2.2 Physical activity among young adults ............................................ 20
  2.3 Cardiovascular risk factors among young adults ....................... 21
  2.4 Exercise and cardiovascular risk factors ...................................... 21
    2.4.1 Exercise, weight loss and changes in body composition ...... 22
    2.4.2 Exercise, lipids and lipoproteins ......................................... 24
    2.4.3 Exercise and blood pressure .............................................. 25
    2.4.4 Exercise and insulin sensitivity .......................................... 25
    2.4.5 Cardiovascular sequelae of exercise training during
      military service ........................................................................ 26
  2.5 Ghrelin ....................................................................................... 27
    2.5.1 Ghrelin and regulation of food intake ................................... 29
    2.5.2 Ghrelin and long-term energy balance ................................ 29
    2.5.3 Ghrelin and body composition ........................................... 30
    2.5.4 Ghrelin and exercise ............................................................ 31
    2.5.5 Ghrelin and glucose metabolism ......................................... 31
    2.5.6 Ghrelin and lipid metabolism .............................................. 32
    2.5.7 Ghrelin and the cardiovascular system ................................. 32
    2.5.8 Ghrelin O-Acetyltransferase .............................................. 33
    2.5.9 The therapeutic potential of ghrelin ..................................... 33
  2.6 Gene variation in type 2 diabetes and insulin resistance ............. 33
    2.6.1 Approaches to identify type 2 diabetes susceptibility loci ...... 34
    2.6.2 Loci associated with type 2 diabetes and insulin resistance .... 40
    2.6.3 Gene-physical activity interaction ....................................... 41
6.2.1 Changes in the levels of unacylated ghrelin in response to exercise
6.2.2 Association of the levels of unacylated ghrelin levels with weight and body composition
6.2.3 Association of the levels of unacylated ghrelin with changes in insulin sensitivity and lipid metabolism
6.3 Gene-physical activity interaction
6.4 Summary of results
6.5 Strength and limitations of the study
6.5.1 Strengths
6.5.2 Limitations
7 Summary of findings and conclusions
7.1 Scientific implications
7.2 Clinical implications
References
Original articles
1 Introduction

Physical inactivity and low cardiorespiratory fitness are among the most important public health problems of the 21st century (Blair 2009, Hellenius & Sundberg 2011). Large prospective cohort studies in the United States have demonstrated that low cardiorespiratory fitness constitutes the largest attributable fraction for all-cause death (Blair 2009). Sedentary lifestyle and physical inactivity have been linked to cardiovascular disease, type 2 diabetes, insulin resistance, hypertension, obesity and overweight, cancer, osteoporosis, dementia and depression (Powell et al. 1989, Hellenius & Eckerlund 2007, Zhang et al. 2010). Physical activity is therefore a cornerstone for the prevention and treatment of chronic diseases, and for longevity.

The rapid increase in overweight and obesity in all age groups during the past 15 to 20 years has been associated with the parallel increase in physical inactivity and sedentary lifestyle (Hellenius & Eckerlund 2007). Physical activity has a key role in the regulation of energy expenditure and body weight (Caspersen et al. 1985). However, the effects of regular physical activity on body composition at the population level are less well understood, but such information would be of great importance for the understanding of the pathophysiology of insulin resistance and type 2 diabetes (Zhang et al. 2010).

Obesity, a chronic imbalance between energy intake and energy expenditure, is of a complex aetiologic origin, which includes some predisposing genetic factors and metabolic disorders (Grundy 1998, Hill & Peters 1998, Wickelgren 1998, Castaneda et al. 2010). Ghrelin is an adipokine implicated in the adipogenesis and provides an attractive target for further research in the pathogenesis of obesity. Previous research efforts have focused predominantly on total and acylated (AG) ghrelin. Recently, the predominant form of ghrelin in the circulation (>90%), unacylated ghrelin (UAG), was also described to be an active form of the hormone. The physiologic role of unacylated ghrelin is not well understood, but emerging evidence suggests it could be, at least in part, antagonistic to the functions exerted by AG (Broglio et al. 2004, Gauna et al. 2004, Gauna et al. 2005, Asakawa et al. 2005). Understanding the role of UAG in adiposity and associated metabolic disturbances, as well as its relationship with exercise-mediated changes in adiposity are likely to further increase the understanding of the pathogenesis of obesity. The enzyme ghrelin-O-acyltransferase (GOAT) plays a key role as a regulator of ghrelin acylation and the UAG-AG-balance. GOAT is a potential and interesting target for the
development of new drugs for obesity and diabetes. Understanding the physiologic functions of UAG increase our knowledge of the functions of GOAT (Kirchner et al. 2009).

Lifestyle interventions including dietary modifications and exercise are key tools for the treatment of obesity and obesity-related disease, such as type 2 diabetes (Pan et al. 1997, Tuomilehto et al. 2001, Knowler et al. 2002). Lifestyle interventions for high risk groups have now been implemented at the population level in Finland (Saaristo et al. 2010a, Saaristo et al. 2010b). The male population is at particularly high risk, and in Finland nearly half of the middle aged men have been reported to suffer from abnormal glucose tolerance (Saaristo et al. 2008). Thus, the prevention of (abdominal) obesity related disturbances in glucose metabolism and cardiovascular disease risk factors may be especially feasible in young men, who have not yet developed metabolic disturbances. In intervention studies an increase in physical activity has been shown to induce favorable changes in body composition and to reduce total and visceral fat mass, even in the absence of weight loss (Ross et al. 2000, Pratley et al. 2000, Kay & Fiatarone Singh 2006). Physical activity decreases substantially from youth to young adulthood, and therefore the role of exercise is highlighted for lifestyle interventions in young adults (Telama & Yang 2000).

Large inter-individual differences in response to exercise interventions have been observed, which are likely to be due to genetic differences. In adults in both the Finnish Diabetes Prevention Study (DPS) and the Diabetes Prevention Program (DPP) differences between genotypes of several type 2 diabetes risk genes were observed for the conversion to type 2 diabetes, and for weight change (Lindi et al. 2002, Florez et al. 2006, Florez et al. 2007, Wang et al. 2007, Kilpelainen et al. 2008a). A direct gene-physical activity interaction was found for variants of PPARG (Kilpelainen et al. 2008a), while others were evaluated for gene-lifestyle (combined physical activity and diet) interaction. Large unselected cohorts with detailed exercise measurements are required to evaluate the gene-physical activity interaction on changes in weight and body composition. Of particular interest are the single nucleotide polymorphisms (SNPs) associated with insulin sensitivity, which are known to be amenable to exercise.
2 Review of the literature

2.1 Overweight and obesity

The increase in the prevalence of obesity is continuous, global and alarming (Wickelgren 1998, International Obesity Task Force 2009). World Health Organization (WHO) reports that overweight and obesity is the fifth leading cause for death globally, and third in the high-income countries (World Health Organisation 2004). Obesity is associated with adverse changes in metabolic and cardiovascular risk factors, including high blood pressure, dyslipidemia, and insulin resistance, thereby leading to an increased risk of morbidity and mortality from cardiovascular disease and type 2 diabetes (Van Gaal et al. 2006).

In Finland, a continuous and gradual increase in overweight during the past 30 years has been reported in the FINRISK surveys. In Finland nearly half (48.5%) of men are overweight and one in five (22.1%) obese. Among women, one in three is overweight and one in five obese (Peltonen & Seal 2008). Significant differences exist between geographical regions in the prevalence of overweight and obesity in Finland (Lahti-Koski et al. 2008).

2.1.1 Overweight among adolescents and young adults

Excess weight and weight gain during adolescence and adult life significantly modify an individual’s risk for cardiovascular disease (CVD) (Berenson et al. 1998, Olshansky et al. 2005), and premature mortality (Hu et al. 2004). An increase in the prevalence of overweight, obesity and obesity-related disorders is observed already among adolescents and young adults (Kautiainen et al. 2002, James et al. 2004, Ogden et al. 2006). Among young men entering military service, an increase in body weight has been observed in Finland, Sweden, Norway and the United States in the recent decades (Rasmussen et al. 1999, Sharp et al. 2002, Dyrstad et al. 2006, Santtila et al. 2006).

2.1.2 Abdominal obesity

Along with increased body weight, a steady increase in central obesity and waist circumference has been observed over during the last 20 years, both among men and women (Peltonen & Seal 2008). An increasing trend in waist circumference
has been reported in young Finnish men between 2001 and 2007 (Raiko et al. 2010).

Central adiposity is associated with an increased predisposition to cardiometabolic disease in comparison to subcutaneous fat deposition. Visceral fat has an independent curvilinear association with mortality (Kuk et al. 2006). Compared to total fat mass, high amounts of visceral fat have been associated more strongly with blood pressure (BP), the HDL/total cholesterol ratio and insulin resistance (Nieves et al. 2003). Central adiposity has also been linked with elevated systolic blood pressure (sBP) levels in the Nurses’ Health Study, and decreased visceral fat has been associated with a reduction in blood pressure (Rexrode et al. 1998).

Obesity and particularly visceral fat are associated with qualitative and quantitative changes in lipids and lipoproteins, such as increases in total cholesterol, very low-density lipoproteins (VLDL), small dense low-density lipoprotein (LDL) particles, total triglycerides (TG) and decreases in high-density lipoprotein (HDL) cholesterol levels (Howard et al. 2003).

### 2.2 Physical activity among young adults

Physical inactivity, a parallel phenomenon, and a causative factor of the obesity epidemic, is the fourth leading risk for death globally, and also fourth immediately after obesity in the high income countries (World Health Organisation 2004). A decrease in physical activity is increasingly common among adolescents and young adults (Tammelin et al. 2007). Merely one in three Finnish men are engaged in the recommended amount of physical activity, and 15% of young men are not physically active at all (Husu P et al. 2011).

A decrease in physical activity has been reported among young adults in Finland, Sweden and Norway in the past decades (Rasmussen et al. 1999, Dyrstad et al. 2006, Santtila et al. 2006), which is reflected in the deterioration of physical fitness among young men entering military service. Deterioration in aerobic performance of conscripts entering military service has been reported in Northern countries (Dyrstad et al. 2006, Santtila et al. 2006).

Sedentary behavior has been shown to be independently associated with metabolic risk factors associated with insulin resistance (Healy et al. 2008).
2.3 Cardiovascular risk factors among young adults

Major risk factors attributing to an increased risk of CVD include high BP, high levels of total cholesterol, low levels of HDL cholesterol, high levels of total TG, insulin resistance and diabetes, abdominal obesity, physical inactivity and aging (Grundy 1998).

Favourable changes in the lipid profile have been observed among young Finnish male adults in the past decades (Viikari et al. 2006, Raiko et al. 2010), at least in part attributable to dietary changes. The Young Finns Study has recently shown that among young Finnish males a mean level of total cholesterol is 5.19 mmol/L, LDL cholesterol 3.28 mmol/L, HDL cholesterol 1.65 mmol/L and total TG 1.21 mmol/L. In contrast, mean BP level is increasing (Raiko et al. 2010). The use of anti-diabetic medication among young Finnish males is also increasing (Raiko et al. 2010).

A recent report from Finnish military conscripts demonstrated a high prevalence of metabolic syndrome components, including impaired fasting glucose (Mikkola et al. 2007). Metabolic syndrome was noted to be particularly prevalent among overweight and obese young men, highlighting the need for targeted interventions.

2.4 Exercise and cardiovascular risk factors

The independent effects of physical activity and adiposity (‘fitness’ versus ‘fatness’) on CVD risk factors are in a focus of intensive research (Blair & Church 2004). Physical activity is known to be protective against premature mortality and morbidity (Wei et al. 1999). However, the independent contribution of physical activity to improved health outcomes has remained unclear in most lifestyle intervention studies combining diet with weight loss and physical activity (Yates et al. 2007). Studies regarding changes in individual CVD risk factors in response to physical activity have been controversial. Findings from recent studies evaluating the effect of exercise on CVD risk factors are shown in Table 1.

Exercise intervention trials have predominantly been carried out in small cohorts, often confounded by co-morbidities. Large-scale population-based studies evaluating the sequelae of long-term physical activity are lacking.
2.4.1 Exercise, weight loss and changes in body composition

Studies evaluating the effects of exercise on weight have often reported only modest weight loss attributable to the independent effect of exercise (Pritchard et al. 1997, Ross et al. 2000, Castaneda et al. 2002, Irwin et al. 2003, Ross et al. 2004, Jakicic 2009). A report by the U.S. National Institutes of Health concluded that isolated exercise interventions resulted in a weight loss of 2–3%, and exercise accounted for only limited additional weight loss in interventions combining exercise and dietary modifications (Jakicic 2009). A large systematic review of exercise trials concluded that the weight change achieved in exercise interventions ranged from +0.2 to −7.5 kg, depending on the length of intervention and type of exercise used in intervention (Kay & Fiatarone Singh 2006). Aerobic exercise is considered to have a greater impact on weight loss than resistance training (Pritchard et al. 1997, Ross et al. 2000, Irwin et al. 2003, Ross et al. 2004).

Even with no or limited weight loss achieved by physical training, beneficial changes in body composition and body fat distribution have been observed. A greater reduction of visceral adipose tissue as compared to subcutaneous fat has been reported with intensive physical activity (Mourier et al. 1997, Pratley et al. 2000). In a systematic review of exercise trials, a significant reduction in visceral fat was observed in seven out of ten trials (Kay & Fiatarone Singh 2006). Smaller exercise trials measuring changes in visceral and total fat mass have reported a reduction in fat mass with endurance (Donnelly et al. 2003, Kondo et al. 2006, Stasiulis et al. 2010, Argus et al. 2010), but not resistance training interventions (Nybo et al. 2010, van der Heijden et al. 2010a).
Table 1. Effects of exercise on cardiovascular risk factors in recent cross-sectional, longitudinal and intervention studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Outcomes</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Cross-sectional</strong></td>
<td></td>
<td></td>
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<tr>
<td>Community Diabetes Prevention Project</td>
<td>369 non-diabetic people</td>
<td>low CRF correlated with insulin resistance</td>
<td>Leite et al. 2009</td>
</tr>
<tr>
<td>Racette et al. 2006</td>
<td>407 older adults</td>
<td>low CRF correlated with insulin resistance</td>
<td>Racette et al. 2006</td>
</tr>
<tr>
<td>Nyholm et al. 2004</td>
<td>20 FDRs of T2D patients</td>
<td>low CRF correlated with insulin resistance</td>
<td>Nyholm et al. 2004</td>
</tr>
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<td>Arsenault et al. 2007</td>
<td>169 healthy men</td>
<td>more visceral adipose tissue, higher TG, apoB and total cholesterol-HDL cholesterol ratio with low CRF</td>
<td>Arsenault et al. 2007</td>
</tr>
<tr>
<td>Lee et al. 2005</td>
<td>297 healthy men</td>
<td>low CRF correlated with adverse lipid profile</td>
<td>Lee et al. 2005</td>
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<td>Konig et al. 2003</td>
<td>127 Finnish men</td>
<td>high CRF associated with low plasma saturated FAs and high polyunsaturated FAs</td>
<td>Konig et al. 2003</td>
</tr>
<tr>
<td>Wong et al. 2004</td>
<td>293 men</td>
<td>more visceral adipose tissue in unfit men</td>
<td>Wong et al. 2004</td>
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<tr>
<td><strong>Longitudinal</strong></td>
<td></td>
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<tr>
<td>4-yr follow-up study</td>
<td>150 Hispanic children</td>
<td>high CRF at baseline predicted lower fat mass gain</td>
<td>Byrd-Williams et al. 2008</td>
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<td>Canadian PA Longitudinal Study</td>
<td>459 adults</td>
<td>higher CRF at baseline associated with lower risk of future obesity at 20-years</td>
<td>Brien et al. 2007</td>
</tr>
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<td>CARDIA</td>
<td>2487 men and women</td>
<td>Inverse association between CRF and incident hypertension</td>
<td>Arnethon et al. 2003</td>
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<td>ACLS</td>
<td>4,884 normotensives</td>
<td>Inverse association between CRF and incident hypertension</td>
<td>Arlow et al. 2006, Chase et al. 2009</td>
</tr>
<tr>
<td>ACLS</td>
<td>4599 men</td>
<td>Each 1-min improvement in treadmill reduced risk of ≥ 5 kg weight gain by 14% in men &amp; 9% in women</td>
<td>Dipietro et al. 1998</td>
</tr>
<tr>
<td>ACLS</td>
<td>724 women</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TWINACTIVE</td>
<td>3233 men free of MetS</td>
<td>Inverse association between muscular strength and metabolic syndrome incidence.</td>
<td>Jurca et al. 2005</td>
</tr>
<tr>
<td>Twin pairs with discordant PA</td>
<td>16 twin pairs</td>
<td>50% greater VFA in physically inactive twins compared to active twins at 32-year follow-up.</td>
<td>Leskinen et al. 2009</td>
</tr>
</tbody>
</table>

23
Study Participants Outcomes Reference

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Participants</th>
<th>Outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT, exercise &amp; diet</td>
<td>217 men &amp; women</td>
<td>increased CRF associated with increased apoB and decreased LDL cholesterol-apoB ratio</td>
<td>Kawano et al. 2009</td>
</tr>
<tr>
<td>ProActive Study RCT</td>
<td>365 high risk individuals</td>
<td>Increased CRF was associated with reduced clustered metabolic risk variables at 1-yr (WC, fasting triacylglycerol, insulin, glucose, BP, and HDL cholesterol)</td>
<td>Simmons et al. 2008</td>
</tr>
<tr>
<td>12-week resistance training program</td>
<td>12 obese adolescents</td>
<td>Unchanged total, visceral, hepatic fat content. Improved hepatic but not peripheral insulin sensitivity.</td>
<td>van der Heijden et al. 2010a</td>
</tr>
<tr>
<td>12-week aerobic training program</td>
<td>29 adolescents</td>
<td>Improved hepatic and peripheral insulin sensitivity in the absence of weight loss.</td>
<td>van der Heijden et al. 2009, van der Heijden et al. 2010b</td>
</tr>
</tbody>
</table>

Abbreviations: ACLS, Aerobics Center Longitudinal Study; apoB, apolipoprotein B; BP, blood pressure; CARDIA, Coronary Artery Risk Development in Young Adults study; CRF, cardiorespiratory fitness; FA, fatty acid; FDR, first-degree relative; MetS, metabolic syndrome; PA, physical activity; RCT, randomized controlled trial; TG, triglycerides; T2D, type 2 diabetes; TWINACTIVE, Finnish Twin Cohort study; VFA, visceral fat area; WC, waist circumference.

2.4.2 Exercise, lipids and lipoproteins

Previous exercise intervention studies have observed an overall benefit of exercise on the lipid profile, particularly on HDL cholesterol, but the effect has been variable and overall small across the studies. Studies with a small number of both normal-weight and obese participants with interventions lasting from 2 to 7 months have consistently reported an increase in HDL cholesterol levels (Kondo et al. 2006, Musa et al. 2009, Stasiulis et al. 2010). A decrease in the levels of LDL cholesterol (Kondo et al. 2006, Stasiulis et al. 2010) and TG (Stasiulis et al. 2010) have been reported in some, but not in all studies (Musa et al. 2009). The dose and intensity of exercise required to normalize the lipid profile remain unclear (Kraus & Slentz 2009). Furthermore, it has also been unclear as to whether these effects are independent of the changes in body composition.
2.4.3 Exercise and blood pressure

Exercise has not lowered BP in some of the previous studies (Church et al. 2007, Ilanne-Parikka et al. 2010). However, a 2-week exercise intervention study of obese men reported a significant reduction in sBP (Whyte et al. 2010). Furthermore, a meta-analysis by Whelton et al., reported that aerobic exercise lowered BP levels both in hypertensive and normotensive individuals (Whelton et al. 2002).

2.4.4 Exercise and insulin sensitivity

A growing body of evidence suggests that both acute and chronic exercise training is associated with improved glucose homeostasis, insulin sensitivity and prevents conversion to overt diabetes in subject with impaired glucose tolerance (IGT). Physical activity and endurance training have been shown to improve insulin-mediated glucose metabolism via improved insulin action on skeletal muscle glucose transport, resulting in increased whole body insulin sensitivity (Henriksen 2002). Resistance training has also been shown to lead to similar improvements in glucose metabolism via enhanced insulin action on skeletal muscle, improved glucose tolerance and decreased glycated haemoglobin concentrations (Ishii et al. 1998, Tabata et al. 1999, Derave et al. 2003, Holten et al. 2004, Tresierras & Balady 2009).

Exercise intervention studies in humans have demonstrated improvements in insulin sensitivity (Hellenius et al. 1995, Dengel et al. 1998, Eriksson et al. 1998, Rice et al. 1999, Kelley & Goodpaster 1999, Ross et al. 2000, Laaksonen et al. 2005) and glucose tolerance (Dengel et al. 1998, Kelley & Goodpaster 1999). Both the Finnish Diabetes Prevention (DPS) and the Diabetes Prevention Programme (DPP) in the U.S. found that exercise as part of the lifestyle intervention together with dietary modifications play an important role in preventing the progression of IGT to type 2 diabetes (Laaksonen et al. 2005, Kriska et al. 2006).

Studies comparing the metabolic sequelae of resistance versus endurance training have reported similar effect on glucose homeostasis (Sillanpaa et al. 2009).
2.4.5 Cardiovascular sequelae of exercise training during military service

Physical training is a key component of military service aiming to prepare soldiers physically for the military environment and subsequent training periods (Santtila 2010). The physical training programme commencing in the first eight-week basic training period lays the foundation for the subsequent more demanding troop and combat training periods. In Finland, physical training accounts for approximately half of the basic training time (total of 141 hours, or 17 hours per week). During six months of service, the total time allocated to physical training is 250 hours, corresponding to the hours of the training of many endurance athletes in the same time period. Thus military service may also be considered as an exercise intervention, and provides an optimal opportunity for evaluating the outcomes in physical performance and cardiovascular health.

The effects of physical training during military service have previously been studied both in countries of compulsory as well as recruited military service.

Physical performance

Changes in physical performance during military service have been measured as aerobic, or endurance performance, and muscular fitness. An overall beneficial effect on aerobic performance has been observed in the U.S., Great Britain and Singapore (Vogel et al. 1978, Patton et al. 1980, Lim & Lee 1994, Bell et al. 2000, Croteau & Young 2000, Kraemer et al. 2004, Williams 2005). However, no changes were observed in aerobic fitness among U.S. cadets with high baseline fitness levels (Daniels et al. 1979), and even a decrease in aerobic fitness was reported among Junior infantry recruits in Britain (Legg & Duggan 1996). Results regarding changes in muscular fitness have been controversial (Bell et al. 2000, Kraemer et al. 2004). Improvements in both aerobic and muscular fitness performance during six months of training have previously been reported in Finland (Mattila et al. 2007).

Weight and body composition

Studies evaluating the changes in weight have consistently observed a reduction in weight of a variable magnitude from 1.1 to 16.1 kg (Lee et al. 1994, Mattila et al. 2007) during military service. Previous results from the Sodankylä cohort, as
well as other studies have consistently shown that the weight loss is most pronounced among those with the greatest BMI at baseline (Lee et al. 1994, Friedl et al. 2001, Mikkola et al. 2009). Beneficial changes in body composition have been observed, with a reduction in fat mass of 1.2 to 4.7 kg and 2.5 to 11.0% (Patton et al. 1980, Lee et al. 1994, Croteau & Young 2000, Friedl et al. 2001, Mattila et al. 2007, Santtila et al. 2008, Wood et al. 2010), and an increase in lean body mass (Mattila et al. 2007).

**Cardiovascular sequelae**

Studies on cardiovascular effects of exercise during military training are limited and conflicting. Lieberman et al. (2008) observed a decrease in total and LDL cholesterol among US female marine trainees in association of beneficial body composition changes during 13 weeks of training. Tähtinen et al. (2000) observed a beneficial increase in the level of HDL cholesterol, but adverse changes on the levels of total and LDL cholesterol, as well as on fasting glucose among Finnish conscripts during training (Tähtinen T 2000).

2.5 Ghrelin

Ghrelin is a 28-amino-acid peptide discovered in 1999 as a natural ligand of the G-protein coupled growth hormone secretagogue receptor type 1a (GHS-R1a) (Kojima et al. 1999). Ghrelin has diverse physiologic functions, and is involved in energy-homeostasis, glucose and lipid metabolism, adipogenesis, food intake as well as in the cardiovascular system (Figure 1) (De & Delporte 2008).

A unique characteristic of the ghrelin peptide is the o-n-octanoylation at the serine 3 residue, which enables receptor binding, stimulation of growth hormone release and is crucial for biological activity of ghrelin (Kojima et al. 1999, Matsumoto et al. 2001). Only the acylated form of ghrelin (AG) was initially considered to be active, while unacylated (UAG, referred also to as des-acyl, non-acylated or non-octanoyl ghrelin) was deemed an inactive form of the peptide (Kojima et al. 1999). Subsequent work has, however, shown that also UAG is a full agonist of the GHS-R1a receptor (Gauna et al. 2007b). Furthermore, UAG has been also reported to exert its effects independently of GHS-R1a (Baldanzi et al. 2002, Bedendi et al. 2003, Muccioli et al. 2004, Thompson et al. 2004, Gauna et al. 2005, Toshinai et al. 2006, Gauna et al. 2007a).
The precursor of ghrelin is a 117 amino acid prepro-ghrelin, from which pro-
ghrelin is derived. The 94 amino-acid pro-ghrelin is further broken down into
ghrelin and obestatin (De & Delporte 2008). Alternative splicing of the ghrelin
gene, and post-translation modification enable the production of several different
forms of the peptide, of which des-acyl ghrelin is the most important (Hosoda et
al. 2003). GOAT, a polytopic membrane-bound enzyme that attaches octanoate to
serine-3 of ghrelin was identified in 2008 as the key enzyme responsible for the
acylation of ghrelin (Yang et al. 2008).

Fig. 1. The physiologic functions of ghrelin (modified from De & Delporte 2008).

Ghrelin is secreted predominantly from the gut and in particular from the X/A
cells of the gastric fundus, but also from small and large intestine, liver, pancreas,
kidney and central nervous system (De & Delporte 2008). The acylated form of
ghrelin accounts for less than 10 percent and UAG for over 90 percent of
circulating ghrelin (Kojima et al. 1999). AG is present in the circulation bound to
lipoproteins whereas UAG circulates unbound (Soares & Leite-Moreira 2008).

Emerging evidence suggests that the two different forms of ghrelin may exert
different physiologic and metabolic effects (Broglio et al. 2004, Gauna et al. 2004,
Gauna et al. 2005, Barazzoni et al. 2007). Due to only recent discovery of GOAT
and biological activity of UAG, previous studies have focused predominantly on
total and/or acylated ghrelin, and thus the physiological role of UAG remains unclear.

2.5.1 Ghrelin and regulation of food intake

Ghrelin is the only circulating orexigenic hormone secreted peripherally from the gut which acts on the hypothalamic arcuate nucleus, the regulatory region for appetite. (Kojima & Kangawa 2010). Ghrelin plays a role in the short-term regulation of food intake, as well as in the long-term energy balance. Concentrations of circulating ghrelin exhibit diurnal variation and are normally raised before meals and suppressed post-prandially (Dostalova & Haluzik 2009). In obese individuals, impaired post-prandial suppression of ghrelin levels have been reported in some, but not in all studies (Dostalova & Haluzik 2009).

Studies evaluating the independent roles of AG and UAG in food intake have hitherto been performed in rodents only. AG, but not UAG, has been reported to stimulate food intake in rodents (Neary et al. 2006). Asakawa et al. reported an inverse effect of UAG to AG on food intake, with decreased food intake and delayed gastric emptying induced by UAG in mice (Asakawa et al. 2005). The orexigenic properties of UAG in humans remain uncertain. Recent data suggests that dietary lipids may in fact play an important role in ghrelin acylation, and GOAT has been suggested to provide a link between ingested lipids, energy expenditure and body composition (Kirchner et al. 2009).

2.5.2 Ghrelin and long-term energy balance

One of the adipokines implicated in adipogenesis is ghrelin, which provides an attractive target for further research into the pathogenesis of obesity. Decreased plasma ghrelin concentrations have been reported in people with obesity, both children and adults (Tschop et al. 2001, Vicennati et al. 2007, Reinehr et al. 2008a). Furthermore, studies of ethnic variation in the levels of ghrelin showed lower levels of ghrelin in Pima Indians, a population suffering from morbid polygenic obesity, as compared to Caucasians (Tschop et al. 2001). Downregulation of ghrelin is suggested to represent a physiological adaptation to positive energy balance associated with human obesity (Tschop et al. 2001, Castaneda et al. 2010).

A regulatory role of UAG in the adipogenesis has been reported in animal studies (Thompson et al. 2004, Zhang et al. 2008). Zhang et al. reported a
reduction of white adipose tissue and resistance to high-fat diet-induced obesity with an increase in plasma UAG concentrations in transgenic fatty-acid binding protein-4 mice (Zhang et al. 2008). In contrast, Thompson et al. observed parallel adipogenic effects of UAG with AG, via a mechanism independent of the GHS-R1a in rats (Thompson et al. 2004). Davies et al. observed that a chronic infusion of AG induced abdominal obesity, whereas UAG had no effect on adiposity in rats (Davies et al. 2009).

Circulating ghrelin has been implicated in long-term weight regulation and is therefore of interest in dynamic changes of body weight. Intervention studies have reported increases in total ghrelin levels after diet-induced weight loss in adults in some (Weigle et al. 2003, Romon et al. 2006), but not in all studies (Bennett et al. 2009). Kim et al. (13) reported a strong inverse correlation of a change in UAG level, but not in AG level, with changes in body weight and BMI during a 3-month exercise intervention in 17 overweight children.

Studies evaluating the predictive value of baseline ghrelin levels for prospective changes in weight are few. Ghrelin did not predict weight gain in an 18-year follow-up study of the Rancho Bernardo Cohort including 1317 men and women aged from 60 to 91 years, (Langenberg et al. 2005).

2.5.3 Ghrelin and body composition

Total ghrelin has been reported to correlate negatively with body fat, waist circumference and fat distribution index (Fagerberg et al. 2003, Makovey et al. 2007). Gender differences in the association of total ghrelin with body composition have also been suggested (Makovey et al. 2007). In mice, ghrelin has been reported to induce abdominal obesity in white-adipose tissue deposits (Davies et al. 2009). Overall, data on body composition and total ghrelin or AG are limited.

Studies in children evaluating the association of ghrelin with body composition changes have shown an inverse association of UAG, total ghrelin, and fat mass during a lifestyle intervention (Kim et al. 2008, Kelishadi et al. 2008). Central adiposity has been associated with total ghrelin, but not with UAG (Kim et al. 2008, Kelishadi et al. 2008), possibly due to a small sample size. Similar studies in adults are lacking. Furthermore, it is not known whether UAG is associated with body weight or body composition in adults.
2.5.4 Ghrelin and exercise

Long-term exercise is associated with changes in long-term energy balance, and adaptations in metabolism and cardiovascular function. Increased levels of total ghrelin have been reported after long-term physical activity in some (Leidy et al. 2004, Foster-Schubert et al. 2005), but not all studies (Ravussin et al. 2001, Morpurgo et al. 2003). Weight loss associated with exercise seems to play an important role in an increase of total ghrelin levels (Leidy et al. 2004, Foster-Schubert et al. 2005). However, most of the long-term exercise intervention studies have measured total ghrelin levels, and UAG and AG levels have been evaluated separately only in overweight children (Kim et al. 2008). In overweight children, exercise intervention increased UAG level, whereas AG remained unchanged (Kim et al. 2008). It is unclear whether the changes in the levels of the different forms of ghrelin associated with exercise are related to changes in weight, body composition, lipid, and glucose metabolism.

2.5.5 Ghrelin and glucose metabolism

Ghrelin has been suggested to have a role in glucose and insulin metabolism and to affect insulin sensitivity (Qader et al. 2005, Sun et al. 2007, Vestergaard et al. 2007, Ukkola 2009). Ghrelin may regulate glucose homeostasis by inhibiting insulin secretion, as reported in rodent studies (Qader et al. 2005). Ablation of ghrelin in mice has been reported to increase glucose-induced insulin secretion and to improve peripheral insulin sensitivity (Sun et al. 2007). Studies in humans have shown that intravenous administration of ghrelin impairs insulin sensitivity. Leptin and insulin have been suggested to regulate ghrelin levels (Erdmann et al. 2003). Interestingly, also glucagon-like peptide-1 (GLP-1) has been reported to suppress ghrelin level supporting the hypothesis that the interaction between ghrelin and incretins may have an effect on food intake (Chelikani et al. 2006, Hagemann et al. 2007).

Recent studies have suggested that the effects of AG on glucose homeostasis may be partially opposite to those of UAG (Gauna et al. 2007b). AG is associated with reduced insulin sensitivity and increases hepatic glucose production via the stimulation of gluconeogenesis in the liver (Poykko et al. 2003). Previous studies suggest that the administration of AG impairs glucose metabolism by increasing glucose concentrations and decreasing insulin sensitivity (Broglio et al. 2004,
Gauna et al. 2004). However, co-administration of UAG with AG seems to improve insulin sensitivity (Gauna et al. 2004).

2.5.6 Ghrelin and lipid metabolism

Ghrelin has been shown to have a significant role in lipid metabolism in the liver, skeletal muscle and adipose tissue (Soares & Leite-Moreira 2008). Total ghrelin favours triglyceride deposition in the liver over skeletal muscle (Barazzoni et al. 2005) and acts directly on adipocytes to stimulate lipogenesis (Patel et al. 2006). Previous studies have reported a positive association between plasma ghrelin and HDL cholesterol levels (Fagerberg et al. 2003, Ukkola et al. 2006). Studies regarding the association of ghrelin with total cholesterol, total TGs and LDL cholesterol have remained controversial (Purnell et al. 2003, Zou et al. 2009). There are no published data on the effects of UAG on changes in the lipid profile.

2.5.7 Ghrelin and the cardiovascular system

Emerging evidence suggests that ghrelin is associated with the development of the metabolic syndrome, and plays an important role in the cardiovascular system. Low levels of total ghrelin and UAG have been reported in the metabolic syndrome, with progressive lowering of total ghrelin level with increasing number of the components of the metabolic syndrome (Ukkola et al. 2006, Ukkola 2009, Pulkkinen et al. 2010).

Ghrelin receptor is expressed in the cardiovascular system, suggesting that ghrelin plays a role in atherosclerosis (Tesauro et al. 2005). In fact, plasma ghrelin levels have been reported to positively correlate with carotid artery atherosclerosis in males (Poykko et al. 2006). In people with the metabolic syndrome an improved endothelial function following the administration of ghrelin has been observed, where proatherogenic changes were prevented, vasodilatation improved, blood pressure decreased and cardiac output increased (Okumura et al. 2002, Cao et al. 2006, Nagaya & Kangawa 2006, Tesauro et al. 2009). Chronic administration of ghrelin in rats has been shown to improve heart failure and left ventricular dysfunction (Pulkkinen et al. 2010).
2.5.8 Ghrelin O-Acetyltransferase

GOAT is a polytopic membrane-bound enzyme attaches octanoate to serine-3 of ghrelin and regulates the balance between UAG and AG (Gutierrez et al. 2008, Yang et al. 2008). GOAT is expressed in gastric mucosa and pancreas (Gutierrez et al. 2008, Kirchner et al. 2009). Importantly, genetic disruption of the GOAT gene has been associated with a complete absence of AG from the circulation in murine studies (Gutierrez et al. 2008). Kirchner et al. recently showed that GOAT functions as a gastric lipid sensor and is required to mediate the impact of dietary lipids on adiposity (Kirchner et al. 2009). The activation of the GOAT system is triggered by a lipid-rich environment and a dietary supply of medium-chain triglycerides thus required for ghrelin acylation (Kirchner et al. 2009). Together with the AG and UAG, GOAT now represents an attractive target for the development of pharmacological treatment for obesity and type 2 diabetes (Pulkkinen et al. 2010, Barnett et al. 2010).

2.5.9 The therapeutic potential of ghrelin

The ghrelin system offers attractive therapeutic potential for the treatment of obesity and type 2 diabetes. GHS-R1a receptor antagonists have been tested, but encountered problems with the crossing of blood-brain barrier and are currently underdeveloped for the treatment of obesity and diabetes (Chen et al. 2009). Targeting an enzyme may be preferential over targeting a receptor and enzyme inhibitors of GOAT provide an attractive possibility to target the acyl-deyl ratio (Barnett et al. 2010) and are thus in a focus of intensive research. However, detailed understanding of the physiological functions of GOAT is required to understand the implications and sequelae of targeting the enzyme. In this, understanding the physiological role of unacylated ghrelin is of primary importance.

2.6 Gene variation in type 2 diabetes and insulin resistance

A strong heritability of type 2 diabetes is supported by a body of evidence from different studies showing significant differences in the prevalence of type 2 diabetes across different ethnic groups, as well as familial aggregation and high concordance of type 2 diabetes in monozygotic twins (Newman et al. 1987, Poulsen et al. 1999, Meigs et al. 2000, Diamond 2003, Das & Elbein 2006). Type
2 diabetes is a polygenic disease where genetic variations in several genes contribute to the risk of developing disease, via the gene-gene interaction and via the interaction with metabolic environment and lifestyle factors, including physical activity and nutrient intake (O'Rahilly et al. 1988, Froguel & Velho 2001, Hansen & Pedersen 2005, Das & Elbein 2006).

2.6.1 Approaches to identify type 2 diabetes susceptibility loci

Approaches used in the identification of susceptibility genes for type 2 diabetes can be divided into three groups: candidate gene approach, linkage studies and genome-wide association studies (GWAS).

Candidate gene studies and genome-wide linkage scans

In the candidate gene approach applied during the past decades, the known pathophysiology of type 2 diabetes is used to identify promising candidate genes. The gene variant, usually a SNP, is then tested for an association with type 2 diabetes (Turner et al. 1995). In linkage studies, variation in the entire human genome was screened with microsatellite markers without a priori knowledge about the genes or gene effects, and genomic regions shared by diabetic relatives more often than expected by chance were analyzed to localize the candidate gene (Ghosh & Schork 1996).

Candidate gene and linkage studies have yielded several potential candidate genes for type 2 diabetes, but only a few them have been consistently replicated in several populations (Risch 2000), mostly due to insufficient power of these studies and therefore highlighting the need for large-scale, biology-agnostic studies. Robust findings include the Pro12Ala variant in the peroxisome proliferator-activated receptor gamma 2 gene (PPARG2) (Deeb et al. 1998, Altshuler et al. 2000), the E23K variant in the potassium inwardly rectifying channel, subfamily J, member 11 gene (KCNJ11) (Gloyn et al. 2003, Florez et al. 2004), both identified by a candidate gene approach, as well as transcription factor 7-like 2 (TCF7L2) (Grant et al. 2006), originally identified by linkage analysis.
**Genome-wide association studies**

Genome-wide association studies, applied since 2007, have revolutionized the search of susceptibility genes for type diabetes, as the GWA studies enable the investigation of common SNPs spread across the entire human genome (Amos 2007). The most significant associations observed in GWAS are confirmed by replicating the findings in other independent populations. While the advantage of GWAS is that they are unbiased by previous hypotheses of candidate genes and pathways, and therefore contribute to improved understanding of disease mechanisms by identification of entirely unexpected genes, they are, however, limited by the modest effect sizes of the common variants (Amos 2007).

Since 2007 over 40 susceptibility variants for type 2 diabetes have been identified by applying the GWAS approach. The previously reported associations of *PPARG*, *TCF7L2* and *KCNJ11* loci have also been confirmed. The identified variants, probable mechanisms and effect sizes are shown in Table 2.

However, only a small fraction of heritability is explained by the known variants and thus many more susceptibility loci remain to be identified. Future studies are likely to be directed from the common variants with modest effect sizes to low-frequency and rare variants, as well as other forms of variation such as epigenetic mechanisms, micro-RNAs and copy number variants (Frayling & McCarthy 2007, Pearson & Manolio 2008, Prokopenko et al. 2008).
<table>
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<th>SNP</th>
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<th>Effect allele frequency</th>
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<td>PPARG2 rs1801281</td>
<td>C/G</td>
<td>0.92</td>
<td>T2D</td>
<td>1.14 (1.08–1.20)</td>
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<td>KCNJ11 rs5219</td>
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<td>TCF7L2 rs7903146</td>
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<td>IGFBP2 rs4402960</td>
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<td>T2D</td>
<td>1.17 (1.10–1.25)</td>
<td>2007</td>
<td>Saxena et al. 2007, Scott et al. 2007, Zeggini et al. 2007, Zeggini et al. 2008</td>
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<td>CDKAL1 rs7754840</td>
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<td>0.31</td>
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<td>Sladek et al. 2007, Dupuis et al. 2010, Voight et al. 2010</td>
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<td><strong>CDC123 / CAMK1D</strong></td>
<td>G/A</td>
<td>0.23</td>
<td>T2D</td>
<td>1.11 (1.07–1.14)</td>
<td>2008</td>
<td>Zeggini et al. 2008</td>
</tr>
<tr>
<td>rs12779790</td>
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<td><strong>KCNQ1 rs2237892, rs231362</strong></td>
<td>C/T</td>
<td>0.52</td>
<td>T2D</td>
<td>1.4 (1.34–1.47)</td>
<td>2008</td>
<td>Yasuda et al. 2008,</td>
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<td><strong>TSPAN8 / LGR5</strong></td>
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<td>0.23</td>
<td>T2D</td>
<td>1.09 (1.06–1.12)</td>
<td>2008</td>
<td>Zeggini et al. 2008</td>
</tr>
<tr>
<td>rs7961581</td>
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<td><strong>IRS1 rs2943641</strong></td>
<td>C/T</td>
<td>0.61</td>
<td>T2D</td>
<td>1.19 (1.13–1.25)</td>
<td>2009</td>
<td>Rung et al. 2009</td>
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<tr>
<td><strong>DUSP9 rs5945326</strong></td>
<td>G/A</td>
<td>0.12</td>
<td>T2D</td>
<td>1.27 (1.18–1.37)</td>
<td>2010</td>
<td>Voight et al. 2010</td>
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<td><strong>PROX1 rs340874</strong></td>
<td>C/T</td>
<td>0.50</td>
<td>FG</td>
<td>0.013 (0.003)</td>
<td>2010</td>
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<td><strong>BCL11A rs243021</strong></td>
<td>A/G</td>
<td>0.46</td>
<td>T2D</td>
<td>1.08 (1.07–1.13)</td>
<td>2010</td>
<td>Voight et al. 2010</td>
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<td><strong>G6PC2 rs560887</strong></td>
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<td>0.70</td>
<td>FG</td>
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<td><strong>ADC Y5 rs2877716</strong></td>
<td>C/T</td>
<td>0.77</td>
<td>FG</td>
<td>0.027 (0.003)</td>
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<td>rs11708067</td>
<td>A/G</td>
<td>0.78</td>
<td>HOBA-B</td>
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<td><strong>GCKR rs1260326</strong></td>
<td>T/C</td>
<td>0.40</td>
<td>2-hr G</td>
<td>0.10 (0.01)</td>
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<td>rs780094</td>
<td>C/T</td>
<td>0.62</td>
<td>FG</td>
<td>0.029 (0.003)</td>
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<td>Dupuis et al. 2010</td>
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<tr>
<td><strong>SLC2A2 rs11920090</strong></td>
<td>T/A</td>
<td>0.85</td>
<td>FG</td>
<td>0.002 (0.004)</td>
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<tr>
<td><strong>WFS1 rs1801214</strong></td>
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<td>0.27</td>
<td>T2D</td>
<td>1.13 (1.07–1.18)</td>
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<td><strong>ZBED3 rs4457053</strong></td>
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<td>1.08 (1.06–1.11)</td>
<td>2010</td>
<td>Voight et al. 2010</td>
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<td>Trait</td>
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<td>References</td>
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<td>DGKB rs2191349</td>
<td>T/G</td>
<td>0.47</td>
<td>FG</td>
<td>0.03 (0.003)</td>
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<td>Dupuis et al. 2010</td>
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<tr>
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<td></td>
<td>T2D</td>
<td>1.06 (1.04–1.08)</td>
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<td>GOK rs4607517</td>
<td>A/G</td>
<td>0.20</td>
<td>FG</td>
<td>0.062 (0.004)</td>
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<tr>
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<td>HbA1C</td>
<td>0.041 (0.005)</td>
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<td></td>
<td>T2D</td>
<td>1.07 (1.05–1.10)</td>
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<tr>
<td>KLF14 rs972283</td>
<td>G/A</td>
<td>0.55</td>
<td>T2D</td>
<td>1.07 (1.05–1.10)</td>
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<td>Voight et al. 2010</td>
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<tr>
<td>TP53INP1 rs896854</td>
<td>T/C</td>
<td>0.48</td>
<td>T2D</td>
<td>1.06 (1.04–1.09)</td>
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<td>Voight et al. 2010</td>
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<tr>
<td>GLIS3 rs7034200</td>
<td>A/C</td>
<td>0.53</td>
<td>FG</td>
<td>0.018 (0.003)</td>
<td></td>
<td>Dupuis et al. 2010</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td>HOMA-B -0.020 (0.004)</td>
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<tr>
<td>TLE4* rs13292136</td>
<td>C/T</td>
<td>0.93</td>
<td>T2D</td>
<td>1.11 (1.07–1.15)</td>
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<tr>
<td>ADRA2A rs10885122</td>
<td>G/T</td>
<td>0.90</td>
<td>FG</td>
<td>0.022 (0.004)</td>
<td></td>
<td>Dupuis et al. 2010</td>
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<tr>
<td>CEN7D2 rs1552224</td>
<td>A/C</td>
<td>0.88</td>
<td>T2D</td>
<td>1.14 (1.11–1.17)</td>
<td></td>
<td>Voight et al. 2010</td>
</tr>
<tr>
<td>CRY2 rs11605924</td>
<td>A/C</td>
<td>0.54</td>
<td>FG</td>
<td>0.015 (0.003)</td>
<td></td>
<td>Dupuis et al. 2010</td>
</tr>
<tr>
<td>FADS1 rs174550</td>
<td>T/C</td>
<td>0.63</td>
<td>FG</td>
<td>0.017 (0.003)</td>
<td></td>
<td>Dupuis et al. 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HOMA-B -0.020 (0.003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MADD rs7944584</td>
<td>A/T</td>
<td>0.69</td>
<td>FG</td>
<td>0.021 (0.003)</td>
<td></td>
<td>Dupuis et al. 2010</td>
</tr>
<tr>
<td>MTNR1B rs10830963</td>
<td>G/C</td>
<td>0.30</td>
<td>FG</td>
<td>0.067 (0.003)</td>
<td></td>
<td>Dupuis et al. 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HOMA-B -0.034 (0.004)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HbA1C</td>
<td>0.024 (0.004)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>T2D</td>
<td>1.09 (1.06–1.12)</td>
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</tr>
<tr>
<td>HMGA2 rs1531343</td>
<td>C/G</td>
<td>0.10</td>
<td>T2D</td>
<td>1.10 (1.07–1.14)</td>
<td></td>
<td>Voight et al. 2010</td>
</tr>
<tr>
<td>HNF1A rs7957197</td>
<td>T/A</td>
<td>0.85</td>
<td>T2D</td>
<td>1.07 (1.05–1.10)</td>
<td></td>
<td>Voight et al. 2010</td>
</tr>
<tr>
<td>IGF1 rs35767</td>
<td>G/A</td>
<td>0.90</td>
<td>FI</td>
<td>0.01 (0.006)</td>
<td></td>
<td>Dupuis et al. 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HOMA-IR 0.013 (0.006)</td>
<td></td>
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</tr>
<tr>
<td>C2CD4B rs11071657</td>
<td>A/G</td>
<td>0.59</td>
<td>FG</td>
<td>0.008 (0.003)</td>
<td></td>
<td>Dupuis et al. 2010</td>
</tr>
<tr>
<td>PRC1 rs8042680</td>
<td>A/C</td>
<td>0.22</td>
<td>T2D</td>
<td>1.07 (1.05–1.09)</td>
<td></td>
<td>Voight et al. 2010</td>
</tr>
<tr>
<td>SNP</td>
<td>Allele</td>
<td>Effect allele frequency</td>
<td>Trait</td>
<td>Effect: OR/Beta (95% CI/SE)</td>
<td>Year</td>
<td>References</td>
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<tr>
<td>VPS13C</td>
<td>G/A</td>
<td>0.42</td>
<td>2-hr G</td>
<td>0.07 (0.01)</td>
<td>Saxena et al. 2010</td>
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<td>ZFAND6</td>
<td>G/A</td>
<td>0.56</td>
<td>T2D</td>
<td>1.06 (1.04–1.08)</td>
<td>Voight et al. 2010</td>
<td></td>
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<tr>
<td>GIPR</td>
<td>A/T</td>
<td>0.18</td>
<td>2-hr G</td>
<td>0.11 (0.01)</td>
<td>Saxena et al. 2010</td>
<td></td>
</tr>
</tbody>
</table>

FG, fasting glucose (mmol/L); FI, fasting insulin (pmol/L); 2-hr G, 2-hour glucose (FG adjusted, mmol/L); HbA1C, Hemoglobin A1C (%). Effect estimates are adapted from the references for each SNP unless otherwise noted.
2.6.2 Loci associated with type 2 diabetes and insulin resistance

Type 2 diabetes is characterized by a state of insulin resistance in the liver, muscle and adipose tissue, and impaired insulin secretion from the pancreatic beta-cells. Both insulin sensitivity and insulin secretion have an important genetic component (Poulsen et al. 1999, Pedersen 1999, Lehtovirta et al. 2000). The vast majority of common variants identified to date are, however, those regulating insulin secretion, as listed in Table 2. Only few common variants have been associated with insulin sensitivity. However, these variants are of great importance for the studies aiming to investigate the effects of physical activity on weight and body composition. In the following the roles of PPARG, TCF7L2 and IRS1 are discussed in detail.

PPARG

PPARG encodes for the peroxisome proliferator-activated receptor gamma (PPARγ) PPARγ2 isoform, a ligand-dependent nuclear receptor, is highly expressed in the adipose tissue (Tontonoz et al. 1994, Fajas et al. 1997, Auwerx 1999, Lehrke & Lazar 2005). PPARγ has regulatory roles in adipocyte differentiation, TG storage, glucose homeostasis and insulin sensitivity (Tontonoz et al. 1994, Fajas et al. 1997, Auwerx 1999, Knouff & Auwerx 2004, Lehrke & Lazar 2005). The Pro12Ala variant of PPARG2 first reported in 1998 has been associated with the regulation of adipocyte differentiation, BMI and risk of type 2 diabetes (Deeb et al. 1998), and these associations have been confirmed in GWAS (Saxena et al. 2007, Scott et al. 2007, Sladek et al. 2007, Zeggini et al. 2007). Despite a modest effect size (1.25-fold increase in the risk of type 2 diabetes in carriers of Pro12Pro genotype), the high frequency of the Pro allele (approximately 85% in Caucasians) accounts for a considerable population-attributable risk (Altshuler et al. 2000).

The association of the Pro12Ala variant of PPARG2 with the risk of type 2 diabetes is at least in part considered to be attributable to its effect on insulin sensitivity. The Pro12Ala variant directly regulates adipose tissue TG synthesis. When PPARγ is downregulated in adipose tissue, free fatty acids (FFAs) are released into the circulation, impairing insulin sensitivity in the liver and skeletal muscle (Spiegelman 1998, Heikkinen et al. 2009). An insulin-sensitizing effect can be produced with synthetic PPARγ agonists, thiazolidinediones, and these
agents have been used in the treatment of patients with type 2 diabetes (Lehmann et al. 1995).

**TCF7L2**

Transcription factor 7-like 2 was identified by linkage analysis as a susceptibility gene for type 2 diabetes. The primary finding has been confirmed by several GWAS (Grant et al. 2006, Zeggini et al. 2008). TCF7L2 encodes for high mobility group box-containing transcription factor, a nuclear receptor for β-catenin, and mediates the WNT signalling cascade (Jin 2008). TCF7L2 regulates the proliferation of pancreatic beta cells and transcription of genes involved in glucose metabolism (including proglucagon and glucagon-like peptides (GLP 1 and 2). Variants in TCF7L2 have been associated with impaired insulin secretion, but also with insulin resistance (Florez et al. 2006, Chandak et al. 2007, Reinehr et al. 2008b, Stancakova et al. 2009).

**IRS1**

A variant of insulin receptor substrate 1 gene (IRS1), identified by GWAS in 2009 has been associated with an increased risk of type 2 diabetes, insulin resistance and hyperinsulinaemia (Rung et al. 2009). A recent evidence suggests that the common variant in IRS1 is primarily associated with distribution of fat, particularly in men (Kilpelainen et al. 2011). Carriers of the diabetogenic allele exhibit a leaner phenotype, but with preferential accumulation of fat in the visceral region, which is associated with an increased risk of insulin resistance (Kilpelainen et al. 2011). Therefore, it is likely that a variant in IRS1 affects primarily fat distribution and secondarily insulin sensitivity.

2.6.3 Gene-physical activity interaction

Large inter-individual variation in response to standardized lifestyle interventions including physical activity and nutritional components is likely to be regulated by gene variants, at least in part. Identification of the key genes and common variants modulating the response to exercise is of crucial importance for the understanding of development of type 2 diabetes and cardiovascular disease, as well as for the development of new, targeted and tailored therapies.
Previous evidence on gene-physical activity interactions evaluating the risk of the development of type 2 diabetes have included GWAS, cross-sectional association studies and lifestyle-intervention studies combining diet intervention and physical activity (Table 3) (Luan et al. 2001, Lakka et al. 2003, An et al. 2005, Weiss et al. 2005, Franks et al. 2007b, Kilpelainen et al. 2008a, He et al. 2011). Lifestyle intervention studies have focused on high-risk individuals and particularly on individuals with IGT (Yamaoka & Tango 2005). Large, unselected cohorts provide significant results for the understanding of the gene-physical activity interaction. Furthermore, in contrast to cross-sectional studies, long-term prospective studies are invaluable for the evaluation of the gene-physical activity interaction on the long-term changes in weight, body composition and metabolism that significantly contribute to the accrued cardiovascular disease risk.

**Intervention studies**

Gene-lifestyle interaction has been evaluated in the first two randomised controlled trials evaluating the efficacy of lifestyle intervention on the prevention of type 2 diabetes among people with IGT.

In the Finnish DPS, lifestyle intervention was observed to successfully prevent progression to type 2 diabetes and result in a significant weight loss (Tuomilehto et al. 2001, Lindstrom et al. 2003). Several SNPs, including PPARγ and TCF7L2 interacted with the lifestyle intervention and the diabetogenic effect of the risk allele was counteracted by the changes in lifestyle (Lindi et al. 2002, Wang et al. 2007). Furthermore, a direct interaction between physical activity and several type 2 diabetes-associated SNPs was observed in the DPS (Table 3) (Kilpelainen et al. 2007, Kilpelainen et al. 2008a, Kilpelainen et al. 2008b).

In the DPP, gene-lifestyle interactions similar to those in the DPS were observed for the progression to glucose intolerance (Florez et al. 2006, Florez et al. 2007). In addition, variants of PPARγ, FTO and INSIG2 interacted with lifestyle on changes in body composition, but no interaction with physical activity was observed (Franks et al. 2007a, Franks et al. 2008).

**Gene-physical activity interaction on glucose homeostasis**

In candidate gene approach studies, an interaction between physical activity and gene variants on insulin sensitivity and parameters of glucose metabolism has been observed (Table 3). The most consistent effects on glucose metabolism have

**Gene-physical activity interaction on weight and body composition**

An interaction between physical activity and several genes have also been observed on changes in weight, BMI and adiposity (Table 3). Only a few of these studies have yielded consistent findings. A gene-physical activity interaction has been observed for the Pro12Ala polymorphism of PPARG (Ostergard et al. 2005) where body weight decreased more in the Ala12 carriers than in others.

In lifestyle intervention studies combining physical activity with diet, a genotype-by-intervention interaction has been observed on weight change (Lindi et al. 2002, Franks et al. 2007a, Haupt et al. 2010, He et al. 2011) and changes in body composition (Franks et al. 2007a) for the Pro12Ala polymorphism of PPARG. Also variants of TCF7L2 have been observed to influence changes in BMI, total, and visceral fat during lifestyle intervention in the TULIP study (Haupt et al. 2010). Whether there is a direct physical activity-gene interaction on the changes of body composition for PPARG, TCF7L2 and other new susceptibility loci associated particularly with insulin resistance, remains unclear.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Intervention</th>
<th>Participants</th>
<th>Body composition outcomes</th>
<th>Metabolic outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCC8</td>
<td>rs3758947</td>
<td>4.2-year intervention with increased physical activity and dietary modifications of</td>
<td>479 Finnish adults with IGT</td>
<td>-</td>
<td>Increased PA removed the effect of the risk genotypes on the risk of T2D.</td>
<td>Kilpelainen et al. 2007</td>
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<tr>
<td>ACE</td>
<td>I/D</td>
<td>6-month aerobic exercise intervention</td>
<td>35 older hypertensive men</td>
<td>-</td>
<td>Greater improvement in insulin sensitivity and decrease in acute insulin response in I/I homozygotes</td>
<td>Dengel et al. 2002</td>
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<tr>
<td>ADRA2B</td>
<td>12Glu9</td>
<td>4.2-year intervention with increased physical activity and dietary modifications</td>
<td>479 Finnish adults with IGT</td>
<td>-</td>
<td>Increased PA did not decrease the risk of T2D in carriers of risk genotype</td>
<td>Laaksonen et al. 2007</td>
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<tr>
<td>ADRB2</td>
<td>Arg16Gly</td>
<td>1-year moderate intensity exercise intervention</td>
<td>251 women</td>
<td>Greater decreases in BMI, fat mass, and fat % in Gly16Gly homozygotes.</td>
<td></td>
<td>Garenc et al. 2003</td>
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<tr>
<td>COMT</td>
<td>Val108/158Met</td>
<td>1-year moderate intensity exercise intervention</td>
<td>173 postmenopausal women</td>
<td>Greater decrease in fat% in Val/Val homozygotes</td>
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<td>TwoRog5 et al. 2004</td>
</tr>
<tr>
<td>CYP19</td>
<td>(TTTA)*n repeat, n = 7–13</td>
<td>1-year moderate intensity exercise intervention</td>
<td>173 postmenopausal women</td>
<td>Greater decrease in fat mass and fat% in response to exercise with two 11-repeat alleles</td>
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<td>TwoRog5 et al. 2004</td>
</tr>
<tr>
<td>ENPP1</td>
<td>K121Q</td>
<td>12-week walking program</td>
<td>84 abdominally obese Korean women</td>
<td>Greater decrease in weight and BMI in KK carriers</td>
<td>Greater decrease in fasting insulin in KK carriers</td>
<td>Park et al. 2008</td>
</tr>
<tr>
<td>Gene</td>
<td>Polymorphism</td>
<td>Intervention</td>
<td>Participants</td>
<td>Body composition outcomes</td>
<td>Metabolic outcomes</td>
<td>Reference</td>
</tr>
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<tr>
<td>FTO</td>
<td>rs8050136</td>
<td>6-month moderate intensity exercise intervention</td>
<td>234 postmenopausal women</td>
<td>Greater weight loss in A/A homozygotes among those meeting/exceeding exercise targets.</td>
<td>-</td>
<td>Mitchell et al. 2010</td>
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<tr>
<td>FTO</td>
<td>rs8050136</td>
<td>20 week endurance training intervention</td>
<td>481 sedentary white subjects</td>
<td>Three-fold greater losses in FM and fat% among carriers of C allele as compared to A/A homozygotes.</td>
<td>-</td>
<td>Rankinen et al. 2010</td>
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<tr>
<td>GNB3</td>
<td>C-825T</td>
<td>Endurance training</td>
<td>255 normotensive men and women</td>
<td>Greater decrease in fat mass and fat% in TT homozygotes</td>
<td>-</td>
<td>Rankinen et al. 2002</td>
</tr>
<tr>
<td>GHRL</td>
<td>rs26802</td>
<td>4.2-year intervention with increased physical activity and dietary modifications</td>
<td>479 Finnish adults with IGT</td>
<td>Responses of BMI to changes in PA differed between genotypes.</td>
<td>Responses of HDL-C to changes in PA differed between genotypes.</td>
<td>Kilpelainen et al. 2008b</td>
</tr>
<tr>
<td>GHRL</td>
<td>rs696217</td>
<td>4.2-year intervention with increased physical activity and dietary modifications</td>
<td>479 Finnish adults with IGT</td>
<td>Responses of BMI to changes in PA differed between genotypes.</td>
<td>Responses of SBP to changes in PA differed between genotypes.</td>
<td>Kilpelainen et al. 2008b</td>
</tr>
<tr>
<td>LEPR</td>
<td>rs1137100</td>
<td>4.2-year intervention with increased physical activity and dietary modifications</td>
<td>479 Finnish adults with IGT</td>
<td>Greater decrease in fat mass, fat% in carriers of X447 in white women.</td>
<td>-</td>
<td>Garenc et al. 2001</td>
</tr>
<tr>
<td>LPL</td>
<td>S447X</td>
<td>20-week endurance training</td>
<td>741 adult white and black subjects</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLIN</td>
<td>13041A&gt;G</td>
<td>6-month endurance training program</td>
<td>101 sedentary older adults</td>
<td>Less change in body composition in non-AA carriers</td>
<td>Improved insulin AUC in non-AA-carrier men</td>
<td>Jenkins et al. 2010</td>
</tr>
<tr>
<td>PLIN</td>
<td>14995A&gt;T</td>
<td>6-month endurance training program</td>
<td>101 sedentary older adults</td>
<td>Less change in body composition in non-AA carriers</td>
<td>Improved insulin AUC in non-AA-carrier men</td>
<td>Jenkins et al. 2010</td>
</tr>
<tr>
<td>PPARG</td>
<td>rs1801281 (Pro12Ala), rs1152003, rs17036314</td>
<td>4.2-year intervention with increased physical activity and dietary modifications</td>
<td>479 Finnish adults with IGT</td>
<td>-</td>
<td>Increased PA decreased the effect of risk alleles of rs17036314 and rs1801282 on the conversion to T2D.</td>
<td>Kilpelainen et al. 2008a</td>
</tr>
<tr>
<td>Gene</td>
<td>Polymorphism</td>
<td>Intervention</td>
<td>Participants</td>
<td>Body composition outcomes</td>
<td>Metabolic outcomes</td>
<td>Reference</td>
</tr>
<tr>
<td>--------</td>
<td>--------------</td>
<td>---------------------------------------------------</td>
<td>--------------</td>
<td>--------------------------</td>
<td>------------------------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>PPARG2</td>
<td>Pro12Ala</td>
<td>3-month exercise intervention</td>
<td>123 healthy</td>
<td>-</td>
<td>HOMA-IR and IRI decreased in Ala12- carriers only</td>
<td>Kahara et al. 2003</td>
</tr>
<tr>
<td>PPARG2</td>
<td>Pro12Ala</td>
<td>6-month endurance training intervention</td>
<td>73 sedentary</td>
<td>-</td>
<td>Improved insulin AUC in Ala12 carriers among men. No differences in women</td>
<td>Weiss et al. 2005</td>
</tr>
<tr>
<td>PPARG2</td>
<td>Pro12Ala</td>
<td>Endurance training</td>
<td>79 healthy first degree relatives of T2D patients</td>
<td>Enhanced weight loss in Ala12 carriers</td>
<td>-</td>
<td>Ostergard et al. 2005</td>
</tr>
<tr>
<td>PPARG2</td>
<td>Pro12Ala</td>
<td>3-months supervised aerobic and resistance training intervention</td>
<td>139 sedentary T2D patients</td>
<td>No differences in body composition changes between Ala12 carriers and others.</td>
<td>Greater improvement in fasting glucose in Ala12 carriers.</td>
<td>Adamo et al. 2005</td>
</tr>
<tr>
<td>SLC2A2</td>
<td>rs5393</td>
<td>4.2-year intervention with increased physical activity and dietary modifications</td>
<td>479 Finnish adults with IGT</td>
<td>-</td>
<td>Increased PA removed the effect of risk genotypes on the risk of T2D.</td>
<td>Kilpelainen et al. 2007</td>
</tr>
<tr>
<td>SLC2A2</td>
<td>rs5394</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs5404</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCF7L2</td>
<td>rs7903146</td>
<td>1-year lifestyle intervention of combined exercise, nutrition and behavioral therapy</td>
<td>236 overweight German children</td>
<td>No differences in ΔBMI between genotypes</td>
<td>Negative effect of T allele on changes in HOMA-IR and QUICKI</td>
<td>Reinehr et al. 2008b</td>
</tr>
<tr>
<td>TCF7L2</td>
<td>rs7903146</td>
<td>2-year combined exercise and diet intervention</td>
<td>309 German subjects at risk for T2D</td>
<td>negative impact of SNP on change in BMI, visceral and non-visceral fat</td>
<td>No association with SNP</td>
<td>Haupt et al. 2010</td>
</tr>
</tbody>
</table>

Abbreviations: ABCC8, ATP-binding cassette, sub-family C (CFTR/MRP), member 8; ACE, Angiotensin-converting enzyme; ADRA2B, adrenergic, alpha-2B-receptor; ADRB2, adrenergic, beta-2-, receptor, surface; AUC, area-under-curve; COMT, Catechol-O-methyltransferase; CYP19, Cytochrome aromatase p450 coding gene; ENPP1, Ectonucleotide pyrophosphatase/phosphodiesterase 1; FTO, Fat mass and obesity-associated; GNB3, guanine nucleotide binding protein (G protein), beta polypeptide 3; GHRL, ghrelin/obestatin prepropeptide; LEPR, leptin receptor; LPL, lipoprotein; PLIN, Perilipins; PPARG, peroxisome proliferator-activated receptor gamma; SLC2A2, solute carrier family 2 (facilitated glucose transporter), member 2; TCF7L2, Transcription factor 7-like 2.
3 The aims of the study

The overall aim of this study was to investigate changes in cardiometabolic risk factors in young adults, and the effect of intensive, long-term exercise performed during military service on the changes in body composition and cardiometabolic risk profile. Moreover, the aim of this study was to determine the underlying endocrine and genetic factors regulating the response to an exercise intervention. More specifically, the aims of the study were:

1. To determine the individual contribution of long-term exercise during military service to changes in common cardiovascular disease risk factors during military service (I).
2. To determine the association of unacylated ghrelin with long-term exercise, during military service and exercise induced changes in weight, body composition and fat distribution (II).
3. To determine the association of unacylated ghrelin with changes in glucose metabolism, insulin sensitivity, and lipid metabolism during long-term exercise in military service (III).
4. To evaluate the gene-physical fitness interaction of common genetic variants associated with insulin resistance on changes in weight and body composition during military service (IV).
4 Methods

4.1 Research Design

A prospective study with an intensive exercise intervention and 6–12 month follow-up was performed on a population of young men entering military service at the Sodankylä Jaeger Brigade, in northern Finland. Data were collected at the beginning and at the end of military service. The follow-up period was 6, 9 or 12 months. The length of military service depends on the tasks and type of military training. Of all study subjects 58% served for 6 months (privates), 9% for 9 months (privates in need of special knowledge and skills) and 33% for 12 months (officers, non-commissioned officers, and privates in need of special professional skills).

4.2 Participants

The study population was representative of the age group since approximately 80% (25,000/year) of the male population complete military service. In 2005, 1,467 men with mean age of 19.2 (SD 1.0 years, range 18–28) mainly from northern Finland attended the Sodankylä Jaeger Brigade. All conscripts (1,467) were invited to participate in the present study, and 79% (1,160) were enrolled, all of Caucasian origin. A total of 140 conscripts discontinued their military service due to physical or psychological reasons. These individuals did not significantly differ from the study cohort (Mikkola et al. 2009). In addition, all female military conscripts (n = 15) attending voluntary military service were invited to participate in this study, but were excluded from statistical analyses due to the small number of cases.

4.3 Ethical considerations

All participants gave a written consent to use the collected data for scientific purposes. The study protocol was approved by the Ethics Committee of Lapland Central Hospital, Rovaniemi, Finland.
4.4 Study protocol

Data were collected at the beginning and at the end of military service. Information about participants’ background, demographics and smoking was obtained by questionnaires. Anthropometric, body composition, BP and exercise performance measurements were taken and venous blood samples collected at baseline and follow-up.

4.4.1 Anthropometric measurements

Weight (to nearest 0.1kg) in light clothing and height (to nearest 0.5cm) were measured. Waist circumference (cm) was measured midway between the lowest rib and the iliac crest. Body mass index (BMI) was calculated dividing body weight (kg) by the square of the height (m²).

4.4.2 Body composition

Body composition was analyzed by multifrequency bioelectric impedance analysis (BIA; InBody720, Biospace Co Ltd, Seoul, Korea). The electrode method of the device consists of a tetrapolar eightpoint tactile electrode system. The outputs of the InBody 720 Body Composition Analyzer are based on segmental multifrequency analysis and values measured from each limb and the trunk. Resistance of the arms, trunk, and legs was measured at frequencies of 1, 5, 50, 250, and 500 kHz. The analysis of body composition is based on a four-compartment model. This assumes that the body is composed of four different elements: total body water, protein, minerals, and body fat. The accuracy of measurement is high in comparison to, for example, the dual-energy x-ray absorptiometry (DEXA) method (Malavolti et al. 2003, Thomson et al. 2007) and especially for assessing total body water (Bedogni et al. 2002). The segmental multifrequency BIA may slightly underestimate body fat percentage in adults with normal weight and slightly overestimate body fat percentage in overweight adults. However, the biological significances of these errors are small (Bedogni et al. 2002, Shafer et al. 2009). The BIA method is also practical in large epidemiological studies with limited time for examining each subject.

During the measurements, the subject’s personal information (measured height, gender, and age) was entered. The measuring device recorded the weight of the subject. During the measurements, the subjects were instructed to grab the
hand electrodes of the device so that the thumb was placed on top of the handgrip while the other fingers were holding the bottom. The subjects were asked to straighten their elbows, leaving some space between the armpits and the body (approximately 15 degree angle). During the measurements, the subjects stood still and were not allowed to talk. The duration of each measurement was 1.5 minutes.

The following body composition indices were derived: fat percentage (fat %), fat mass (FM, kg), skeletal mass (SM), lean body mass (LBM) and visceral fat area (VFA, cm²). VFA was calculated by the InBody720 which has been previously validated against a cross-sectional area of VFA measured by CT (Somaton Plus 24, Siemens, Germany). In the validation study including 332 subjects the correlation between these measurements was $r = 0.922$.

4.4.3 Blood pressure

Systolic and diastolic BP levels were measured with an automated BP measuring device (Omron Healthcare, model HEM-757, Japan) by a trained observer after 5–10 min in a sitting position.

4.4.4 Biochemical measurements

Venous blood samples were drawn by physicians or medical personnel after 12 hours of overnight fasting, centrifuged immediately at 1500 x g for 15 minutes, and frozen at $-80^\circ$C. Biochemical assessments were performed in the laboratory of Oulu Deaconess Institute by using commercially available, glucose-6-hexokinase assay for plasma glucose, homogeneous enzymatic test for HDL cholesterol, enzymatic colorimetric test for total triglycerides and enzymatic tests for total and LDL cholesterol (Konelab™ analyzers, Thermo Electron Oy, Vantaa, Finland) according to national quality standards. Serum insulin was determined using the AxSym Insulin assay (Abbot Laboratories). Plasma concentration of UAG was determined using commercially available enzyme immunoassay (EIA) Kit, from SPI-BIO, Bertin Technologies. The lower limit of detection for the UAG assay was 2pg/mL and intra- and inter-assay coefficients of variation (CVs) were 11.8 and 13.2%, respectively. Insulin resistance was evaluated by the homeostasis model assessment (HOMA-IR) (Wallace et al. 2004).
4.5 Genotyping

DNA was isolated from whole blood using commercial DNA isolation kits. Genotyping of all SNPs was performed at the University of Eastern Finland, with Sequenom iPLEX (Sequenom, Inc, San Diego, CA), in accordance of the standard operating procedures of the Sequenom iPLEX.

4.6 Exercise performance

Aerobic performance was measured by the Cooper 12-minute running test (Cooper 1968). The test was performed outdoors and controlled by educated supervisors. The test timing and circumstances were standardized. Participants were instructed to run 12 minutes with a maximal effort, and the test result was reported by the distance run with 10 meters’ accuracy. The Cooper 12-minutes running test was developed for military use, and it provides a fairly good estimation for maximal oxygen uptake (VO₂max) without treadmill testing, which is considered as a “gold standard” (correlation coefficients are 0.84–0.92 with the treadmill VO₂max) (Cooper 1968, Grant et al. 1995).

Muscle fitness was measured by 5 tests: sit-ups, a back-muscle test (testing endurance of abdominal, back, and hip-flexor muscles), push and pull-ups (testing upper extremities), and standing long jump (testing explosive muscle strength), as described in detail elsewhere (Santila et al. 2006). Participants were asked to perform a maximum number of repetitions of concentric muscle actions possible during 60 seconds, with a 5 minute break for recovery between each component. Results were recorded to the accuracy of the nearest repetition and for the long jump, to the nearest 1cm. Muscle fitness performance was graded for each component (0 = poor, 1 = satisfactory, 2 = good, 3 = very good), and a sum of scores of individual components was calculated to determine the total muscle fitness index (MFI, 0–4 = poor, 5–8 = satisfactory, 9–12 = good and 13–15 = very good).

4.7 Amount of physical exercise

Physical activity during military training consists of exercise training (includes running, strength training, martial arts, orienteering, swimming, cross-country skiing, and recovery training), as well as combat training and marching. The estimated amount of physical activity during the 8 week basic training period in
the beginning of the military service corresponds on average to approximately 4h of sports-related physical activity and 12h of military-related physical training (such as combat training and marching) per week. After the basic training period, the total amount of physical activity varies slightly during subsequent special training and unit training periods, and also depending on the branch of service.

4.8 Diet

The intended content of energy in the food served to every conscript by the military forces is 3200–3600 kcal per day, of which 30–35% consist of fat (Tähtinen T 2000). In addition, the service men are allowed to use optional food items which account for approximately 25% of their daily calorie intake (Bingham C 2004). Hence it was not possible to control for the exact daily calorie intake.

Dietary habits were assessed by a dietician-developed questionnaire. The used questionnaire formula consists of 38 questions about the eating habits, alcohol consumption, smoking, and exercise of the participants.

4.9 Smoking

Smoking habits were assessed by a questionnaire. Study subjects answered a question “Are you currently smoking?” with six alternatives to answer. Those who answered that they do not smoke at all/smoke one day a week or occasionally were categorized as non-smokers. Those who reported smoking 2–7 days a week were categorized as smokers.

4.10 Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or range, or median and the interquartile range, as appropriate. Paired t-test was used to evaluate the differences between the mean values at baseline and at follow-up. P < 0.05 was considered as statistically significant. Statistical analysis was performed using SPSS Statistics 18 for Windows, SPSS Inc, Chicago, IL, and SAS 9.1.3. for Windows, SAS Institute Inc. Gary NC).
Cardiometabolic changes (Study I)

Associations between the changes in dependent and independent variables (changes in weight, VFA, 12-min running test or MFI) were assessed by Pearson correlation coefficients. Linearity assumptions of the model were evaluated by means of standard scatter plots, with linear and nonlinear fit. Multivariate linear regression analysis, adjusting for length of service, smoking, and baseline value of the independent variable, was used to estimate the association of independent variables with the dependent variables. A change in exercise performance as an independent variable was adjusted by change in weight and vice versa.

Ghrelin and body composition (Study II)

Weight loss of 2.5% or greater has been previously shown to be clinically significant (Saaristo et al. 2010b). Therefore, weight change was divided into three groups of weight loss \( \leq -2.5\% \) \((n = 166)\), stable weight \((n = 155)\) and weight gain \( \geq 2.5\% \) \((n = 143)\). ANOVA was used to compare the differences between the groups (Table 7). Log transformation was performed for the levels of UAG at baseline because of skewed distribution. Associations between dependent variables and UAG level, and the changes in these variables were assessed by Spearman correlation coefficients. Univariate linear regression analysis was used to estimate the association of a change in UAG level with changes in waist circumference and fat percentage (Model 1). Multivariate linear regression analysis was used to estimate the association of a change in UAG level with changes in 1) waist circumference and 2) fat percentage, adjusted for weight at baseline (Model 2), weight at baseline and weight change (Model 3), weight at baseline and change in MFI (Model 4), and weight at baseline, weight change and change in MFI (Model 5).

Ghrelin, glucose and lipid homeostasis (Study III)

Associations between dependent variables and UAG level, and the changes in these were assessed by Spearman correlation coefficients. ANOVA was used to compare the differences in UAG levels between the groups in Figure 3. Multivariate linear regression analysis (Table 11) was used to estimate the association of level of UAG at baseline and a change in UAG level with separate dependent variables (fasting glucose and insulin, HOMA-IR, total, HDL and LDL.
cholesterol and total triglycerides). Log transformation, because of skewed distribution, was performed for the following measurements at baseline: UAG, fasting glucose, fasting insulin, and HOMA-IR. In Model 1 the associations between a change in UAG level and dependent variables were adjusted for baseline UAG level. In Model 2 the associations of UAG and dependent variables at baseline were adjusted for baseline weight and current smoking. Similarly the associations between the changes in UAG levels and changes in dependent variables were adjusted for weight change, current smoking and baseline UAG level (log).

**Gene-exercise interaction (Study IV)**

Paired t-test or ANOVA where appropriate was used to evaluate the differences between groups. Interaction between SNP and change in exercise performance was evaluated with the general linear model and adjusted for the baseline value of the respective trait.
5 Results

Characteristics of the cohort and mean changes during the follow-up are described in Table 4. At baseline the participants had a mean BMI (23.9 kg/m$^2$, range from 16.3 to 46.0 kg/m$^2$). Improvements in both aerobic and strength performance were observed during the follow-up. During the follow-up, significant reductions were observed in weight, fat mass, fat percentage, and VFA. Systolic BP decreased and HDL cholesterol increased significantly. In contrast, diastolic BP, total and LDL cholesterol and triglycerides increased.

Table 4. Characteristics of participants at baseline and changes in cardiovascular risk factors from baseline to follow-up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Baseline</th>
<th>Change from baseline to follow-up</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean, range)</td>
<td>1070</td>
<td>19.3 (18–28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg) (mean, range)</td>
<td>1070</td>
<td>75.1 (47.2–140.0)</td>
<td>−0.42 (5.19)</td>
<td>0.008</td>
</tr>
<tr>
<td>BMI (kg/m$^2$) (mean, range)</td>
<td>1070</td>
<td>23.9 (16.3–46.0)</td>
<td>−0.30 (1.69)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>1056</td>
<td>81.6 (10.2)</td>
<td>0.10 (5.8)</td>
<td>0.589</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>1030</td>
<td>13.4 (9.2)</td>
<td>−1.3 (4.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fat %</td>
<td>1030</td>
<td>16.7 (8.2)</td>
<td>−1.1 (4.6)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>1030</td>
<td>58.3 (6.9)</td>
<td>0.8 (2.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Skeletal mass (kg)</td>
<td>1030</td>
<td>34.9 (4.4)</td>
<td>0.6 (1.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Visceral Fat Area (cm$^2$)</td>
<td>1030</td>
<td>65.7 (50.8)</td>
<td>−28.4 (33.3)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>1060</td>
<td>128.8 (13.5)</td>
<td>−2.1 (13.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>1061</td>
<td>69.8 (9.8)</td>
<td>2.0 (9.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>998</td>
<td>3.89 (0.83)</td>
<td>0.43 (0.79)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>986</td>
<td>2.23 (0.74)</td>
<td>0.18 (0.64)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>998</td>
<td>1.29 (0.30)</td>
<td>0.08 (0.26)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1000</td>
<td>0.79 (0.36)</td>
<td>0.42 (0.76)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>12-min running test (m) (mean, range)</td>
<td>1146</td>
<td>2482 (1000–3910)</td>
<td>170 (269)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Muscle Fitness Index (points)</td>
<td>1137</td>
<td>8.0 (1–15)</td>
<td>1.5 (2.3)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are mean, SD unless stated otherwise.
5.1 Association of changes in cardiovascular risk factors with changes in exercise performance and body composition (Study I)

Weight loss correlated strongly with the reduction of waist circumference, FM, fat percentage, and VFA (Table 5). Weight loss also correlated with a decrease in both systolic and diastolic BP, with a decrease in total and LDL cholesterol and triglycerides, and an increase in HDL cholesterol. Decrease in VFA correlated with reductions in BP and lipid levels similarly to those of weight loss, with the exception of triglycerides (p=NS).

Improvement in endurance and muscle performance significantly correlated with weight loss, decrease in waist circumference, and decreases in FM, fat percentage and VFA. Correlations for the change in the 12-min running test were substantially higher than for the change in MFI. Favorable changes in levels of BP and serum lipids correlated better with change in aerobic rather than strength performance.

Table 5. Pearson correlations between changes (Δ) in weight, visceral fat area (VFA), 12-min running test (Cooper test) and muscle fitness index (MFI), and body composition and cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Change in variable</th>
<th>Δ Weight</th>
<th>Δ VFA</th>
<th>Δ Cooper test</th>
<th>Δ MFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>1.000</td>
<td>0.645</td>
<td>−0.31</td>
<td>−0.173</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.977</td>
<td>0.664</td>
<td>−0.305</td>
<td>−0.163</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.877</td>
<td>0.695</td>
<td>−0.296</td>
<td>−0.178</td>
</tr>
<tr>
<td>Fat %</td>
<td>0.779</td>
<td>0.631</td>
<td>−0.266</td>
<td>−0.187</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>0.435</td>
<td>0.042</td>
<td>−0.093</td>
<td>−0.013</td>
</tr>
<tr>
<td>Skeletal mass (kg)</td>
<td>(&lt; 0.001)</td>
<td>(0.176)</td>
<td>(0.004)</td>
<td>(0.690)</td>
</tr>
<tr>
<td>Visceral Fat Area (cm²)</td>
<td>0.459</td>
<td>0.073</td>
<td>−0.114</td>
<td>−0.02</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.203</td>
<td>0.248</td>
<td>−0.217</td>
<td>−0.155</td>
</tr>
</tbody>
</table>

58
5.2 **Comparison of the effects of exercise versus weight loss on CVD risk factors**

Associations between the changes in weight, endurance performance, blood pressure levels, and lipids were linear (data not shown), and therefore linear regression analysis was applied in the subsequent statistical analysis.

Multiple linear regression analysis showed that the improvement in BP and lipid levels with increased aerobic capacity was attributable to weight loss (Figure 2). Additional adjustment for changes in either aerobic or muscle performance did not alter the effect of weight loss on CVD risk factors. The association of weight loss with changes in BP and lipid levels remained significant after the adjustment for baseline weight, changes in both exercise parameters, length of follow-up and smoking (Table 6).

The effects of the reduction in VFA on changes in BP and lipid levels were smaller than that of weight loss (Table 6), but remained significant for BP, LDL and total cholesterol levels after adjustment for confounding factors. Effects of the improvement in 12-min running test as a modifier of CVD risk factors were small, and were significant only for diastolic BP after the adjustment for confounding factors. Increase in muscle performance was associated with the reduction of diastolic BP and triglyceride levels, even after the adjustment for confounding factors.

<table>
<thead>
<tr>
<th>Change in variable</th>
<th>Δ Weight</th>
<th>Δ VFA</th>
<th>Δ Cooper test</th>
<th>Δ MFI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure(mmHg)</td>
<td>0.155</td>
<td>0.106</td>
<td>−0.065</td>
<td>−0.005</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(0.042)</td>
<td>(0.887)</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.251</td>
<td>0.128</td>
<td>−0.074</td>
<td>−0.059</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(0.024)</td>
<td>(0.0770)</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.339</td>
<td>0.236</td>
<td>−0.115</td>
<td>−0.063</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(0.059)</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>−0.165</td>
<td>−0.152</td>
<td>0.04</td>
<td>0.031</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(0.219)</td>
<td>(0.349)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.043</td>
<td>−0.058</td>
<td>0.029</td>
<td>−0.083</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>(0.174)</td>
<td>(0.073)</td>
<td>(0.370)</td>
<td>(0.012)</td>
</tr>
<tr>
<td>12-min running test</td>
<td>−0.31</td>
<td>−0.469</td>
<td>1.000</td>
<td>0.216</td>
</tr>
<tr>
<td>(m)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Muscle Fitness Index</td>
<td>−0.173</td>
<td>−0.181</td>
<td>0.216</td>
<td>1.000</td>
</tr>
<tr>
<td>(points)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
<td>(&lt; 0.001)</td>
</tr>
</tbody>
</table>

P values are in parentheses.
Table 6. Associations of changes in weight, visceral adiposity, aerobic and muscle fitness, with changes in cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \Delta ) Weight B (SE)</th>
<th>( \Delta ) VFA B (SE)</th>
<th>( \Delta ) Cooper B (SE)</th>
<th>( \Delta ) MFI B (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \beta ) P value</td>
<td>( \beta ) P value</td>
<td>( \beta ) P value</td>
<td>( \beta ) P value</td>
</tr>
<tr>
<td>Crude</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.3935 (0.0774)</td>
<td>0.0416 (0.0123)</td>
<td>-0.003 (0.0016)</td>
<td>-0.026 (0.1865)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.3882 (0.0578)</td>
<td>0.0740 (0.0091)</td>
<td>-0.008 (0.0012)</td>
<td>-0.676 (1.382)</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol/L)</td>
<td>0.0379 (0.0047)</td>
<td>0.0030 (0.0008)</td>
<td>-0.000 (0.0001)</td>
<td>-0.020 (0.0112)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.0417 (0.0037)</td>
<td>0.0046 (0.0006)</td>
<td>-0.000 (0.0001)</td>
<td>-0.017 (0.0092)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>-0.008 (0.0015)</td>
<td>-0.001 (0.0002)</td>
<td>0.0000 (0.0000)</td>
<td>0.0034 (0.0037)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.0062 (0.0046)</td>
<td>-0.001 (0.0007)</td>
<td>0.0001 (0.0001)</td>
<td>-0.026 (0.0105)</td>
</tr>
<tr>
<td>Variable</td>
<td>Δ Weight</td>
<td>Δ VFA</td>
<td>Δ Cooper</td>
<td>Δ MFI</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------</td>
<td>-------</td>
<td>----------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>B (SE)</td>
<td>β</td>
<td>P value</td>
<td>B (SE)</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.7223 (0.1133)</td>
<td>0.2746 &lt; 0.001</td>
<td>0.0703 (0.0238)</td>
<td>0.1707 (0.0020)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.2168 (0.0831)</td>
<td>0.1100 0.009</td>
<td>0.0449 (0.0173)</td>
<td>0.1440 (0.0015)</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol/L)</td>
<td>0.0475 (0.0067)</td>
<td>0.3083 &lt; 0.001</td>
<td>0.0042 (0.0015)</td>
<td>0.1731 (0.0001)</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>0.0426 (0.0053)</td>
<td>0.3400 &lt; 0.001</td>
<td>0.0038 (0.0012)</td>
<td>0.1923 (0.0001)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>−0.006 (0.0022)</td>
<td>−0.1111 0.013</td>
<td>−0.000 (0.0005)</td>
<td>−0.0483 (0.0000)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.0202 (0.0065)</td>
<td>0.1390 0.002</td>
<td>0.0007 (0.0014)</td>
<td>0.0290 (0.0001)</td>
</tr>
</tbody>
</table>

Effect size is β coefficient (standard error) calculated by linear regression. * Analyses were adjusted for baseline value of the independent variable, length of follow-up and smoking. Changes in 12-min running test and muscle fitness index were adjusted for changes in weight. Changes in weight and visceral fat were adjusted for change in 12-min running test and muscle fitness index.
Fig. 2. Associations between change (\( \Delta \)) in weight and endurance performance (12-minute running test), and changes in cardiovascular risk factors.
5.3 Unacylated ghrelin, exercise and changes in body composition (Studies II and III)

An increase in UAG was observed during the follow-up, which significantly associated with the magnitude of weight loss (Figure 3). Increase in UAG level during the follow-up was pronounced among the men with significant weight loss (weight loss ≥ 2.5%), compared to the weight stable and weight-gaining groups. Similarly, increase in UAG level was pronounced among men with the largest reduction in central adiposity.

Fig. 3. Comparison of the changes in the levels of unacylated ghrelin during the 6-month follow-up between the different groups formed according to the changes in weight and waist circumference. P-value is for the differences between the groups. ** indicates p-value < 0.01 and *** p-value < 0.001. 1st tertile = greatest reduction (≤ −3.0 cm) and 3rd tertile = smallest change (≥ 2.5 cm) in waist circumference.
Table 7. Unacylated ghrelin levels, body composition and exercise parameters at baseline and their changes during the 6-month follow-up by the groups of weight change.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Weight loss ≥ 2.50%</th>
<th>Stable weight</th>
<th>Weight gain ≥ 2.50%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Baseline measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unacylated ghrelin (pg/mL)*</td>
<td>28.6</td>
<td>7.8–54.4</td>
<td>33.9</td>
<td>13.7–66.2</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>27.7</td>
<td>4.3</td>
<td>23.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>86.2</td>
<td>15.4</td>
<td>71.7</td>
<td>9.6</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.8</td>
<td>11.5</td>
<td>79.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>22.2</td>
<td>10.5</td>
<td>11.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Fat %</td>
<td>24.7</td>
<td>7.9</td>
<td>15.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>64.0</td>
<td>7.6</td>
<td>60.1</td>
<td>6.4</td>
</tr>
<tr>
<td>Visceral fat area (cm²)</td>
<td>112.6</td>
<td>54.2</td>
<td>57.2</td>
<td>37.2</td>
</tr>
<tr>
<td>12-min running test (m)</td>
<td>2189</td>
<td>326</td>
<td>2420</td>
<td>511</td>
</tr>
<tr>
<td>Muscle fitness index (points)</td>
<td>5.4</td>
<td>3.4</td>
<td>8.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Change during the follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unacylated ghrelin (pg/mL)</td>
<td>19.1</td>
<td>40.5</td>
<td>13.6</td>
<td>43.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>−7.2</td>
<td>4.2</td>
<td>0.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>−6.2</td>
<td>5.4</td>
<td>0.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>−6.7</td>
<td>4.6</td>
<td>−0.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Fat %</td>
<td>−5.9</td>
<td>3.8</td>
<td>−1.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>−0.50</td>
<td>2.4</td>
<td>1.0</td>
<td>1.8</td>
</tr>
<tr>
<td>Visceral fat area (cm²)</td>
<td>−60.1</td>
<td>32.6</td>
<td>−26.7</td>
<td>22.4</td>
</tr>
<tr>
<td>12-min running test (m)</td>
<td>311</td>
<td>276</td>
<td>214</td>
<td>454</td>
</tr>
<tr>
<td>Muscle fitness index (points)</td>
<td>2.1</td>
<td>2.2</td>
<td>1.2</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Values are mean and standard deviation, except for *median (interquartile range) for baseline UAG values. P value is for ANOVA. P values for differences at baseline are for log transformed where appropriate.
Characterisation of the group with significant weight loss of 2.5% or greater showed that they also had the lowest UAG levels at baseline (Table 7). Moreover, the group with the greatest weight loss had the highest mean BMI, weight, waist circumference, fat mass and fat percentage, VFA at baseline. Their body composition changes showed the greatest reduction in all adiposity parameters, compared to the other groups. Regarding exercise performance, the groups with the greatest weight loss had the poorest endurance and strength performance at baseline, but subsequently improved in their exercise performance the most.

The level of UAG correlated inversely with weight, waist circumference, FM, and fat percentage, and FFM at baseline (Table 8). The changes in UAG level correlated strongly and inversely with changes in weight, waist circumference, FM and fat percentage, but not with FFM. The level of UAG did not correlate with exercise performance at baseline. The change in the level of UAG correlated with the change in MFI, but not 12-min running test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline measurements</th>
<th>Change during 6-month follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>−0.192 &lt; 0.001</td>
<td>−0.130 0.005</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>−0.244 &lt; 0.001</td>
<td>−0.224 &lt; 0.001</td>
</tr>
<tr>
<td>Waist-Hip-Ratio</td>
<td>−0.084 0.062</td>
<td>0.015 0.742</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>−0.176 &lt; 0.001</td>
<td>−0.164 &lt; 0.001</td>
</tr>
<tr>
<td>Body fat %</td>
<td>−0.166 &lt; 0.001</td>
<td>−0.164 &lt; 0.001</td>
</tr>
<tr>
<td>Visceral fat area (cm²)</td>
<td>0.007 0.881</td>
<td>0.043 0.358</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>−0.129 0.004</td>
<td>0.012 0.791</td>
</tr>
<tr>
<td>12 min running test (m)</td>
<td>0.006 0.900</td>
<td>0.020 0.663</td>
</tr>
<tr>
<td>Muscle fitness index (points)</td>
<td>0.062 0.176</td>
<td>0.113 0.013</td>
</tr>
</tbody>
</table>

5.3.1 Independent contribution of changes in the level of UAG to body fat distribution

Multiple linear regression analysis showed that an inverse association of the level of UAG with changes in waist circumference and fat percentage remained statistically significant after the adjustment for weight at baseline, weight change, and change in MFI (Table 9). The association with the change in waist
circumference after the adjustments was significantly greater than the association with the fat percentage.

Table 9. Associations of a change (Δ) in the level of unacylated ghrelin (UAG) with a changes (Δ) in waist circumference and fat percentage.

<table>
<thead>
<tr>
<th>Model</th>
<th>Δ Waist circumference</th>
<th>Δ Fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>-0.210</td>
<td>-0.029</td>
</tr>
<tr>
<td>2</td>
<td>-0.142</td>
<td>-0.019</td>
</tr>
<tr>
<td>3</td>
<td>-0.105</td>
<td>-0.015</td>
</tr>
<tr>
<td>4</td>
<td>-0.133</td>
<td>-0.017</td>
</tr>
<tr>
<td>5</td>
<td>-0.109</td>
<td>-0.014</td>
</tr>
</tbody>
</table>

Values are standardized beta (β), and effect size (B) and standard error.

Univariate regression analysis was used for Model 1 and multivariate linear regression analysis for Models 2 to 5.

Model 1: association of ΔUAG with Δ waist and Δ fat%
Model 2: association of ΔUAG with Δ waist and Δ fat% adjusted for weight at baseline analysis)
Model 3: association of ΔUAG with Δ waist and Δ fat% adjusted for weight at baseline and Δ weight
Model 4: association of ΔUAG with Δ waist and Δ fat% adjusted for weight at baseline and Δ muscle fitness index (MFI)
Model 5: association of ΔUAG with Δ waist and Δ fat% adjusted for weight at baseline, Δ weight and Δ MFI

5.4 Correlation of UAG with glucose and lipid metabolism

Correlations between UAG, insulin sensitivity and lipids at baseline

UAG correlated strongly and inversely with glucose homeostasis variables at baseline (Table 10.) Particularly strong inverse correlations were observed for UAG level and fasting insulin, fasting glucose, and HOMA-IR. UAG correlated inversely with total triglycerides and LDL cholesterol and positively with HDL cholesterol.

Correlations of changes in UAG levels with insulin sensitivity and lipids during the follow-up

Changes in UAG level correlated strongly and inversely with changes in fasting glucose, fasting insulin, and HOMA-IR (Table 10). Additionally, changes in UAG level correlated inversely with changes in total triglycerides and total cholesterol.
Table 10. Spearman correlations between the levels of unacylated ghrelin and cardiovascular risk factors at baseline, and correlation between the change in UAG level with the changes in cardiovascular risk factors during 6-month follow-up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline measurements</th>
<th>Change during the 6-month follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>-0.132 (0.003)</td>
<td>-0.181 (&lt; 0.001)</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>-0.181 (&lt; 0.001)</td>
<td>-0.200 (&lt; 0.001)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-0.185 (&lt; 0.001)</td>
<td>-0.207 (&lt; 0.001)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>-0.085 (0.06)</td>
<td>-0.142 (&lt; 0.001)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>-0.090 (0.046)</td>
<td>-0.076 (0.098)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.095 (0.035)</td>
<td>-0.062 (0.175)</td>
</tr>
<tr>
<td>Total triglycerides (mmol/L)</td>
<td>-0.189 (&lt; 0.001)</td>
<td>-0.169 (&lt; 0.001)</td>
</tr>
</tbody>
</table>

P-values in parentheses.

Effects of weight loss and changes in UAG levels on insulin sensitivity

Multiple linear regression analysis showed that an inverse association between the levels of UAG and levels of fasting glucose and insulin, and HOMA-IR at baseline remained significant after adjustment for weight and smoking (Table 11.) The inverse association between the changes in UAG level and changes in fasting glucose, fasting insulin and HOMA-IR remained significant after the adjustment for weight change, smoking and baseline UAG level (log). An inverse association between the changes in UAG levels and HOMA-IR also remained significant after the adjustment for changes in waist circumference, smoking and baseline UAG level (p = 0.039). The association between UAG levels at baseline and total TG remained significant after the adjustment for weight and smoking, but the association between UAG levels and total cholesterol did not. The associations between the change in UAG level and the changes in total cholesterol and total triglyceride levels were not significant after the adjustment for weight change, smoking and baseline UAG level.
Table 11. Associations of baseline UAG level and changes in UAG level with baseline measurements and changes in fasting parameters of glucose metabolism and lipid levels.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Baseline measurements</th>
<th>Changes during the follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1 (unadjusted)</td>
<td>Model 2 (adjusted for baseline weight and smoking)</td>
</tr>
<tr>
<td></td>
<td>β (B (SE)) P–value</td>
<td>β (B (SE)) P–value</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>−0.158 (0.010) &lt; 0.001</td>
<td>−0.135 (0.010) 0.003</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>−0.159 (0.054) &lt; 0.001</td>
<td>−0.100 (0.047) 0.010</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>−0.159 (0.055) &lt; 0.001</td>
<td>−0.100 (0.048) 0.011</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>−0.082 (0.025) 0.071</td>
<td>−0.061 (0.026) 0.191</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>−0.057 (0.040) 0.211</td>
<td>−0.025 (0.040) 0.582</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>−0.012 (0.027) 0.786</td>
<td>−0.050 (0.026) 0.247</td>
</tr>
<tr>
<td>Total triglycerides (mmol/L)</td>
<td>−0.140 (0.044) 0.002</td>
<td>−0.112 (0.044) 0.013</td>
</tr>
</tbody>
</table>

Effect size is B coefficient (standard error) and standardized beta (β), calculated by linear regression.
5.5 Gene-exercise interaction

Effects of PPARG2 rs1801281, IRS1 rs2943641, and TCF7L2 rs7903146 on changes in adiposity by groups of exercise performance are shown in Figure 4. A significant SNP × exercise (Δ 12-min running test) interaction, adjusted for the baseline value of the respective trait, was observed on changes in waist circumference for PPARG2 (p = 0.011), on change in weight for IRS1 (p = 0.019) and on changes in weight and fat percentage for TCF7L2 (p = 0.021 and 0.006, respectively).

Carriers of the Ala12-allele of PPARG2 had a significantly greater increase in the waist circumference in the ΔCooper equal to or below median group, but no difference was observed between carriers of the Pro12Pro genotype and Ala12 carries in the ΔCooper above median group. A similar, non-significant trend was observed for weight. No differences were observed between the Pro12Pro and Ala12/X genotypes on fat percentage.

Homozygote carriers of the minor allele of IRS1 gained significantly more weight in the ΔCooper equal to or below median group, as compared to the heterozygote carriers and the homozygote carriers of the major allele. No significant differences were observed between the different genotypes of IRS1 on waist circumference and fat percentage.

A trend for an increase in waist circumference and fat percentage in homozygote carriers of the minor allele of TCF7L2 rs7903146 was observed in the ΔCooper equal to or below median group. However, in the ΔCooper above median group, trend for greater reduction in waist circumference and fat percentage among the homozygotes of minor allele was observed (p = NS).
Fig. 4. Effect of diabetes risk SNPs of PPARG2, IRS1 and TCF7L2 on changes in weight, waist circumference and fat percentage according to exercise performance (Cooper test).
6 Discussion

6.1 Exercise, adiposity and changes in cardiovascular risk factors

In this study a regular 6 to 12 month physical exercise program during military service significantly improved CVD risk profile, providing evidence that physical activity leads to the improvement of CVD risk factors levels in young men. Particularly beneficial was aerobic exercise resulting in the reduction of visceral fat area.

6.1.1 ‘Fitness versus fatness’ and the independent contribution of exercise

Both endurance and strength performance improved during the 6 to 12 month exercise intervention, and significantly reduced weight, central adiposity, total fat mass, and visceral fat. Improvement in endurance performance was associated with greater weight loss and more beneficial body composition changes than improvement in strength performance. Similar body composition changes have previously been reported with moderate to vigorous exercise (Slentz et al. 2009). While the mean total weight loss in our cohort was modest, as previously published, it was more pronounced in the overweight and obese young men (−3.2kg for BMI category of 25–29.9 kg/m², and −8.9kg for BMI of ≥30 kg/m², respectively) (Mikkola et al. 2009). The relatively modest overall improvement in physical performance in our cohort is likely to reflect the heterogeneous physical activity background of study participants entering military service.

Physical activity is known to be protective against premature mortality and morbidity (Wei et al. 1999), but with most lifestyle intervention studies combining physical activity with changes in diet and weight loss (Yates et al. 2007), the independent contribution of physical activity to improved health outcomes has remained unclear. In our study both weight loss and reduction in central adiposity, as well as improvement in physical performance were associated with reduced BP levels and beneficial changes in the lipid profile. The effect of exercise on BP and lipid levels was, however, attributable mainly to weight loss and reduced VFA.
6.1.2 Blood pressure

Studies regarding the changes in BP in response to physical activity have been controversial. Exercise has not lowered blood pressure in some of the previous studies (Church et al. 2007, Ilanne-Parikka et al. 2010). However, a meta-analysis by Whelton et al. reported that aerobic exercise lowered BP levels both in hypertensive and normotensive individuals (Whelton et al. 2002). Also Whyke et al. reported a significant decrease in BP levels among young adults (Whyte et al. 2010). The modest reduction in BP levels with intensive long-term exercise observed in this study is in agreement with the previous reports (Whelton et al. 2002, Whyte et al. 2010).

6.1.3 Lipid profile

Previous exercise intervention studies have observed an overall beneficial effect of exercise on the lipid profile, particularly with respect to HDL cholesterol and also total TGs. However, the effect has been variable and overall small across the studies, and the dose and intensity of exercise required for substantial changes in the lipid profile remain unclear (Kraus & Slentz 2009). It also remains unclear as to whether these effects are independent of the changes in body composition. In this study an increase in HDL and decreases in total and LDL cholesterol levels were observed, which were attributable to weight loss and reduced visceral fat. Findings from the present study therefore suggest that the effect of exercise on lipid profile is attributable to the changes in weight and body composition.

6.1.4 Central adiposity

Central adiposity provides a plausible link between the improvement in exercise performance and reduction in CVD risk factors. Visceral fat has an independent curvilinear association with mortality (Kuk et al. 2006). Compared to total fat mass, visceral fat have been associated more strongly with BP levels, the HDL/total cholesterol ratio and insulin resistance (Nieves et al. 2003). Central adiposity has also been linked with elevated sBP levels in the Nurses’ Health Study, and reduction in visceral fat has been associated with decreased BP levels (Rexrode et al. 1998). Obesity and particularly visceral fat are associated with qualitative and quantitative changes in lipids and lipoproteins, such as increased total cholesterol, very low-density lipoproteins (VLDL), small dense LDL
particles, total TG and decreased HDL cholesterol levels (Howard et al. 2003). Findings from the present study showing that exercise intervention reduced VFA and resulted in less atherogenic changes in lipid and lipoprotein levels are in agreement with the previous studies.

6.1.5 Cardiometabolic changes and military service

An increase in the prevalence of obesity among men entering military service has been reported in the U.S. and Scandinavia (Rasmussen et al. 1999, Sharp et al. 2002), together with a simultaneous gradual decline in aerobic fitness (Santtila et al. 2006). However, our results demonstrate weight loss, beneficial body composition changes and improved cardiovascular risk factors in association with improved exercise performance during the military service. Albeit these changes on average were modest, they were pronounced in overweight and obese men. If maintained, these relatively small changes could potentially translate into substantial health benefits over time.

6.2 Unacylated ghrelin: effects on weight, body composition and metabolism

6.2.1 Changes in the levels of unacylated ghrelin in response to exercise

A significant increase in the level of UAG was observed in association with the exercise program during 6 months of military service in young men. Increase in UAG level was strongly associated with weight loss and reduction in central adiposity. This is the first study where an increase in the levels of UAG has been reported during a long-term exercise intervention in young adults.

Data on UAG and exercise has been very limited and changes in UAG levels have previously been evaluated with long-term exercise in children only. A non-significant increase in the levels of UAG has been previously reported during a 12-week exercise programme in a study of 17 overweight children (Kim et al. 2008). In contrast, with acute exercise in adults an increase in the levels of AG was reported whereas the levels of UAG remained unchanged among 9 young men (Shiiya et al. 2011). In the present substudy of 552 young men, an increase in the levels of UAG was observed during a 6-month physical activity
programme, which was especially pronounced among those with weight loss and reduction in central adiposity. These findings from young men are in agreement with previous reports of increased levels of total ghrelin after an exercise intervention accompanied by weight loss in women (Leidy et al. 2004, Foster-Schubert et al. 2005).

**6.2.2 Association of the levels of unacylated ghrelin levels with weight and body composition**

The role of UAG with weight, body composition and adiposity in adults has been unclear. Previous studies have reported in part opposite effects of AG and UAG on appetite control, glucose metabolism and insulin sensitivity (Broglio et al. 2004, Gauna et al. 2004, Gauna et al. 2005, Neary et al. 2006). Whether these findings also hold for adiposity has not been previously known.

In the present study a significant increase in the levels of UAG during 6 months of follow-up were associated with weight loss and reduction in surrogate markers of central and total adiposity. The relationship of UAG with central obesity was stronger than with total fat mass, suggesting an association with the distribution of fat. Furthermore, UAG was associated with the distribution of fat independent of weight at baseline, weight loss and improvement in exercise performance.

A regulatory role of UAG on adipogenesis has been reported in animal studies (Thompson et al. 2004, Zhang et al. 2008). In humans, an inverse association of both UAG and total ghrelin with fat mass has been reported in children during lifestyle intervention (Kim et al. 2008, Kelishadi et al. 2008). Central adiposity has been associated with total ghrelin, but not UAG (Kim et al. 2008, Kelishadi et al. 2008), possibly due to a small sample size of the earlier studies. Our results show that the association of the changes in the levels of UAG with the change in total and abdominal fat is independent of weight at baseline, weight change and also change in exercise performance.

Importantly, the present study demonstrates a stronger association between the change in the level of UAG and central obesity, than with total fat mass. As discussed above, a large amount of visceral fat predicts mortality (Kuk et al. 2006) and it is strongly associated with elevated BP levels, the low HDL/total cholesterol ratio, insulin resistance (Nieves et al. 2003), and changes in lipids and lipoproteins (Howard et al. 2003).
The association of the orexigenic properties of UAG and its other functions remains uncertain. AG, but not UAG, has been reported to stimulate food intake in rodents (Neary et al. 2006). In humans, the orexigenic properties of UAG remain unclear. Further studies are needed to ascertain whether the association of high levels of UAG associated with weight loss and reduced fat mass reflect the effect of UAG on appetite. GOAT has been suggested to provide a link between ingested lipids, energy expenditure and body composition (Kirchner et al. 2009).

6.2.3 Association of the levels of unacylated ghrelin with changes in insulin sensitivity and lipid metabolism

Glucose metabolism

Previous studies suggest that UAG is involved in glucose metabolism in the liver. UAG can act as a potent insulin secretagogue in glucose stimulated conditions, resulting in enhanced insulin action on target tissues, especially in the liver (Gauna et al. 2007a). The levels of UAG have been reported to correlate with insulin sensitivity in an opposite manner to AG.

The present study was the first to investigate the role of UAG on changes in insulin sensitivity in response to an exercise intervention. Increased levels of UAG were strongly associated with an improvement in insulin sensitivity, independent of weight loss or reduction in central adiposity.

Previous studies have reported a positive correlation between the levels of UAG and insulin sensitivity (Gauna et al. 2004, Barazzoni et al. 2007, St-Pierre et al. 2007, Soares & Leite-Moreira 2008), but the role of UAG in modulating insulin sensitivity achieved by means of lifestyle intervention remains unclear. An inverse correlation between the levels of UAG and insulin, and insulin resistance measured by HOMA-IR has been reported in individuals with the metabolic syndrome in the National Cholesterol Education Program. Similarly, a positive correlation was reported between the levels of UAG and insulin sensitivity measured by the clamp technique in non-diabetic overweight and obese women (St-Pierre et al. 2007). Both studies concluded that the AG/UAG ratio regulates insulin sensitivity (Barazzoni et al. 2007, St-Pierre et al. 2007). However, an inverse correlation of the levels of both AG and UAG levels with insulin sensitivity has also been reported (Akamizu et al. 2005). Exercise intervention studies have not reported a correlation between total ghrelin or UAG with
changes in insulin levels or insulin resistance in women and children, respectively, possibly due to a small sample size (Foster-Schubert et al. 2005, Kim et al. 2008).

The present study is in agreement with earlier reports showing an inverse correlation between the levels of UAG and fasting insulin, glucose and insulin sensitivity (Gauna et al. 2004, Barazzoni et al. 2007, St-Pierre et al. 2007, Soares & Leite-Moreira 2008).

Lipid metabolism

The association of UAG with changes of lipid profile in response to lifestyle interventions or exercise has not been previously reported. Total ghrelin has been shown to have a significant role in lipid metabolism in the liver, skeletal muscle and adipose tissue (Soares & Leite-Moreira 2008), to favour TG deposition in the liver over skeletal muscle (Barazzoni et al. 2005), and to act directly on adipocytes to stimulate lipogenesis (Patel et al. 2006). Previous studies have reported a positive association between plasma total ghrelin and HDL cholesterol levels (Fagerberg et al. 2003, Ukkola et al. 2006). Studies regarding the association of ghrelin with total cholesterol, total TGs and LDL cholesterol have remained controversial (Purnell et al. 2003, Zou et al. 2009). Studies on the effects of UAG on changes of lipid profile in response to lifestyle interventions are lacking. In our cohort, inverse correlations between the baseline levels of UAG and total TGs and LDL cholesterol, as well as a positive correlation between the levels of UAG and HDL cholesterol were observed. Furthermore, physical activity-induced increases in the levels of UAG were associated with reduced levels of total cholesterol and TGs, which were attributable to weight loss.

6.3 Gene-physical activity interaction

Previous lifestyle intervention studies have shown significant gene-lifestyle interactions for variants in PPARG and TCF7L2 for weight loss and progression to type 2 diabetes both in the DPS and DPP (Lindi et al. 2002, Florez et al. 2006, Florez et al. 2007, Kilpelainen et al. 2008a). Gene-lifestyle interaction has been observed for PPARG and TCF7L2 on body composition changes (Franks et al. 2007a, Haupt et al. 2010). In the DPS, a significant gene-physical activity interaction was observed for several SNPs in PPARG on progression to type 2 diabetes (Kilpelainen et al. 2008a).
As physical activity modifies predominantly insulin sensitivity rather than insulin secretion, the SNPs affecting insulin sensitivity are of primary interest for the evaluation of the gene-physical activity interaction. Therefore SNPs of \textit{PPARG}, \textit{IRS1} and \textit{TCF7L2} were selected for evaluation in our cohort. Previous studies evaluating a gene-physical activity interaction have relied on self-reported estimates of physical activity per week (Laaksonen et al. 2005, Kilpelainen et al. 2008a). A more detailed evaluation of gene-physical activity interaction particularly in healthy individuals with good baseline exercise performance does, however, require an objectively measured exercise performance which differentiates between endurance and strength performance. Thus the Cooper 12-min running test as an estimate of VO2 max and strength performance measurements from the military tests were used in the current study.

For the prevention of diabetes, it is of importance whether a gene-physical activity interaction exists in normoglycaemic healthy individuals, who have not yet developed abnormal glucose tolerance. In the present study we evaluated whether the SNPs \textit{PPARG2} rs1801282, \textit{IRS1} rs2943641 and \textit{TCF7L2} rs7903146 interact with changes in physical performance during 6 to 12 months of training on changes in weight and body composition in normoglycaemic young men.

Significant gene-physical activity interactions were observed for \textit{PPARG2} on the change of waist circumference, for \textit{IRS1} on the change of weight, and for \textit{TCF7L2} on the changes of weight and fat percentage. Significant differences were observed between different genotypes of \textit{PPARG2} on the waist circumference and between different genotypes of \textit{IRS1} on weight change among those with no or limited improvement in physical performance, but not among the group with significant improvement in physical performance (\(\Delta\) Cooper test equal to or above the median).

Our findings are in agreement with those previously observed in high risk groups in combined lifestyle intervention studies for variants of \textit{PPARG} and \textit{TCF7L2}. Our findings on the role of \textit{IRS1} to modify weight change have not been previously reported. Importantly, these results highlight the role of intensive long-term physical activity in reducing adiposity in young men who are carriers of risk alleles predisposing type 2 diabetes or abdominal obesity.

\section*{6.4 Summary of results}

Key results of the study are summarised in Figure 5. An overall increase in both aerobic and strength performance was observed during military service, which
was associated with an overall weight loss, reduction in visceral adiposity, and reduction in body fat percentage. Improvement in exercise performance was associated with improvement lipid profile and BP, but the association was largely explained by weight loss and reduction in central adiposity.

Fig. 5. Summary of key results.

An increase in the levels of UAG was observed during follow-up, which was associated with weight loss, reduction of total and abdominal fat, and distribution of fat with a particularly strong association with waist circumference. Moreover, an increase in the levels UAG was associated with improved insulin sensitivity and favourable changes in the lipid profile. The association between the changes of the levels of UAG and insulin sensitivity was independent of the changes in weight or waist circumference. In contrast, the association between the change in the levels of UAG and changes in the lipid profile were explained by changes in weight and waist circumference.

A significant gene-physical activity interaction was observed for PPARG2 rs1801281, IRS1 rs2943641, and TCF7L2 rs7903146, whereby the effects of the risk allele on weight, waist circumference or fat percentage was observed in young men with no or little improvement in physical performance. However, a significant improvement in physical performance counteracted the adipogenic effect of the risk allele.
6.5 Strength and limitations of the study

6.5.1 Strengths

The study population of military conscripts was a large, representative sample of young healthy Finnish men as approximately 80% of the age groups complete military service. The study population evaluated here corresponds to the Finnish population of 19-year old males well. Mean weight of all Finnish conscripts was 75.2 kg in 2004 (Santtila et al. 2006) and 75.1 kg in the present study. Mean Cooper test result of all Finnish conscripts was 2434 m in 2004 (Santtila et al. 2006) and 2482 m in the present study. Thus, the results of our study are likely to be applicable to the entire population of healthy young men.

A slight selection bias is possible as approximately 10% of young men are exempt from military service after physical and mental medical examinations, and 7% of eligible young men opt for non-military service. This, however, is unlikely to lead to overestimates in the prevalence of smoking, obesity and cardiometabolic risk factors as those with the most serious physical and psychiatric disorders are exempted from military service.

Contribution of intense physical activity and exercise to the cardiometabolic risk profile was possible to evaluate since living circumstances, main meals, sleeping, and exercise behaviors are standardized during military service. Conducting similar standardized, equally representative prospective population level studies on exercise, body composition and cardiovascular risk factors would be very challenging in other settings.

Physical performance was objectively measured and aerobic and strength performance evaluated separately, allowing the comparison of their independent contributions to cardiometabolic and body composition changes. Both aerobic and anaerobic performance was measured in standardized circumstances and in accordance with the protocol of the Finnish Defence Forces. All supervisors of the fitness tests were educated and experienced.

In contrast to self-reported physical activity, objectively measured exercise performance allowed the much more detailed evaluation of gene-endurance performance interaction. Therefore, albeit the sample size of approximately 1,100 men is rather small for genetic studies, we were able to observe significant gene-physical performance interactions on changes in body composition in a prospective setting.
This study yielded novel data on the association of the levels of UAG with changes in body composition, and glucose and lipid metabolism in response to physical activity. In contrast to previous studies on exercise and ghrelin which have been performed in smaller study populations, we were able to observe strong associations in large, unselected cohort of healthy young adults.

6.5.2 Limitations

This study was carried out in the military setting and therefore we did not have a control group, albeit each of the participants served as his own control. The inevitable changes in diet, other lifestyle factors and environment associated with military service may have also had some effect on body weight. The findings of this study are also limited to young men and as such cannot be generalized to other age groups.

The amount of exercise was estimated and not directly and individually measured. The estimated amount of physical activity during the 8-week basic training period in the beginning of the military service corresponds on average to approximately 4h of sports-related physical activity and 12 h of military-related physical training (Santtila et al. 2008). Training outcome records from Defence Forces confirm the average duration of exercise performed to be 4h/week and the minimum amount of 12h military-related training to have occurred during the basic training period.

Aerobic performance was assessed with the Cooper-12-minutes running test (Cooper 1968), which has been in use by the Finnish Defence Forces for decades. The Cooper test has been developed for military use and its prediction for VO\textsubscript{2max} is good (correlation coefficient 0.84–0.92 (Cooper 1968, Grant et al. 1995). However, it provides only an estimate of VO\textsubscript{2max}, as treadmill testing was not possible to carry out in our relatively large study population.

This study did not include a dietary intervention used in many lifestyle interventions, due to the military setting. The intended content of energy in the food served to every conscript by the military forces is 3200–3600 kcal per day, of which 30–35% consist of fat (Tähtinen T 2000). Furthermore, there is inter-individual variation in the intake of optional food items, which may also have an effect on weight during military service. Hence, the importance of exercise as a modifier of CVD risk factors is highlighted in this setting with no caloric restriction. Caloric restriction has been reported to have an independent effect and
also a combined effect with exercise to improve CVD risk in healthy non-obese individuals (Lefevre et al. 2009).

Due to military setting where the data was collected, it was not possible to measure the levels of AG and thus we were not able to evaluate and compare the two forms of ghrelin and the AG/UAG ratio in our study. In view of the current research into the physiological importance of the AG/UAG ratio, and also obestatin, it is recommended the similar future studies would measure all peptides of the ghrelin family, as well as GOAT.
7 Summary of findings and conclusions

The main findings of Studies I-IV were:

Study I – An improvement in both endurance and strength performance was observed during military service, which correlated with the reduction in weight, total and abdominal fat. Improvement in exercise performance was associated with reduction in BP levels and less atherogenic changes the lipid profile. Endurance performance correlated with changes in CVD risk factors better than strength performance. Associations between improved exercise performance and changes in CVD risk factors were attributable to reduced weight and central adiposity.

Study II – An increase in the plasma level of UAG was observed with long-term intensive exercise, which was associated with weight loss and less adipogenic changes in body composition and body fat distribution. The association of the levels of UAG with changes in central obesity was stronger than that with total fat mass.

Study III - The exercise-associated increase in the levels of UAG was associated with decreases in fasting insulin and glucose levels, and an increase in insulin sensitivity, via a mechanism independent of weight loss. Both the baseline levels of UAG and the changes in the levels of UAG were associated with less atherogenic changes in the lipid profile which were attributable to weight loss.

Study IV – A significant gene-physical performance interaction was observed for the Pro12Ala polymorphism rs1801282 of PPARG2 on central adiposity, IRS1 rs2943641 on weight and TCF7L2 rs7903146 on changes in weight and fat percentage.

7.1 Scientific implications

Our study is in agreement with previous findings that an improvement in both endurance and strength performance leads to the reduction in weight, total and abdominal fat mass, as well as leads to favourable changes in blood pressure and lipid profile. Endurance performance correlated with the changes in CVD risk factors better than strength performance.
The novel findings of the changes in the levels of UAG in adults during long-term exercise, and the associated favorable changes in body composition, glucose and lipid metabolism increase the current knowledge on the role of UAG to modify CVD risk factors. It is likely that the AG:UAG ratio is of importance for glucose metabolism, but also possibly for lipid metabolism and body composition. Studies investigating AG, UAG, total ghrelin and GOAT with respect to metabolic changes and body composition are warranted to improve the current understanding of the physiologic role of ghrelin. Such studies are likely to add new information on the role of GOAT, a promising target for developing obesity and diabetes medications.

Significant interactions between endurance performance and gene variants associated with insulin resistance on changes in weight and body composition were found in our study. Many of the associations observed did not reach statistical significance, warranting similar, larger studies to further investigate the gene-exercise interaction.

7.2 Clinical implications

Cardiovascular benefits of long-term moderate to intensive exercise, such as reported in this study, add encouraging evidence for the population-level approach to improve cardiovascular health in young men by means of an exercise intervention. Studies of the exercise behaviours and cardiovascular health of these men after the military service are warranted to determine the long-term significance and maintenance of such changes.

The prevalence of abnormal glucose tolerance and cardiometabolic risk factors are high in Finland. For the primary prevention of cardiovascular disease and type 2 diabetes it is necessary that interventions are targeted to adolescents and young adults, in order to prevent the current decline in physical activity. The findings add encouraging evidence that substantial cardiovascular health benefits can be achieved by exercise and physical activity undertaken during military service.

Subgroup analysis demonstrated that those with a significant weight loss of 2.5% or greater were those with the highest BMI and waist circumference, and lowest fitness levels at the beginning of military service. These findings suggest that overweight and obese young men who have several cardiometabolic risk factors are likely to benefit most from lifestyle intervention in non-military settings.
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RELATIONSHIP OF PHYSICAL ACTIVITY, UNACYLATED GHRRELIN AND GENE VARIATION WITH CHANGES IN CARDIOVASCULAR RISK FACTORS DURING MILITARY SERVICE