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**PATHOGENESIS OF TYPE 2
DIABETES WITH EMPHASIS
ON THE MECHANISM OF
INSULIN RESISTANCE**

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“Life moves forward, but is better understood backward”
Soren Kierkegaard (1813-1855)

ABSTRACT

Type 2 diabetes is characterized by chronic hyperglycemia and develops slowly through stages of early impairments of glucose metabolism. The disease is often associated with obesity, physical inactivity, decreased insulin sensitivity and impaired insulin responsiveness. An increase in the muscle concentration of malonyl CoA, an inhibitor of fatty-acid oxidation, has been observed in association with insulin resistance and type 2 diabetes. The thesis aimed to evaluate the influence of sex and diabetes heredity on the prevalence and pathogenesis of early abnormalities of glucose metabolism, as well as the impact of exercise training on phenotype characteristics and insulin sensitivity in people with or without mild type 2 diabetes. The impact of exercise training on cellular and molecular mechanisms regulating insulin sensitivity in muscle was also investigated.

The subject-material comprised of approximately 8,000 men and women, with or without family history of type 2 diabetes (FHD). They participated in the Stockholm Diabetes Prevention Program (SDPP). The participants were categorized according to an oral glucose tolerance test (OGTT) and the homeostasis model assessment (HOMA) was used to determine insulin sensitivity and beta cell function. Thirteen men with type 2 diabetes and 17 sex-, age-, and body mass index-matched control subjects were evaluated to compare phenotype characteristics, insulin sensitivity and malonyl CoA levels in biopsies from the vastus lateralis muscle. Furthermore, eleven healthy middle-aged controls and twelve patients with type 2 diabetes were investigated before and after a 12 week training program to explore molecular mechanisms mediating training improved insulin sensitivity.

Prevalence of early abnormalities of glucose metabolism was two to three times higher in subjects with FHD and two to three times higher in men compared to women.

In these subjects both waist circumference and systolic blood pressure were increased and insulin sensitivity and beta cell function were decreased. Subjects with impaired fasting glucose (IFG) had more pronounced impairment of beta cell function and insulin sensitivity than subjects with impaired glucose tolerance (IGT).

Patients with type 2 diabetes had significantly lower physical fitness (VO_{2max}) and higher truncal fat mass compared to healthy controls. The plasma concentration of free fatty acids and the rate of fatty acid oxidation during the hyperinsulinemic euglycemic clamp, were higher in the diabetic subjects than in the controls. During the high-dose insulin clamp, the increase in cytosolic citrate and malate in muscle, which parallels and regulates malonyl CoA levels, was significantly less in patients. Despite this, a similar increase in the concentration of malonyl CoA was observed in the two groups, suggesting an abnormality in malonyl CoA regulation in the patients with type 2 diabetes.

Long-term exercise training decreased intra-abdominal fat mass and improved VO_2 max in both controls and patients. Subjects with type 2 diabetes improved insulin sensitivity by 77%, when measured by hyperinsulinemic euglycemic clamp, whereas no significant change was seen in the controls. Furthermore, after training the basal concentration of malonyl CoA in muscle was significantly decreased in both groups. This was accompanied by increased activity of malonyl CoA decarboxylase (MCD). In patients, the basal diacylglycerol (DAG) levels decreased after training whereas MCD mRNA and abundance of peroxisomal-proliferator-activated receptor (PPAR) γ co-

activator 1 α (PGC-1 α) increased in controls. No changes in the phosphorylation of AMP-activated protein kinase (AMPK) or acetyl CoA carboxylase (ACC) were seen.

In conclusion, type 2 diabetes heredity and male sex increased the prevalence of early abnormalities of glucose homeostasis. Insulin sensitivity is decreased in mild type 2 diabetes which closely correlates with an increase in truncal fat mass, decrease in physical fitness and unexpectedly high levels of malonyl CoA in muscle. We propose that high levels of muscle malonyl CoA, in combination with decreased suppression of plasma FFA, constitute a crucial mechanism behind insulin resistance in type 2 diabetes. Physical training markedly improved insulin sensitivity and decreased intra-abdominal fat area in patients with type 2 diabetes. This study demonstrates for the first time in man that physical training increased activity of MCD in muscle. We believe that the latter mechanism significantly accounts for the decrease of muscle malonyl CoA and DAG, resulting in improved insulin sensitivity.

Keywords: Acetyl CoA carboxylase; AMP-activated protein kinase; Glucose intolerance; Insulin secretion, Insulin sensitivity; Malonyl CoA, Malonyl CoA decarboxylase; Peroxisome proliferator activated receptor (PPAR) γ coactivator-1 α ; Type 2 diabetes heredity

SVENSK SAMMANFATTNING

Typ 2 diabetes kännetecknas av kronisk hyperglykemi och utvecklas långsamt genom olika stadier av störningar i glukosmetabolismen och ses vanligtvis flera år innan sjukdomen diagnosticerats. Sjukdomen associeras ofta med fetma, fysisk inaktivitet, minskad insulinkänslighet och nedsatt förmåga att frisätta insulin. Förhöjda intracellulära koncentrationer i skelettmuskel av malonyl CoA, en indirekt hämmare av fettoxidationen, har observerats i samband med insulinresistens och typ 2 diabetes. Syftet för avhandlingsarbetet är att studera betydelsen av kön respektive ärftlighet för prevalens och patogenes till tidiga störningar i glukos metabolismen samt även studera tränings effekt på fenotypa karakteristika och insulinkänslighet hos individer med och utan typ 2 diabetes. Vidare studerades tränings effekt på cellulära och molekylära mekanismer som reglerar insulinkänsligheten i muskel.

Individmaterialet bestod av ca 8 000 män och kvinnor, med respektive utan ärftlighet för typ 2 diabetes och alla deltog i Stockholms Diabetes Preventiva Program (SDPP). Deltagarna kategoriserades enligt en oral glukos tolerans test (OGTT). Både insulinkänsligheten och betacells funktionen beräknades utifrån homeostasis model assessment (HOMA). Vidare studerades 13 st män med typ 2 diabetes och 17 st köns-, ålders- och BMI-matchade kontroller för att kunna jämföra fenotypa karakteristika, insulinkänslighet och malonyl CoA nivåer i muskel. I syfte att utvärdera tränings effekt på olika molekylära mekanismer av betydelse för ökad insulinkänslighet studerades 11 friska medelålders kontrollpersoner och 12 patienter med typ 2 diabetes, före och efter 3 månaders strukturerad träning.

Resultaten visade att prevalensen av tidiga störningar i glukos metabolismen var två till tre gånger högre hos individer med ärftlighet för typ 2 diabetes och två till tre gånger högre hos män jämfört med kvinnor. Generellt sett hade individer med störd glukos omsättning större bukomfång och högre systoliskt blodtryck samt försämrad insulinkänslighet och betacells funktion. Vidare hade individer med nedsatt fasteglukos en mer uttalad nedsättning av både insulinkänsligheten och betacells funktionen jämfört med individer med ett förhöjt 2 timmars värde vid OGTT.

Patienter med typ 2 diabetes visade sig vara sämre fysiskt tränade och ha ökat buk fett jämfört med friska kontroller. Både plasma koncentrationen av fria fettsyror (FFA) och grad av fett oxidation under hyperinsulinemisk klamp var högre hos diabetikerna än hos kontrollerna. Under högdos insulin klamp, var ökningen i muskel av citrat och malat, som parallellt reglerar malonyl CoA koncentrationen, signifikant lägre hos diabetikerna. Trots detta hade båda grupperna samma ökning av malonyl CoA, vilket sannolikt tyder på en störd reglering av malonyl CoA hos individer med typ 2 diabetes. Långvarig fysisk träning minskade intraabdominellt fett samt ökade fysisk arbetsförmåga (VO_{2max}) hos både kontroller och friska. Diabetikerna ökade insulinkänsligheten, som bestämdes genom hyperinsulinemisk euglykemisk klamp, med 77% medan man inte fann någon signifikant förändring hos kontrollerna. Vidare sågs en signifikant minskning av malonyl CoA i muskel i båda grupperna som ett resultat av träningen. Denna minskning åtföljdes av en ökad aktivitet av malonyl CoA dekarboxylas (MCD). Efter träning sågs också en minskning av diacylglycerol (DAG) nivåerna hos diabetikerna medan man hos kontrollerna fann en ökning av MCD mRNA och en ökning av peroxisomal-proliferator-activated receptor γ co-activator 1 α (PGC-

1 α) protein. Inga signifikanta förändringar sågs i fosforyleringen av AMP-activated protein kinase (AMPK) (Thr 172) eller acetyl CoA carboxylase (ACC).

Sammanfattningsvis ökar ärftlighet för typ 2 diabetes och manligt kön prevalensen av tidiga störningar i glukosomsättningen. Vid mild typ 2 diabetes ses en minskad insulinkänslighet som är starkt kopplad till ökad bukfetma, minskad fysisk prestationsförmåga och oförväntat höga koncentrationer av malonyl CoA i muskel. Dessa förhöjda nivåer av malonyl CoA i kombination med en minskad förmåga att supprimera både plasmanivåer och oxidation av FFA föreslås därför vara en viktig mekanism bakom insulinresistens vid typ 2 diabetes. Tre månaders strukturerad träning, i form av Friskis och Sveltis, ökade signifikant insulinkänsligheten och minskade intraabdominell fettmängd hos individer med typ 2 diabetes. Studien visar för första gången på människa att fysisk träning ökar aktiviteten av MCD i muskel. Denna mekanism föreslås vara av central betydelse för minskning av malonyl CoA och DAG i skelettmuskel, och bidra till en ökad insulinkänslighet.

LIST OF PUBLICATIONS

This thesis is based on the following papers which will be referred to by their Roman numerals:

- I. Kuhl J, Hilding A, Östenson CG, Grill V, Efendic S, Båvenholm P.
Characterisation of subjects with early abnormalities of glucose tolerance in the Stockholm Diabetes Prevention Programme: the impact of sex and type 2 diabetes heredity
Diabetologia, 48: 35–40, 2005

- II. Båvenholm P, Kuhl J, Pigon J, Saha A K, Ruderman N, Efendic S.
Insulin Resistance in Type 2 Diabetes: Association with Truncal Obesity, Impaired Fitness and, Atypical Malonyl Coenzyme A Regulation
J Clin Endocrinol Metab, 88:82-87, 2003

- III. Kuhl J, Ruderman N, Musi N, Goodyear L, Patti ME, Crunkhorn S, Dronamraju D, Thorell A, Nygren J, Ljungkvist O, Degerblad M, Ståhle A, Brismar T, Andersen K, Saha A, Efendic S, Båvenholm P.
Exercise training decreases the concentration of malonyl-CoA and increases the expression and activity of malonyl-CoA decarboxylase in human muscle
Am J Physiol Endocrinol Metab 290:1296-1303, 2006

- IV. Kuhl J, Ruderman N, Musi N, Goodyear L, Patti ME, Thorell A, Nygren J, Ljungkvist O, Degerblad M, Ståhle A, Brismar T, Saha A, Efendic S, Båvenholm P.
Effects of Exercise Training on Phenotype Characteristics, Insulin Sensitivity and Malonyl CoA Regulation in Subjects with Type 2 Diabetes
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LIST OF ABBREVIATIONS

ACC	Acetyl CoA carboxylase
ADA	American Diabetes Association
AICAR	5-aminoimidazole-4-carboxamide ribonucleoside
AMPK	AMP-activated protein kinase
BMI	Body mass index
CaMK-IV	Calcium/calmodulin-dependent protein kinase-IV
CGI	Combined glucose intolerance
CPT 1	Carnitine palmitoyltransferase 1
CVD	Cardiovascular disease
DAG	Diacylglycerol
DEXA	Dual-energy X-ray absorptiometry
DM	Diabetes mellitus
FFA	Free fatty acids
FHD	Family history of type 2 diabetes
GLUT-4	Glucose transporter 4
HDL	High-density lipoprotein
HOMA	Homeostasis model assessment
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IRS-1	Insulin receptor substrate 1
LBM	Lean body mass
LCFA CoA	Long chain fatty acyl CoA
LDL	Low-density lipoprotein
Malonyl CoA	Malonyl Coenzyme A
MAP-kinase	Mitogen-activated kinase
MCD	Malonyl Coenzyme A decarboxylase
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
PFK-2	Phosphofructokinase-2
PGC1- α	Peroxisome proliferator activated receptor γ coactivator-1 α
PI	Proinsulin
PI3-K	Phosphatidylinositol 3-kinase
PKC	Protein kinase C
PPAR α	Peroxisome proliferator-activated receptor α
RPE	Rate of perceived exertion
RQ	Respiratory quotient
SDPP	Stockholm Diabetes Prevention Program
VO _{2max}	Maximal oxygen consumption

1 INTRODUCTION

1.1 GENERAL BACKGROUND

There is a dramatic global increase in the prevalence of diabetes mellitus (DM), a disease characterized by chronic hyperglycemia¹. The disease DM has been known for more than 2000 years and the word “diabetes” describes the typical signs of a patient producing excessive amounts of urine and drinking compensatory amounts of fluids. The word “mellitus” means honey sweet.

There are two major forms of DM. Type 1 is characterized by the presence of islet cell auto-antibodies that lead to impairment and ultimately cessation of insulin secretion². In type 2 diabetes hyperglycemia results from a combination of impaired insulin secretion and insulin resistance³. Insulin resistance decreases the ability of the skeletal muscle, adipose tissue and liver to respond to insulin. When the β -cells are no longer able to compensate for the tissue resistance to insulin action, hyperglycemia becomes manifest.

Type 2 diabetes accounts for approximately 90% of all cases of DM and is a slow-onset, heterogeneous disorder, resulting from interactions between environmental factors and genetic inheritance^{4,5}. Type 2 diabetes is associated with an 2-4 fold increased risk of cardiovascular disease (CVD)^{6,7}. Around 75% of all diabetic patients die of CVD⁸. Microvascular complications, i.e. retinopathy, nephropathy, and neuropathy also contribute to an increased morbidity and mortality in people with diabetes.

The prevalence of type 2 diabetes is rising in most countries and by 2010 the prevalence world-wide is estimated to be 220 million and by the year 2025 it is expected to exceed 300 million^{9,10}. In Sweden the prevalence of type 2 diabetes is approximately 300 000 and the cost is estimated to around 6-7% of the total healthcare expenditures, which is mainly related to treatment costs of macro and micro-vascular complications¹¹.

Thus, from both a health economic and a health care perspective it is important to identify individuals who are at risk of developing diabetes. Life-style prevention programs, including increased physical activity and improved diet habits as well as weight loss, can successfully prevent, or delay the onset of the disease^{12,13}.

1.2 EARLY DISTURBANCES OF GLUCOSE METABOLISM

Type 2 diabetes develops slowly through stages of early disturbances of glucose metabolism, including impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or both IFG and IGT, i.e. combined glucose intolerance (CGI)^{14,15}. People with IFG and/or IGT have an increased risk of developing type 2 diabetes and CVD¹⁶. The risk is highest among those who have CGI. A majority of individuals who develop type 2 diabetes have either IFG or IGT or both approximately 5 years before the onset of the disease¹⁶.

In most populations, IGT is more prevalent than IFG, although an limited overlap between the two conditions exists¹⁶⁻¹⁸. IFG seems to be more common in middle-aged men, whereas IGT is slightly more common in women. The prevalence of IGT

increases with age and the association with CVD is greater¹⁶. The pathogenesis of IFG and IGT, however, is not clear¹⁹⁻²².

1.3 GENETICS

An interplay between environmental factors and a genetic predisposition play an important role in the pathogenesis of type 2 diabetes²³. There is no genetic test to diagnose type 1 or type 2 diabetes. However, certain susceptibility genes and genetic variations can be found. Familial clustering and twin studies have suggested a genetic component for type 2 diabetes²⁴. Concordance between monozygotic twins is approximately 80% while in the case of dizygotic twins concordance is 40% for type 2 diabetes. Concordance appears to be significantly greater in type 2 diabetes in comparison to type 1, and in monozygotic than in dizygotic twins²⁵.

First degree relatives of patients with type 2 diabetes have a four-fold risk of developing type 2 diabetes compared to subjects with first degree relatives without diabetes heredity²⁶. Some populations, like the Pima Indians, have exceptionally high prevalence of type 2 diabetes, 10-20% as compared to 3-5% in Caucasians²⁷. The most likely explanation is that when populations with a hereditary predisposition for type 2 diabetes abandoned their traditional lifestyle due to migration to modern societies with altered diets, physical inactivity, obesity and stress they develop the disease²³.

Little is known about the impact of gender and diabetes heredity on the development of early abnormalities of glucose metabolism, nor is the impact of maternal or paternal diabetes heredity, in males and females, on the development of glucose abnormalities clarified.

1.4 ENVIRONMENTAL FACTORS

Increased rates of obesity due to low levels of physical activity and high-energy diets are contributing factors to the global development of type 2 diabetes²⁸. Obesity, particularly abdominal obesity, promotes the development of insulin resistance and the “metabolic syndrome” (or insulin resistance syndrome) and ultimately, type 2 diabetes^{3,29,30}. There are several existing definitions of the syndrome. The individuals who fulfill the criteria are generally characterized by insulin resistance, obesity, hypertension, dyslipidemia, glucose intolerance, inflammation and atherosclerosis. Approximately one quarter of the population of the developed world, per definition, are believed to have the metabolic syndrome^{30,31}. Other environmental factors contributing to an increased risk of developing type 2 diabetes are smoking and psychosocial stress^{32,33}.

1.5 INSULIN RESISTANCE

1.5.1 General background

Insulin resistance is defined as a decreased ability of insulin to produce its normal biological effects at circulating concentrations that are effective in healthy subjects³⁴. Insulin resistance leads to both increased hepatic glucose output, and lipolysis, and to decreased peripheral glucose uptake after meals³⁴. Insulin resistance is generally defined on the basis of high levels of plasma insulin, either fasting or during a

glucose tolerance test, or by a decreased rate of glucose disappearance during a hyperinsulinemic-euglycemic clamp³⁵.

1.5.2 Insulin resistance and risk factors for vascular disease

Insulin resistance is a common finding in patients with type 2 diabetes³⁶, and may also be present in otherwise healthy subjects with normal glucose tolerance³⁷. In both groups insulin resistance correlates with low physical fitness and abdominal obesity³⁵. Central obesity plays a special role on development of insulin resistance by causing free fatty acid (FFA) levels to increase in the portal and peripheral circulations. Increases in plasma FFA levels inhibit insulin-stimulated glucose utilization and glycogen synthesis in human skeletal muscle^{38,39}. Dyslipidemia associated with insulin resistance comprises reduced high-density lipoprotein (HDL)-cholesterol and raised triglyceride concentrations, as well as a preponderance of small, dense low-density lipoprotein (LDL) particles. These lipid alterations predispose strongly to coronary heart disease⁴⁰.

Hypertension is a common feature in individuals with type 2 diabetes⁴¹. Insulin resistance is associated with hypertension due to activation of the sympathetic nervous system, renal sodium retention and arterial wall thickening^{42,43}. More than 50% of subjects with essential hypertension are insulin resistant⁴⁴.

1.5.3 Insulin resistance, exercise and prevention of diabetes

There is evidence that diminished physical fitness strongly correlates with insulin resistance^{35,45}. Numerous observational and prospective studies indicate that physical inactivity increase the risk of developing type 2 diabetes⁴⁶⁻⁴⁸. Large studies also show that low physical activity level and low aerobic fitness predict increased risk of overall and CVD mortality in people with type 2 diabetes⁴⁹.

Life-style modification programs that include diet and/or exercise interventions significantly reduce the incidence of type 2 diabetes among subjects with IGT^{12,13,50,51}. The molecular mechanisms behind the beneficial effects of exercise on glucose homeostasis are incompletely understood⁵².

1.5.4 Cellular and molecular mechanisms behind insulin resistance in skeletal muscle

During normal conditions in skeletal muscle, insulin affects intracellular functions after binding to its receptor by activating tyrosine kinase⁵³. In short, this results in the phosphorylation of insulin receptor substrate 1 (IRS-1) and activation of phosphatidylinositol 3-kinase (PI3-K) (Fig.1). Glucose uptake is facilitated through translocation of glucose transporter 4 (GLUT-4) from an intracellular pool to the cell surface.

It has been proposed that diminished insulin mediated activation of receptor tyrosine kinase depend on an altered intramuscular lipid metabolism^{54,55}. In the presence of increased concentrations of long chain fatty acyl CoA (LCFA CoA), the insulin signaling cascade will be impaired due to increased diacylglycerol (DAG) levels. This leads to an activation of protein kinase C (PKC) isoforms in muscle which in turn promotes the serine phosphorylation of IRS-1 with a decreased insulin sensitivity as a result⁵⁴⁻⁵⁷.

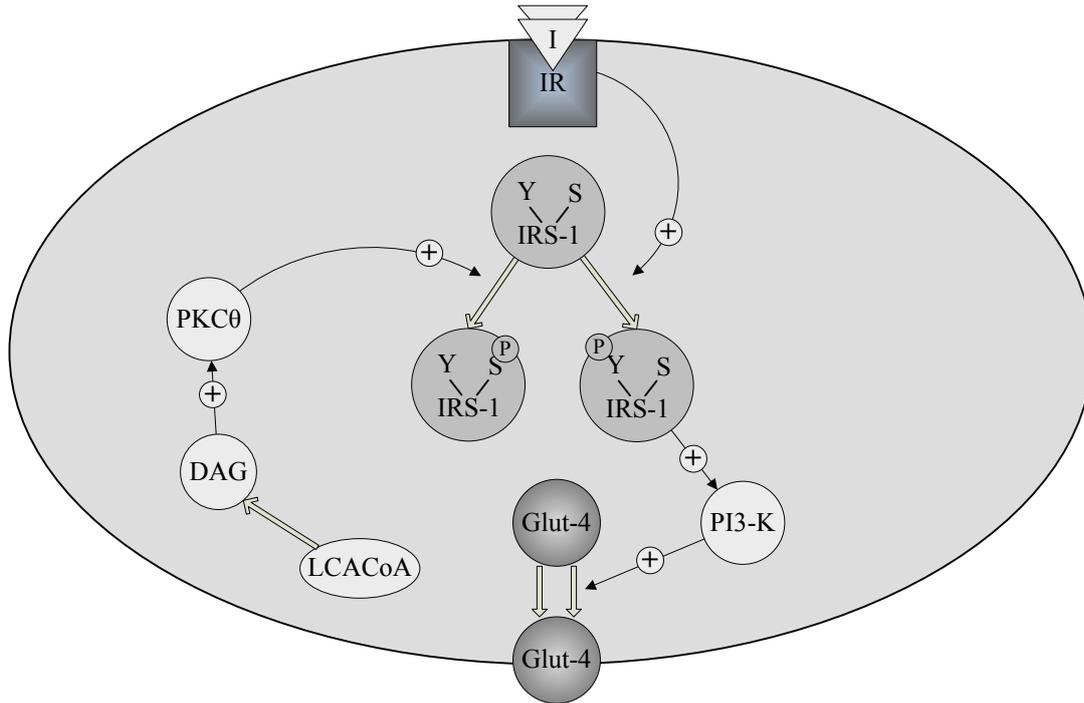


Fig.1 Insulin signaling and glucose uptake in skeletal muscle.

During normal conditions, insulin (I) binds to its receptor (IR) which causes phosphorylation of the IRS-1 on a tyrosine residue which in turn activates phosphatidylinositol 3-kinase (PI3-K). Glut-4 translocates to the membrane which in turn leads to glucose uptake.

During conditions of increased levels of LCFA CoA in the cytosol, the insulin signaling cascade follows an abnormal pathway. Increases in LCFA CoA, generates higher levels of diacylglycerol (DAG) which in turn activates PKC and promote phosphorylation of IRS-1 on a serine residue which results in impaired signaling and decreased glucose uptake.

Data from insulin-resistant rodents have demonstrated a link between concentrations of LCFA CoA (and other lipid metabolites, such as DAG) and level of malonyl Coenzyme A (malonyl CoA) in the cytosol and activation of protein kinase C^{56,58,59}. Malonyl CoA is a key molecule for the regulation of mitochondrial fatty acid oxidation (β -oxidation) and lipid partitioning and is both an intermediate in the de novo synthesis of fatty acids and an allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT 1) (Fig. 2). In skeletal muscle, its role in the fatty acid synthesis is minimal. An increase in malonyl CoA inhibits CPT 1 and decreases the transport of LCFACoAs into the mitochondria where they are oxidized⁶⁰, which results in an accumulation of LCFA CoA in the cytosol⁶¹. Studies in animals^{58,62,63} and humans⁶⁴ show that disturbances in the regulation of malonyl CoA are associated with insulin resistance and obesity.

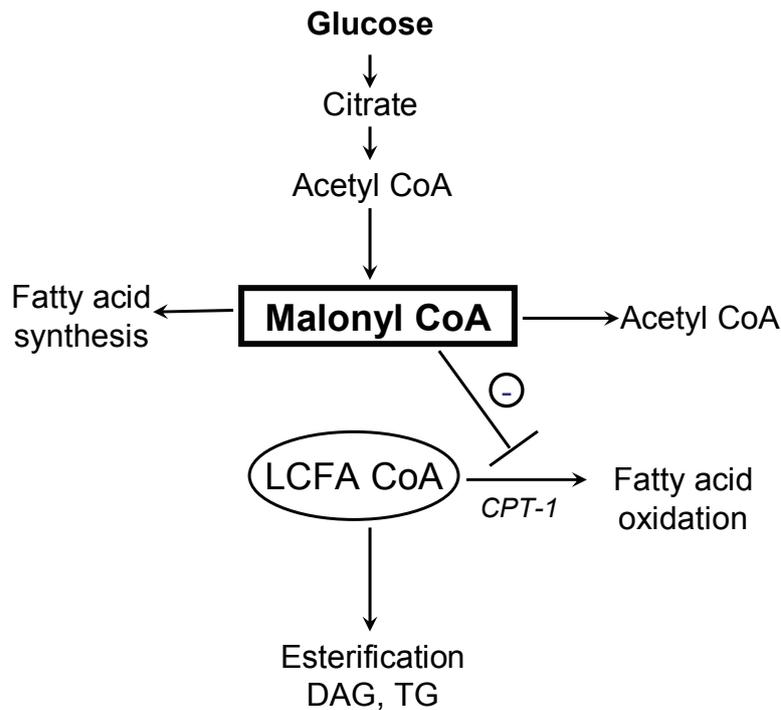


Fig. 2 Malonyl CoA is a key molecule for the regulation of fatty acid oxidation and lipid partitioning and is both an intermediate in the de novo synthesis of fatty acids and an allosteric inhibitor of carnitine palmitoyltransferase 1 (CPT 1). In skeletal muscle its primary role is inhibition of CPT1 and fatty acid oxidation.

1.5.5 Regulation of malonyl CoA in muscle

The formation of malonyl CoA is acutely regulated by changes in the activity of acetyl CoA carboxylase (ACC), the enzyme that catalysis malonyl CoA synthesis from acetyl CoA. ACC activity is regulated by both changes in the concentration of citrate and an AMP-activated protein kinase (AMPK) mediated mechanism (Fig. 3). Citrate is both an allosteric activator of ACC and a source of its precursor, cytosolic acetyl CoA. The concentration of malonyl CoA in skeletal muscle respond to acute changes in cellular fuel availability and energy expenditure⁶⁵. Increases in malonyl CoA have been observed in muscle after provision of glucose, such as occurs during an euglycemic-hyperinsulinemic clamp⁶⁴ or refeeding after a fast⁶⁶. In contrast, exercise (or starvation) decreases the concentration of malonyl CoA by activating AMPK which phosphorylate and inhibit ACC⁶⁷⁻⁷⁰. During these conditions decreases in malonyl CoA concentration increases the activity of CPT 1 which in turn promotes the transfer of LCFA CoA from the cytosol into the mitochondria where they are oxidized (Fig 3)⁶⁰. In addition, exercise-mediated increases in AMPK may also affect the glucose metabolism by increasing the activity of phosphofructokinase-2 (PFK-2) and thereby glycolysis⁷¹ (Fig 3). It has been suggested that the concentration of malonyl CoA in skeletal muscle also might be regulated by AMPK-induced increases in the activity of malonyl CoA decarboxylase (MCD), an enzyme that degrades malonyl CoA to acetyl CoA⁷²⁻⁷⁴ (Fig. 3). It has been reported in studies in rats that AMPK can acutely phosphorylate and activate MCD^{72,73}, but this has been questioned by others⁷⁵.

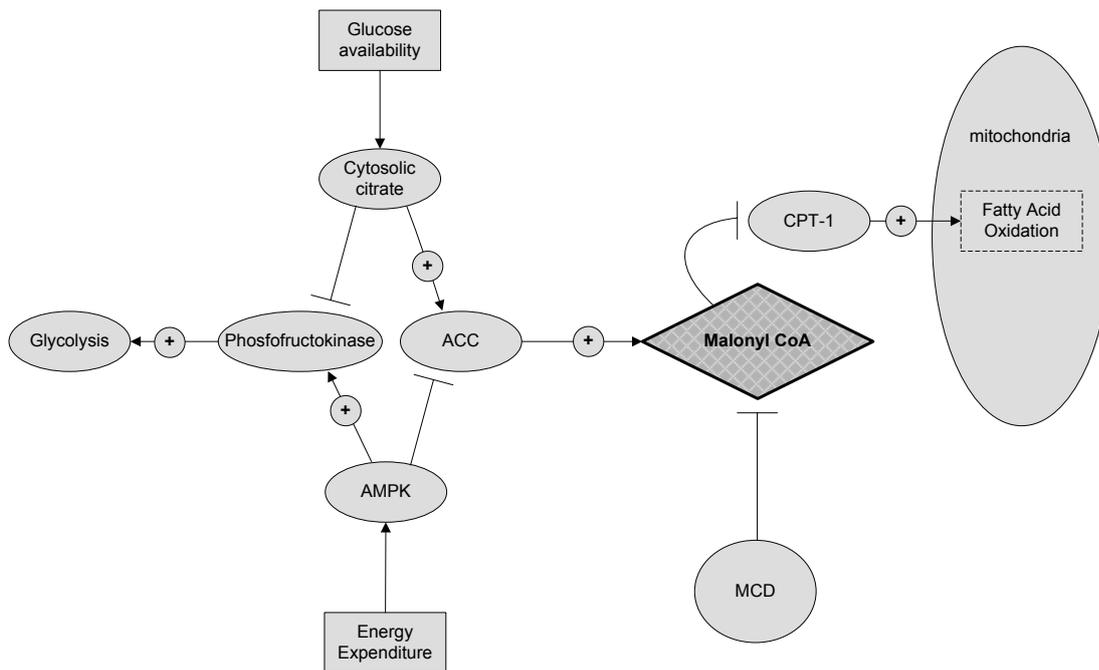


Fig. 3 Regulation of malonyl CoA levels in muscle

1.5.6 Acute exercise and regulation of malonyl CoA in muscle

In the beginning of 1970 it was shown that well-trained athletes had lower plasma insulin levels and improved glucose tolerance ⁷⁶. It was later shown that physical training increases insulin-mediated glucose uptake in human muscle and that high insulin sensitivity in well-trained athletes decreases 2-3 days after the last bout of exercise ⁷⁷. In parallel, it was demonstrated in rat muscle that an increased translocation of GLUT-4 from an intracellular location to the cell-membrane constitutes the molecular basis for increased insulin sensitivity in this situation ⁷⁸. Today there is evidence for separate pools of GLUT-4 that mobilize during physical training ⁷⁹.

Acute exercise (muscle contraction) increases glycogenolysis in muscle. Immediately after exercise there is a compensatory increase in glucose uptake and synthesis of glycogen in the muscle. The latter results from an increased activity of glycogen synthase ⁸⁰ (Fig 4). It has been suggested that low glycogen depots in itself generate an increased pool of cytosolic glucose transporters, GLUT-4, which transport glucose into the cell ⁸¹. Decreased glycogen depots also increases the intracellular quotes of AMP/ATP and creatine/phosphocreatine, which in turn increases AMPK activity and stimulates PFK-2 and thereby glycolysis ⁷¹ (Fig 3,4). Activation of AMPK also plays a central role in translocation of GLUT-4 during acute exercise. This can be shown by the administration of an activator of AMPK, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), that increases insulin sensitivity through increased expression

and translocation of GLUT-4^{82,83}. This effect does not involve early steps in insulin-signaling which indicate that AMPK increases insulin uptake independent or possibly by down-stream PI3-kinase⁸⁴.

Acute exercise increases the translocation of GLUT-4 to the cell membrane by an insulin independent mechanism^{85,86}. Several studies indicate that this process is related to activation of intracellular signaling by mitogen-activated kinase (MAP-kinase), especially p38 MAP-kinase, but also as a result of acute activation of AMPK⁸⁷ (Fig. 4). Muscle contraction may also increase insulin sensitivity and thus enhance glucose uptake through rapid activation of AMPK. As discussed above, this leads to decreased activity of ACC and increased activity of MCD resulting in decreased malonyl CoA levels and increased fatty acid oxidation rates (Fig 4).

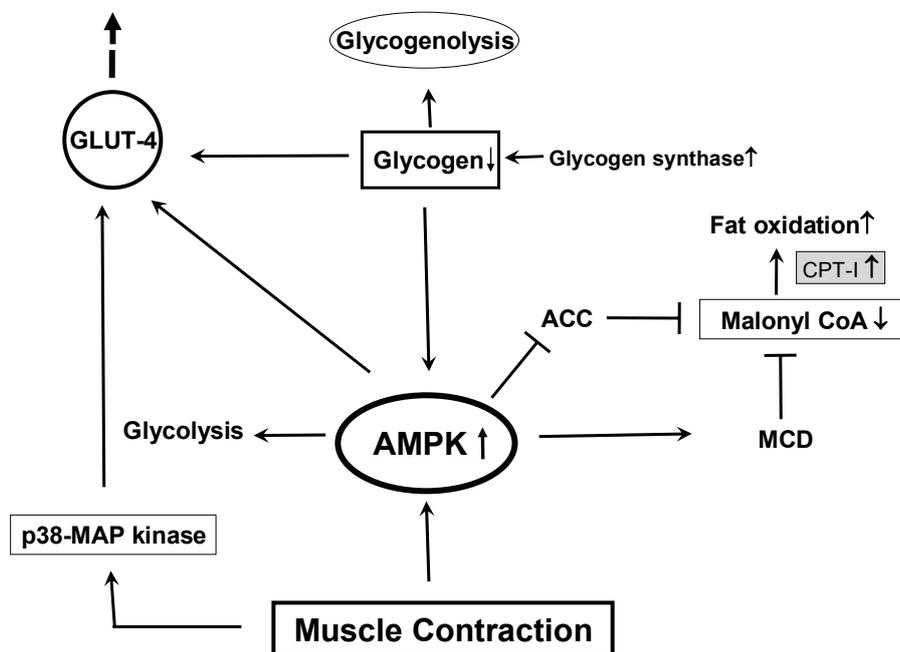


Fig. 4 Glucose uptake during muscle contraction.

1.5.7 Insulin resistance and long-term exercise training

During long-term exercise training AMPK activation is essential for mitochondrial biogenesis and adaptation of the oxidative capacity in muscle (Fig.5)⁸⁸. This effect is possibly mediated through an up-regulation of calcium/calmodulin-dependent protein kinase-IV (CaMK-IV) and peroxisome proliferator activated receptor (PPAR) γ coactivator-1 α (PGC1- α), which are key-factors in the regulation of mitochondrial biogenesis. CaMK-IV and PGC1- α are important in the context of long-term exercise training since they induce transformation of fast to slow, more insulin sensitive, muscle-fibers⁸⁹.

It has recently been shown that insulin resistance is associated with down regulation of genes that are involved in the regulation of mitochondrial function, i.e. oxidative phosphorylation^{90,91}. These abnormalities are associated with down-regulation of PGC1- α ^{90,91} that has been linked to the mediation of MCD expression by co-activation of PPAR α ^{92,93}. Furthermore, it has also been suggested that insulin resistance might depend on an inherited mitochondrial dysfunction⁹⁴ and it is unclear if these mitochondrial defects are reversible or not by exercise training.

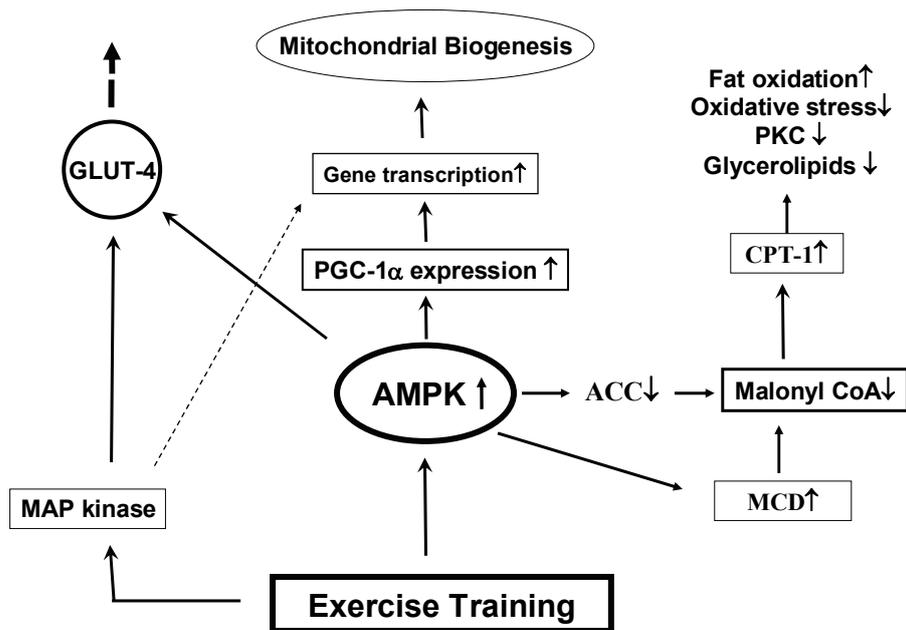


Fig. 5 Glucose uptake and insulin sensitivity in skeletal muscle after exercise training

During long-term exercise training repeated activation of AMPK may also increase insulin sensitivity through both ACC and MCD dependent mechanisms and by GLUT-4 translocation. The latter may occur either directly by activated AMPK, or through activation of intracellular signaling by MAP-kinase dependent pathways (discussed above).

Physical activity thus corrects the fundamental defects of insulin resistance, i.e. an increase in malonyl CoA decreases fat oxidation and facilitates an accumulation of LCFA CoA and DAG in the cytosol. This in turn leads to activation of PKC isoenzymes, increases in ceramide synthesis, increased activation of NF κ B-system, and oxidative stress, all of which lead to insulin resistance and, in some tissues, mitochondrial damage⁹⁵.

2 AIMS OF THE STUDY

To study the impact of sex and type 2 diabetes heredity on the prevalence and pathogenesis of early abnormalities of glucose metabolism in healthy subjects

To study impact of exercise training on phenotype characteristics in people with or without type 2 diabetes

To study impact of exercise training on cellular and molecular mechanisms regulating insulin sensitivity in muscle in people with or without type 2 diabetes

3 SUBJECTS AND METHODS

Subjects

Paper I. The study consisted of 3,128 men and 4,821 women, aged 35–56 years, with or without family history of diabetes (FHD), and without previously diagnosed type 2 diabetes. All subjects were living in five municipalities situated in the outskirts of Stockholm and belonged to the Stockholm Diabetes Prevention Program (SDPP)^{32,96-98}. The data collection was performed in two steps. A questionnaire was sent to all inhabitants in the relevant age group asking about country of birth, presence of type 2 diabetes or heredity for the disease. The questionnaire was returned by 79% (10,236 of 12,952) of the men and 85% (16,481 of 19,416) of the women. Individuals with known type 2 diabetes or who had foreign origin or who provided incomplete answers, or presented insufficient FHD, were excluded from the study (4,801 men and 8,178 women). Among remaining participants, all subjects with FHD, defined as at least one first-degree relative (mother, father, sister or brother) or two second-degree relatives (grandparents, uncles or aunts) with diagnosed type 2 diabetes (2,106 men and 3,583 women) were invited to participate in a health examination at a primary health care centre. Of the responders, 20% of the men and 24% of the women had FHD. An age-stratified sample of subjects without FHD, 2,424 men and 3,497 women (together with 424 women reporting gestational diabetes), were also invited to participate in the examination. In total, 3,162 (70%) men and 4,946 (66%) women participated in the examination. After the examination, additionally 33 (1%) men and 129 women (2%) were excluded due to uncertain FHD or foreign origin or to breast-feeding, pregnancy, certain medication. The final sample consisted of 3,128 men and 4,821 women.

Paper II. Thirteen Swedish Caucasian men with mild type 2 diabetes and 17 matched control subjects participated in the study. Duration of diabetes was less than 2 years. The two study groups were strictly matched by age, body mass index (BMI) and reported similar physical activity level. None of the subjects participated in any regular exercise program. Individuals who had a BMI between 23 and 32 kg/m² were considered eligible for the study. None of the subjects had chronic diseases (except diabetes); none were on lipid-lowering, anti-diabetic, or antihypertensive medications; and none had a resting blood pressure in excess of 160/95 mm Hg. None of the control subjects had a family history of diabetes. All diabetic patients had been given dietary information before the studies.

Paper III. Eleven Swedish Caucasian subjects (seven women and four men) participated in the study. The individuals had BMI between 22 and 32 kg/m². None of the subjects participated in any regular exercise program with more than one training session per week and they were free of chronic disease. Three subjects did not complete the study, one because of an injury of the ankle joint and two because of poor compliance with the training program. Compliance to the training sessions was 86%.

Paper IV. The study included twelve Swedish Caucasian patients with type 2 diabetes (seven men and five women). They were on diet treatment alone and had BMI between

23 and 32 kg/m². None of the study subjects participated in a regular exercise program or had a chronic disease (except diabetes). One individual was on anti-hypertensive and one on lipid-lowering medication. None had a resting blood pressure in excess of 150/95 mmHg. Compliance to the training sessions was 92%.

Methods

3.1 PAPER I

Study protocol. The participants visited the primary health care centre, where they filled out an extensive questionnaire that included questions on birth weight, physical activity, dietary habits, health status, alcohol and tobacco use and psychosocial conditions. Body height, weight and waist circumference were measured with the subjects wearing light indoor clothes and no shoes. BMI was calculated as the ratio of body weight in kilograms to the square of height in meters (kg/m²).

Criteria for abnormalities of glucose metabolism. A standard 75-g oral glucose tolerance test (OGTT) was administered to the subjects¹⁴. Criteria for IFG was a fasting plasma glucose level of ≥ 6.1 to < 7.0 mmol/l and a 2-h plasma glucose level at OGTT < 7.8 mmol/l; for IGT a fasting plasma glucose level < 6.1 mmol/l and a 2-h plasma glucose level at OGTT of ≥ 7.8 to < 11.1 mmol/l; and for CGI a fasting plasma glucose level ≥ 6.1 and < 7.0 mmol/l and 2-h plasma glucose level at OGTT ≥ 7.8 and < 11.1 mmol/l¹⁵. Type 2 diabetes was defined as having a fasting plasma glucose value ≥ 7.0 mmol/l and/or a 2-h plasma glucose value at OGTT ≥ 11.1 mmol/l^{14,15}.

Calculations for insulin resistance and β -cell function. The homeostasis model assessment (HOMA) was used to assess insulin sensitivity and beta cell function, based on fasting insulin and glucose levels and according to published algorithms: HOMA resistance = $(\text{insulin} \times \text{glucose}) / 22.5$, and HOMA beta cell function = $20 \times \text{insulin} / (\text{glucose} - 3.5)$ ⁹⁹.

Plasma analyses. Plasma glucose was assayed in duplicate using a glucose oxidase method with a Yellow Spring Glucose Analyzer (Yellow Springs, Inc., Yellow Springs, OH, USA). Insulin was assayed by RIA, using our own antibodies, human insulin as a standard, and charcoal addition to separate antibody-bound and free insulin¹⁰⁰. Proinsulin (PI) cross-reacts in this assay by about 80%. PI was determined in men by an ELISA with a broad specificity comprising, in addition to intact PI, the four PI conversion intermediates, reacting with 65–99% efficiency relative to intact PI. The limit of detection was 0.25 pmol/l, and the total interassay coefficient of variation was 7.6–5.3% at 1.1–8 pmol/l.

3.2 PAPER II-IV

Study protocol and metabolic investigations. In paper II, metabolic investigations were performed after a 12-h fast and started at 8 a.m. All subjects were free of symptoms of infectious disease during the preceding 4 weeks. The participants were asked to maintain their normal physical activity and diet during the 3 days prior to the studies. All clinical tests and metabolic investigations in each subject were generally performed

within a 4- to 6-week period and were evenly distributed over the year. They were never performed the first day after a weekend or holiday.

In paper III-IV, all subjects were investigated before and after a 12 week program of combined aerobic and dynamic strength training (Fig. 6). Metabolic investigations were performed at 8 a.m. after a 12-h overnight fast (baseline). After the training period, the insulin clamp studies and muscle biopsies were performed 24-36 h after the last bout of exercise. The subjects were instructed not to engage in any physical activity after the last bout of exercise, i.e. during the 24-36 hours prior to the biopsy and they were instructed to eat their usual balanced diet during this time. They were reminded of this individually, as well as during three separate group meetings, one before entering the study, a second during the course of study and the third before final examinations. Other clinical tests and metabolic investigations were generally performed within a week or on the same day as the clamp study. They were never performed the first day after a weekend or holiday.

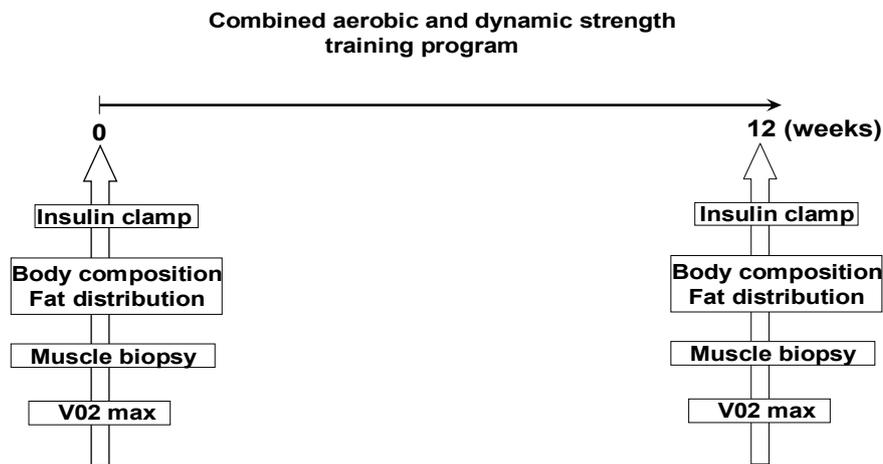


Fig. 6 Study protocol for paper III and IV

Oral glucose tolerance test (OGTT) and plasma analyses. In paper II, an oral glucose tolerance and plasma insulin were assessed over 120 min during a 75-g OGTT¹⁴. The insulin response was calculated as the incremental insulin area (above fasting insulin concentration) between 0–30 and 0–120 min.

Plasma glucose was determined by the glucose oxidase method (see paper I), and plasma insulin¹⁰¹ and C-peptide¹⁰² were determined by RIA using an antibody developed in this laboratory and a commercial kit (Novo Nordisk A/S, Bagsværd, Denmark), respectively. Interassay and intra-assay coefficients of variation were, respectively, less than 3.9% and less than 3.1% for insulin, and 4.5% and 3% for C-peptide. Cross-reactivity with PI was 100% in the insulin assay and approximately 80% in the C-peptide assay. Very low-density lipoprotein, low-density lipoprotein, and high-density lipoprotein were determined by a combination of preparative ultracentrifugation and precipitation¹⁰³. Plasma nonesterified fatty acids (i.e. FFAs) were determined using a commercially available kit (Wako Pure Chemical Industries Ltd., Richmond, VA).

Hyperinsulinemic euglycemic clamp. In paper II, sequential two-step hyperinsulinemic euglycemic clamps and in paper III and IV, one-step hyperinsulinemic euglycemic clamps were performed after an overnight fast. At the onset of the experiments, a basilic vein of each arm was cannulated, one for sampling and the other for infusion.

In sequential two-step hyperinsulinemic euglycemic clamps a third catheter was introduced into the cephalic vein of the arm used for infusions, for continuous sampling of arterialized blood for glucose measurement by a Biostator (Glucose-Controlled Insulin Infusion System, Miles Laboratories, Inc., Life Science Instruments, Elkhart, IN). The two levels of hyperinsulinemia of 150-min duration each were induced by iv infusion of 0.25 and 1.0 mU/kg body weight•min human rapid-acting insulin (Actrapid, Novo Nordisk A/S, Bagsværd, Denmark; 0.2 IU/ml with 4 mg/ml of human albumin in saline). Euglycemia was maintained by using the Biostator, which calculated glucose infusion rate from the reading of blood glucose measurements during the previous 4 min according to an algorithm¹⁰⁴. Potassium (0.15 mmol/g of glucose) was added to the infusate.

In paper III and IV one-step hyperinsulinemic euglycemic clamps were performed. During the first ten minutes insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was infused at decreasing rates and in a stepwise manner and adjusted for body weight and then at 1.0 mUkg⁻¹min⁻¹ for 110 min. Glucose (20%) was simultaneously infused intravenously at a variable rate to maintain the blood glucose concentration at 4.8 mmol/l¹⁰⁵. Blood glucose was analyzed every five minutes.

Indirect calorimetry. The Deltatrac II Metabolic Monitor (Datex-Ohmeda, Helsinki, Finland) was used to measure oxygen consumption and carbon dioxide production and from these data the respiratory quotient (RQ) and the rates of fatty acid and glucose oxidation were calculated¹⁰⁶. For this purpose, 45 min before the two insulin infusion periods (paper II), a transparent plastic hood was placed over the subject's head for 30 min to determine O₂ consumption and CO₂ production. In paper III, the measurement was performed 30 min before the clamp and 30 min at the end of the clamp. Timed samples of urine were collected for analysis of urinary urea excretion, and from this, changes in urea pool size were calculated to correct for amino acid oxidation¹⁰⁷.

Body composition. Lean body mass (LBM), total body fat, and total truncal (abdominal and thoracic regions) fat mass were calculated using DXA (Lunar DPX-L x-ray bone densitometer, version 1.3Z, Lunar Corp., Madison, WI). Computerized tomography (Siemens Somatom Plus, Siemens Corp., New York) was used to determine intra-abdominal fat mass. All subjects were examined in the morning prior to the insulin clamp. One 10-mm slice was exposed and examined at the level of the upper part of the iliac crest, which coincided with the disc between the fourth and fifth lumbar vertebrae and the umbilicus. A density range between -150 and -40 Hounsfield units was used to define fat mass. Total abdominal adipose tissue (subcutaneous plus intra-abdominal fat mass, measured in square centimeters) in this interval was calculated by the computer. The intra-abdominal cavity, including the retroperitoneal space, was outlined, and the area was estimated separately. Subcutaneous fat mass was calculated as the difference between total and intra-abdominal fat mass area. Total adipose tissue in this interval was calculated by the computer.

Physical fitness. Maximal oxygen consumption (VO_{2max}) was determined during an exercise test performed on an electrically braked cycle ergometer. In paper II, after a

short period of exercise at 30 W, the load was increased in a stepwise manner by 30 W every minute until exhaustion or dyspnea. In paper III and IV, the initial resistance was set at 40 W/ 50 W (females/males) and was increased stepwise every minute by 10 W/ 20 W¹⁰⁸. Subjects reported their rate of perceived exertion (RPE) according to Borg's RPE-scale at the end of every second step increase¹⁰⁹. The test was terminated due to dyspnea or exhaustion, after attaining an RPE-score > 15 of maximum 20. A 12-lead electrocardiogram was recorded during the test. Expired gases were measured for oxygen content, and calculations were performed by a Jaeger Oxycon computer software program (Jaeger, Hoechberg, Germany).

Exercise training. All subjects participated in a combined aerobic and dynamic strength training group program three times a week over a period of three month. As described previously¹¹⁰, each training session lasted 50 minutes and included warm up (6 minutes), jogging (4 minutes), flexibility exercises (stretching) (4 minutes), strength training of the arms and back (4 minutes), abdomen (4 minutes), and large muscle groups (3 minutes). This was followed by jogging (3 minutes), flexibility exercises (3 minutes), strength exercises of large muscle groups (7 minutes), jogging (3 minutes), further strength exercises of large muscle groups (4 minutes), flexibility exercises, stretching and cool down (5 minutes)¹¹⁰. The lower extremities (including the vastus lateralis muscle) were active during at least two thirds of the training session. A specialized physiotherapist supervised all of these activities. To increase adherence to the program, four training sessions at different times during the week were offered. Two specific exercise targets were used during the program: a) An exercise intensity of >50% of the individuals maximal exercise capacity (based on the maximal heart rate reached during the baseline exercise test), maintained for at least 40 minutes. b) An exercise intensity >80% of the individuals maximal exercise capacity during three periods of 3-4 minutes each, engaging large muscle groups for training and adaptation of the central circulation¹¹¹. The intensity of exercise during the training program was individually adjusted and based on the performance at the baseline exercise test. The subjects were encouraged to reach an exertion of 13-15/20 rated on Borg's RPE-scale to obtain the exercise target of > 80 % of maximal working capacity during the more intense parts of the training program, and 9-11/20 on the RPE-scale (corresponding to > 50% of maximal exercise capacity) during the remainder of the session. The complete program was supported by music, which guided the intensity of the performance during the session. The training sessions were followed by 10 minutes of relaxation, also supported by music. To ascertain that the target exercise intensity was reached, heart rate was assessed at one of the three weekly training sessions during weeks 1 and 12 of the training period with a portable heart rate recorder, (Sport Tester, Polar Electro Oy, Kempele, Finland). The subjects also rated their perceived exertion using the RPE-scale during the more intense parts of the program.

Muscle biopsies. In paper II, muscle biopsies (75–100 mg) were obtained from the vastus lateralis portion of the quadriceps femoris muscle using a Weil-Blakesley's conchotome¹¹². After anesthetic (Xylocaine (lidocaine) 10 mg/ml) was given and a small incision (1 cm) was made three biopsies were taken from each individual from the same area of the vastus lateralis: one at the end of the equilibration period, and the others at the end of the low-dose and high-dose insulin infusions. In paper III and IV, open muscle biopsies were taken by a trained surgeon. The initial muscle biopsy was

obtained immediately after the indirect calorimetric studies and prior to the insulin infusion, both before and after training. After instillation of 5-15 ml of prilocain (Citanest[®]) 5 mg/ml for local anaesthesia of the skin and subcutaneous tissue, an open biopsy of the vastus lateralis muscle (approximately 1g) was performed through a 4 cm incision approximately 12 cm above the knee joint, as described previously¹¹³. After closure of the fascia and skin, the insulin infusion was started. The second muscle biopsy was done through the same incision immediately after the insulin infusion was stopped. The tissue was taken from an area close to, but distinct from, the muscle biopsy taken during the first biopsy. All tissues were immediately frozen (within seconds) in liquid nitrogen and stored at -70 C for further analysis. Citrate and malate were determined by standard spectrophotometric methods¹¹⁴. For measurement of malonyl CoA, muscle was homogenized and deproteinized with 10% perchloric acid, and the filtrate neutralized as described previously¹¹⁵. Malonyl CoA was determined radioenzymatically in the neutralized filtrate by a modification of the method of McGarry et al^{65,116}. Malonyl CoA decarboxylase was assayed spectrophotometrically as described previously⁷². The measurement of DAG content was determined as described earlier¹¹⁷. Lipids were extracted from biopsies of muscle using chloroform:methanol:PBS + 0.2% SDS (1:2:0.8). Diacylglycerol kinase and (γ -³²P)ATP (15 μ Ci/ μ mol cold ATP) were added to extracts, and the reaction was stopped using chloroform:methanol (2:1). Samples were run on thin-layer chromatography plates in chloroform:acetone:methanol:acetic acid:water (100:40:20:20:10). The DAG bands were counted in a Beckman LS 6500 (Beckman Instruments). When measuring peroxisome proliferator activated receptor (PPAR) γ coactivator-1 α (PGC-1 α) protein, fifty micrograms of crude muscle homogenate was electrophoresed and transferred to poly (vinylidene difluoride) membrane (Bio-Rad). After transfer, the membranes were blocked with 5% BSA in TBS (25 mM Tris/135 mM NaCl/2. MM KCl)/0.05% Tween 20 (TBST) for 1 h at room temperature. The membrane were incubated with PGC-1 antibody purchased from CALBIOCHEM (San Diego, CA, USA) and then with secondary antibodies conjugated to horseradish peroxidase from Amersham Pharmacia. Bands were visualized by enhanced chemiluminescence and were quantitated by laser densitometry.

The measurement of AMPK and ACC phosphorylation and protein was determined by homogenize freeze-clamped muscle samples as previously described and lysates were used for Western blotting¹¹⁸. For Western blotting analysis, proteins (40 μ g) from the lysates were separated by 8% SDS-PAGE and transferred to nitrocellulose membranes. After blocking the membranes with 2.5% BSA/TBST, they were probed overnight at 4°C with antibodies against phospho-Thr¹⁷² AMPK (1:1000, Cell Signaling, Beverly, MA), anti-phospho-acetyl CoA carboxylase (ACC) (recognizes both the Ser⁷⁹ of ACC-1 and Ser²¹⁸ of ACC-2) (1:1000, Upstate, Waltham, MA), anti-AMPK α 2 (raised against the sequence CMDDSAMHIPPGLKPH-NH₂ of α 2)¹¹⁸, anti-AMPK α 1/ α 2 (raised against the sequence CAEKQKHDGRVKIGHY-NH₂ of both α 1 and α 2), or streptavidin for ACC detection (2 μ g/ml, Roche, Indianapolis, IN). Bound antibodies were detected using anti-rabbit immunoglobulin-horseradish peroxidase-linked antibody and ECL reagents (NEN Life Science Products, Boston, MA). The bands were quantified using ImageQuant software (Molecular Dynamics, Inc).

Gene expression. Muscle biopsies were homogenized in Trizol. Total RNA was purified with RNeasy (Qiagen). Expression of MCD and PGC-1 α were determined

using two-step real-time quantitative PCR (ABI Prism 7700, Applied Biosystems). Total RNA was treated with DNase I; cDNA was synthesized using random hexamer primers (Advantage, BD Biosciences). Primer and probe sequences were selected using Primer Express. Target gene and endogenous control amplicons were labeled with FAM and VIC, respectively. Expression was normalized to that of cyclophilin. Primer and probe sequences are available on request.

Statistical analysis. In paper I, data are presented as means \pm SEM or geometric mean (SE range). Plasma glucose, plasma insulin, HOMA values and plasma PI were log-transformed to improve skewness. Analysis of variance was used, and when controlling for covariates, analysis of covariance, both followed by Scheffé's post-hoc test, was performed to analyze differences in continuous variables between groups. Systolic and diastolic blood pressure, fasting and 2-h insulin values, HOMA measures, PI and PI insulin ratios are presented after adjustment for age, FHD, waist circumference, sex and when appropriate also for current smoking, physical activity at work and during leisure time. When a significant interaction term between glucose tolerance groups and age or waist circumference was found results are presented with age or waist circumference at their means. Multiple logistic regressions were performed to calculate prevalence odds ratios (POR), accompanied by 95% CI. Including them one by one in the logistic regression model tested for potential confounders. They were retained in the model if they contributed to at least a 10% change of the age-adjusted (age in three categories 35–42, 43–50, 51–56 years) crude estimate. Statistical significance was set at $p < 0.05$.

In paper II-IV, all values are presented as means \pm SEM. Logarithmic transformation was performed on all skewed variables to obtain a normal distribution before statistical computations and significance testing were undertaken. In paper II, a Student's unpaired *t* test was used to evaluate statistical differences when two sets of data were compared. A paired *t* test was used to determine the significance of the stepwise increase in malonyl CoA, citrate, and malate levels during clamps, and repeated measurements. ANOVA was used to assess the significance of the increase in carbohydrate oxidation and the decrease in fatty acid oxidation. Scheffé test was used as the post hoc test. Regression analysis was performed to identify variables that correlated with M-values or VO₂ max (adjusted for age). Multiple stepwise linear regression analysis was used to study independent determinants of M-values. The models have been checked by inspection of residuals. Partial correlation coefficients were calculated using age as a forced variable in the equations.

In paper III-IV, a paired *t* test was used, to compare first, parameters measured during basal conditions before and after training; second, parameters measured before and after insulin infusion during the clamps; and third, insulin-stimulated samples before and after training. The non-parametric Mann-Whitney U test was used for skewed variables that did not obtain a normal distribution after logarithmic transformation. Statistical significance was set at $p < 0.05$.

Ethical considerations. The experimental protocols in all studies were approved by the Ethics Committee at the Karolinska University Hospital and were carried out in accordance with the Declaration of Helsinki. All participants received written and oral information regarding the nature and potential risks of the studies and gave their informed consent.

4 RESULTS

4.1 PAPER I

Table 1 shows influence of sex and diabetes heredity on the prevalence of glucose abnormalities in 3,128 men and 4,821 women that were investigated in paper I.

In total, 90.6% men and 94.4% women had normal glucose tolerance (NGT). The corresponding figures for IFG were 1.9% men and 1.0% women, and for IGT 4.6% men and 3.0% women, and for CGI 0.8% men and 0.3% woman, and finally 2.1% men and 1.3% woman had type 2 diabetes.

The prevalence of having abnormalities of glucose metabolism was approximately two to three times higher in individuals with FHD. In total, 12.3% of men and 7.2% of women with FHD had disturbances of glucose metabolism, whereas the corresponding figure for men without FHD was 6.2% and for women 3.8%.

The prevalence of having abnormalities of glucose tolerance was approximately two-fold higher in men compared to women. There was a partial overlap between IFG and IGT. In men, 15% with IGT also had IFG and 31% with IFG had IGT. The corresponding figures for women were 9 and 24%, respectively. The prevalence of type 2 diabetes was similar in both sexes in subjects without FHD. Importantly, in subjects with FHD the prevalence was nearly two-fold higher in men than in women.

Table 1. Glucose tolerance in subjects with or without heredity for type 2 diabetes

	Sex	FHD positive		FHD negative	
		Number	%	Number	%
NGT	Male	1,422	87.7	1,413	93.8
	Female	2,398	92.8	2,152	96.2
IFG	Male	38	2.3	21	1.4
	Female	33	1.3	15	0.7
IGT	Male	91	5.6	52	3.5
	Female	95	3.7	50	2.2
CGI	Male	19	1.2	7	0.5
	Female	12	0.5	3	0.1
DM 2	Male	51	3.1	14	0.9
	Female	45	1.7	18	0.8
Total	Male	1,621	100.0	1,507	100.0
	Female	2,583	100.0	2,238	100.0

CGI, combined glucose intolerance; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; NGT, normal glucose tolerance; DM 2, type 2 diabetes; FHD positive, with family history of type 2 diabetes; FHD negative, without family history of type 2 diabetes

Impact of parental diabetes heredity on the prevalence of glucose abnormalities. The prevalence odds ratio (POR) for having early abnormalities of glucose metabolism was similar in those who had family history of type 2 diabetes on father's and mother's side in both males and females. Maternal heredity, but not paternal heredity was associated with an increased risk of having type 2 diabetes in women. In men, both family history of type 2 diabetes on father's and mother's side were associated with type 2 diabetes. See paper I, Table 2, for additional details.

Basic characteristics. The subjects had similar age. BMI and waist circumference were significantly higher in both men and women with abnormalities of glucose tolerance. Individuals with IFG, IGT, CGI and type 2 diabetes had higher systolic blood pressure than NGT subjects. However, only IGT had significantly higher diastolic blood pressure than NGT. See paper I, Table 3, for additional details.

Insulin and PI levels, insulin sensitivity and beta cell function. Insulin levels, both fasting and 2 h plasma insulin levels, were higher in subjects with abnormalities of glucose tolerance compared to NGT subjects. In male subjects with abnormalities of glucose metabolism fasting plasma PI levels were significantly higher compared to NGT subjects. Interestingly, individuals with IFG and CGI had significantly higher fasting PI levels and PI / immuno-reactive insulin (IRI) ratios compared to individuals with IGT. The HOMA model revealed that both beta cell function and insulin sensitivity were decreased in IFG, IGT, CGI and type 2 diabetes, as compared to NGT. Notably, individuals with IFG and CGI had both decreased beta cell function and decreased insulin sensitivity compared to IGT subjects. See paper I, Table 4, for additional details.

4.2 PAPER II

In paper II, 13 controls and 17 patients with type 2 diabetes were studied. They were closely matched by age and BMI (Table 2). Patients had increased waist-to-hip ratio, total fat mass and truncal fat mass. Physical fitness, measured as $\dot{V}O_2$ max per total body weight, was significantly higher in controls compared to patients. However, the difference was not significant when $\dot{V}O_2$ max was calculated against kilograms of LBM. Subjects with type 2 diabetes had significantly higher fasting plasma insulin levels and blood glucose levels. The early insulin response (0–30 min) and total incremental insulin release (0–120 min) during an OGTT tended to be lower in patients despite greater increases in plasma glucose concentration. See paper II, Table 1, for additional details.

Substrate utilization rates and insulin sensitivity during two-step clamps. Fat and carbohydrate oxidation and plasma FFA levels were similar between the two groups during basal conditions. However, during the high-dose insulin clamp, fat oxidation rates and plasma FFA levels were significantly higher and carbohydrate oxidation rates lower in subjects with type 2 diabetes. The glucose infusion rates (M-value) required to maintain euglycemia (plasma glucose, 5.1 mM) during the clamp, a measure of whole body insulin sensitivity, were higher in controls during both low- and high-dose insulin clamps. There were no significant differences in insulin levels during clamps in patients and controls. See paper II, Table 2, for additional details.

Table 2. Basic characteristics of controls and patients

	Controls (n = 17)	Type 2 diabetes (n = 13)	P
Age (yr)	47.9 ± 1.6	49.2 ± 0.9	NS
BMI (kg/m ²)	26.2 ± 0.5	26.5 ± 0.5	NS
Waist-to-hip ratio	0.93 ± 0.01	0.98 ± 0.02	0.04
DEXA			
Lean weight (kg)	61.8 ± 1.1	59.5 ± 1.4	0.2
Total fat (kg)	24.1 ± 1.6	27.8 ± 1.5	NS
Truncal fat (kg)	13.1 ± 0.9	16.5 ± 0.9	0.02
Computerized tomography			
Abdominal fat area (cm ²)	356 ± 29	407 ± 19	NS
Intra-abdominal area	137 ± 15	186 ± 12	0.02
Subcutaneous fat area	216 ± 79	225 ± 40	NS
VO ₂ max (ml/min/kg)	33.4 ± 1.4	29.4 ± 1.0	0.03

Values are mean ± SEM. BMI, body mass index; VO_{2max}, maximal oxygen consumption; DEXA, dual-energy X-ray absorptiometry; NS, Not significant; Unpaired two-tailed *t* test was performed to identify differences between controls and patients. Skewed variables were log transformed before statistical computations.

Malonyl CoA, citrate and malate. The concentrations of citrate, malate, and malonyl CoA were similar in the muscle of patients and controls during basal conditions (Table 3). During the high-dose insulin clamp, the increases in muscle citrate ((controls, 31 nmol/g (*P* < 0.001) and patients, 15 nmol/g (*P* < 0.05)) and malate ((controls, 47 nmol/g (*P* < 0.001) and patients, 24 nmol/g (*P* < 0.001)) were less in patients compared to controls. Furthermore, the sum of citrate and malate (i.e. a presumed index of cytosolic citrate) were less in the patients than in controls (Table 3). However, the increase in malonyl CoA concentration during the high-dose insulin clamp was significant and nearly the same in the two groups.

Table 3. Malonyl CoA, citrate, and malate levels in human muscle

	Controls (n = 17)	Type 2 diabetes (n = 13)	P
Malonyl CoA (nmol/g)			
Initial biopsy	0.198 ± 0.01	0.199 ± 0.02	NS
Final biopsy	0.241 ± 0.01 ^b	0.238 ± 0.02 ^a	NS
Citrate (nmol/g)			
Initial biopsy	106 ± 7	100 ± 5	NS
Final biopsy	137 ± 7 ^b	115 ± 9 ^a	0.08
Malate (nmol/g)			
Initial biopsy	79 ± 7	76 ± 5	NS
Final biopsy	126 ± 9 ^b	90 ± 5 ^b	0.02
Δcitrate + malate (nmol/g)	77 ± 10 ^b	39 ± 10 ^b	0.01

Values are mean ± SD or SE. NS, Not significant. Unpaired two-tailed *t* test was performed to identify differences between patients and controls. A paired *t* test was used to determine the significance of the stepwise increase in malonyl CoA, citrate, and malate levels during clamps. Nine patients had citrate analyzed, and seven had malate analyzed. ^a *P* < 0.05; ^b *P* < 0.001.

4.3 PAPER III

In paper III, 11 healthy middle aged and slightly overweight subjects were recruited to study the impact of 12-week combined aerobic and dynamic strength training. As shown in Table 4, exercise training significantly increased physical fitness (VO_{2max}) by 13% and decreased intra-abdominal fat area by 17% but did not significantly change total body weight, BMI or waist-to-hip ratios. There was no significant change in total or truncal fat mass, or lean body mass. Furthermore, no significant change was seen in insulin sensitivity measured by hyperinsulinemic euglycemic clamp before and after training. See paper III, Table 1, for additional details.

Table 4. Phenotype of subjects before and after exercise.

	Before (n=11)	After (n=8)	P
Age (yrs)	51.0±2.2		
Weight (kg)	72.4±3.7	73.8±4.1	NS
BMI (kg/m ²)	25.3±0.7	25.4±0.6	NS
Waist-to-hip ratio	0.88±0.02	0.86±0.03	NS
M-value (mg/kg/min)	6.1±0.5	6.4±0.9	NS
VO_{2max} (ml/min/LBM)	45.7±1.2	51.3±0.8	0.02
Intra-abdominal fat area (cm ²)	113±11	94±10	0.006

Values are means±SEM. BMI, body mass index; VO_{2max} , maximal oxygen consumption; LBM, lean body mass; DEXA, dual-energy X-ray absorptiometry; NS, not significant.

P, Paired t-test was performed to evaluate significance of differences between subjects before and after exercise. Only subjects who had completed the study (n= 8) were used for this comparison.

Substrate utilization rates and plasma FFA levels. There were no significant changes during basal conditions in either fat or carbohydrate oxidation or in energy expenditure or in plasma FFA levels after training. However, both before and after exercise, plasma FFA levels significantly decreased and fat oxidation was significantly suppressed (34-38%) during clamp. Respiratory quotient (RQ) and glucose oxidation were increased during clamps even though only significantly before training. See paper III, Table 2, for additional details.

AMPK and ACC. There were no significant changes in protein content or phosphorylation of AMPK or ACC after physical training. However, the phosphorylation of ACC was decreased by 28-48% after the clamps, suggesting an increase in its activity, even though the only change was statistically significant after training. See paper III, Table 3, for additional details.

Malonyl CoA. Exercise training significantly decreased the basal concentration of malonyl CoA by 26% (Fig.7). Furthermore, insulin infusion significantly increased the concentration of malonyl-CoA both before (26%) and after (29%) training, which support the findings in paper II.

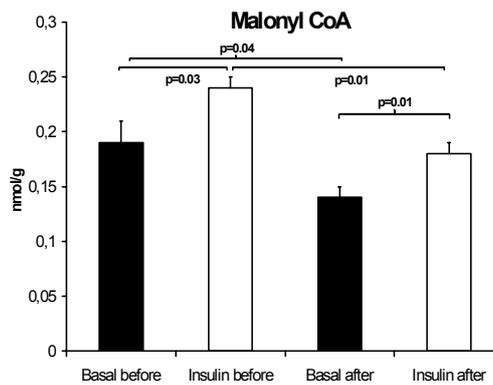


Fig. 7. The basal concentration of malonyl CoA was significantly decreased after training. Insulin infusion significantly increased malonyl-CoA both before and after training. Results are means±SEM for 8 subjects. Statistical significance was assessed by a paired t-test.

MCD. The decreased concentration of malonyl CoA after training was associated with 88% increase in MCD activity and 51% increase in MCD mRNA expression (Fig. 8).

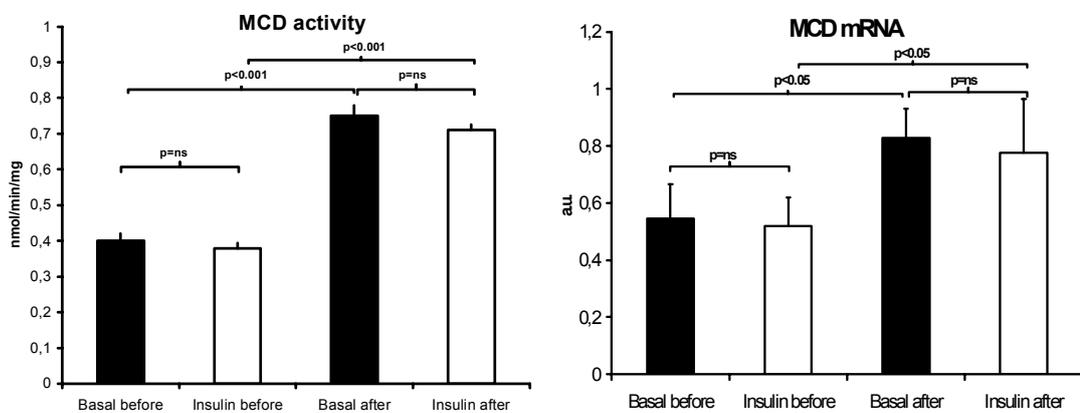


Fig. 8. The basal malonyl CoA decarboxylase (MCD) activity and mRNA expression were significantly increased after training. Insulin infusion had no effect in this respect. Data are means±SEM for 5 subjects. Statistical significance was assessed by a paired t-test.

PGC-1 α . There was a 3-fold increase in basal PGC-1 α protein after training (Fig. 9), and also a 45% increase in PGC-1 α mRNA expression, although not significant (data not shown).

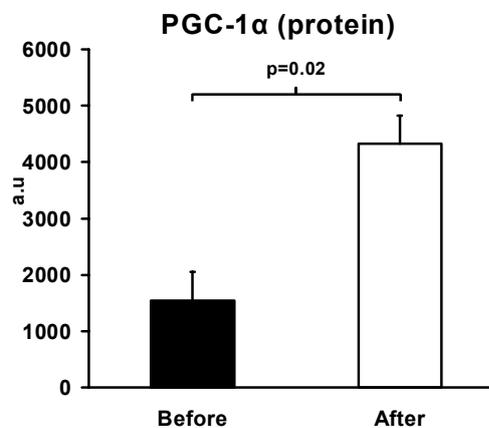


Fig. 9. The basal peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1 α) protein significantly increased after exercise. Data are means±SEM for five subjects. Statistical significance was assessed by a paired t-test. a.u., arbitrary units.

4.4 PAPER IV

In paper IV, twelve middle aged and slightly overweight patients with type 2 diabetes were investigated before and after training. As shown in Table 5, physical training significantly decreased intra-abdominal fat area by 15% and increased insulin sensitivity as reflected by increased glucose infusion rate (M-value) by 77% during clamps. No significant effect was seen on weight, BMI, waist-to-hip ratios, total or truncal fat mass, or lean body mass, VO_{2max} or HbA1c. Furthermore, there was no significant change in fasting-glucose and fasting-insulin. See paper IV, Table 1, for additional details.

Table 5. Phenotype of patients with type 2 diabetes before and after exercise.

	Before (n=12)	After (n=12)	P
Age (yrs)	52.1±1.2		
Weight (kg)	76.8±2.3	75.8±2.6	NS
BMI (kg/m ²)	25.6±0.9	25.2±0.9	NS
Waist-to-hip ratio	0.91±0.02	0.91±0.02	NS
M-value (mg/kg/min)	3.0±0.5	5.3±0.9	0.002
VO_{2max} (ml/min/LBM)	42.7±1.3	45.7±1.6	NS
Intra-abdominal fat area (cm ²)	145±15	123±14	0.02
HbA1c	5.5±0.3	5.3±0.2	NS

Values are means±SEM. BMI, body mass index; VO_{2max} , maximal oxygen consumption; LBM, lean body mass; DEXA, dual-energy X-ray absorptiometry; NS, not significant. P, Paired t-test was performed to evaluate significance of differences between patients before and after exercise.

Substrate utilization rates and plasma FFA levels. Exercise training had no effect on basal substrate utilization rates, systemic energy expenditure or plasma FFA levels. There was a significant increase in carbohydrate oxidation and decrease in plasma FFA levels during clamp both before and after training. Furthermore, insulin infusion led to a 24% suppression of fat oxidation and a significant increase in RQ after but not before training. See paper IV, Table 2, for additional details.

Malonyl CoA. Three month of exercise training significantly decreased the malonyl CoA content by 28% in patients with type 2 diabetes, which supports the findings in healthy controls, paper III (Fig.10). Compared to basal values, the concentration of malonyl-CoA was increased after the clamp, both before (16%) and after (31%) training.

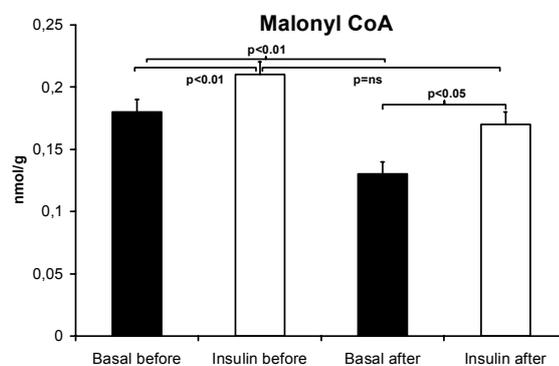


Fig. 10. The basal concentration of malonyl CoA was significantly decreased after training.

Insulin infusion significantly increased malonyl-CoA both before and after training.

Results are means±SEM for 10 subjects. Statistical significance was assessed by a paired t-test.

ACC and AMPK. There were no significant changes observed in protein content or phosphorylation of ACC or AMPK after training. There were also no significant changes in the activities of AMPK or ACC during insulin clamps, although the abundance of both pAMPK and pACC, in all instances, tended to decrease. See paper IV, Table 3, for additional details.

MCD. As shown in Fig. 11, physical training increased the activity of basal MCD by 23% and the data confirm the results shown in paper III, where a decrease in malonyl CoA was associated with an increase in MCD activity. However, in patients, training had no significant effect on MCD mRNA expression in muscle (data not shown).

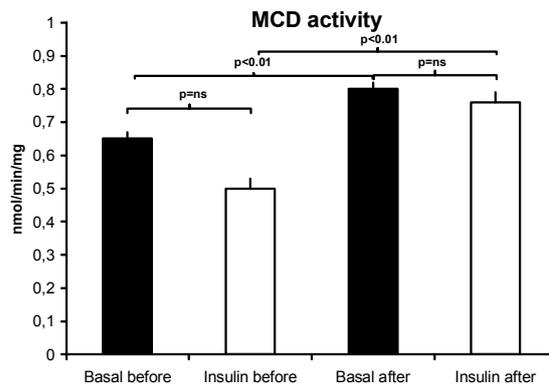


Fig. 11. The basal malonyl CoA decarboxylase (MCD) activity was significantly increased after training. Insulin infusion had no effect in this respect. Data are means±SEM for 5 subjects. Statistical significance was assessed by a paired t-test.

DAG. The decrease in Malonyl CoA after physical training was also associated with a decrease in DAG levels by 46% after training (Fig 12).

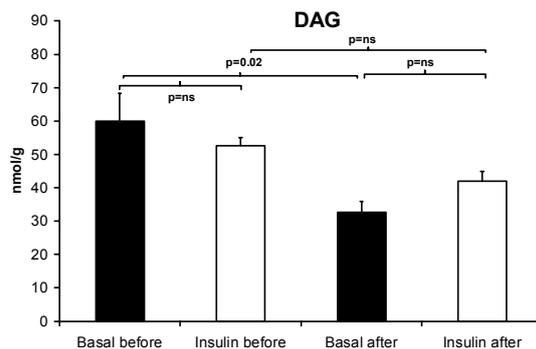


Fig. 12. The basal diacylglycerol (DAG) levels significantly decreased after exercise. Insulin infusion had no effect in this respect. Data are means±SEM for 5 subjects. Statistical significance was assessed by a paired t-test.

5 DISCUSSION

Type 2 diabetes develops slowly through stages of early abnormalities of glucose metabolism such as IFG, IGT and CGI. In the present study we have investigated pathogenesis of these abnormalities using subjects participating in the SDPP^{32,96-98}. Specifically, we evaluated the impact of sex and diabetes heredity in this context. Therefore, in the study we included about 50% of subjects with type 2 diabetes heredity. The findings are relevant for the age-group 35-56 included in the SDPP cohort.

The first important new finding was that the prevalence of IFG, IGT, CGI and type 2 diabetes was 2-3 times higher in individuals with FHD as compared to subjects without FHD. Second, prevalence of milder abnormalities of glucose metabolism was 2-3-times higher in men than in women irrespective of diabetes heredity. Thus, the data supports the finding in other studies that IFG is more common in men¹⁶, but contradicts other European studies claiming higher prevalence of IGT in women^{16,119}. Third, the prevalence of type 2 diabetes was similar in both sexes in the groups without diabetes heredity. In contrast, in subjects with diabetes heredity the prevalence of diabetes was more than two times higher in men as compared to women. The mechanisms behind this difference are not clear. It may be speculated that genetic defects related to male sex precipitate development of diabetes. Another possibility is that estrogens protect or delay the development of manifest diabetes. It is sufficiently documented that estrogens exert a strong anti-diabetic action¹²⁰.

The present study also investigated the impact of maternal and paternal type 2 diabetes heredity on the prevalence of early disturbances of glucose metabolism and type 2 diabetes. It has previously been suggested that type 2 diabetes offspring have the possibility of unprecedented maternal or paternal inheritance of different type 2 diabetes phenotypes¹²¹. In our study, maternal and paternal inheritance had a similar influence on the risk of having early disturbances of glucose metabolism in both sexes. However, in women, maternal diabetes heredity, but not paternal, was associated with an increased risk of having type 2 diabetes, whereas in men, maternal and paternal heredity had a similar influence on the risk.

Data from previous demonstrates indicates poor concordance between the diagnosis of IFG and IGT, and half or less of the people with IFG have IGT, and even a lower proportion (20-30%) with IGT also have IFG¹⁶. In our study, irrespective of FHD, 28% of individuals with IFG also had IGT (Fig. 13) and 13% of subjects with IGT were diagnosed by their fasting glucose levels (Fig. 14). Thus, our results show that 87% of subjects with IGT had normal fasting glucose levels. People with the risk of developing type 2 diabetes would be overlooked if only the fasting glucose level were considered, which has been recommended by the American Diabetes Association (ADA)¹²². Therefore, our data demonstrates that an OGTT is mandatory for identification of subjects at risk of developing type 2 diabetes.

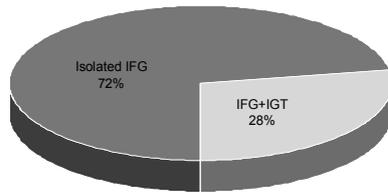


Fig. 13. IFG - isolated and in combination with IGT

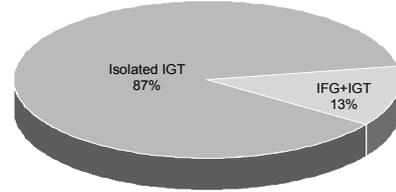


Fig. 14. IGT - isolated and in combination with IFG

The waist circumference, BMI and blood pressure were higher in subjects with early disturbances of glucose metabolism compared to individuals with normal glucose tolerance which is supported by another study that also reported higher blood pressure in IFG and/or IGT compared to NGT²². It is reasonable to assume that an increase in CVD risk among IFG, and/or IGT¹⁶, is partly mediated by an increase in blood pressure.

Insulin sensitivity and beta cell function were assessed using the HOMA. The model has its obvious limitations since it is based on fasting values of glucose and insulin. However, the HOMA model has been validated against euglycemic-hyperinsulinemic and hyperglycemic clamps as well as fasting insulin concentrations⁹⁹. In both men and women, subjects with early abnormalities of glucose metabolism were more insulin resistant and had poorer beta cell function compared to individuals who had normal glucose tolerance. This was particularly obvious among subjects with IFG who showed to be more insulin resistant than subjects with IGT, which agrees with some studies^{19,20,22} but not with others²¹. Furthermore, the HOMA analysis also revealed that individuals with IFG had greater impairments of beta cell function than IGT subjects. This finding is supported by additional studies^{21,22} but not by others^{19,20}. It is not clear why individuals with isolated IFG had normal 2 h plasma glucose during OGTT, despite a marked impairment of insulin sensitivity and beta cell function. Fasting glucose concentrations mainly depend on glucose production from the liver¹²³, whereas postprandial plasma glucose values are determined by hepatic and extra-hepatic insulin sensitivity and insulin secretion¹²⁴. The current study did not permit direct measurements of insulin sensitivity¹²⁵, nor were glucose effectiveness, hormones from the gut or alpha cell responsiveness measured, factors that may be of importance for determining normal glucose patterns during OGTT in IFG^{126,127}. Finally, increased pro-insulin levels in males with IFG as compared to IGT, support the results from the HOMA analysis and also suggest a more pronounced impairment of beta cell function in IFG subjects¹²⁸.

The cellular and molecular mechanisms underlying insulin resistance in muscle are not completely understood. The studies presented in paper II-IV strongly support the hypothesis that a combination of increased levels of FFA in plasma and a reduced capability to suppress fatty acid oxidation (“metabolic inflexibility”)¹²⁹, as well as

increased malonyl CoA levels in muscle, could play a fundamental role in insulin resistance in people with type 2 diabetes^{58,61}. Increased levels of malonyl CoA contribute to insulin resistance through its role in the accumulation of lipids in the cytosol. As discussed earlier, malonyl CoA inhibits CPT 1, and thereby decreases mitochondrial oxidation^{60,62,130,131}. As a result, LCFA CoA and DAG accumulate in the muscle cytosol which may inhibit insulin signaling through activation of PKC isoforms, which in turn inhibits insulin-receptor tyrosine kinase and glycogen synthase^{56,57,131,132}. The above data are generated from studies in animal models of type 2 diabetes where increases in malonyl CoA and LCFA CoA levels as well as, changed PKC distribution have been shown to parallel decreases in insulin action in muscle⁶². The study of normal individuals shows that insulin resistance generated in muscle by increasing plasma FFA levels (by fat infusion plus heparin) during an euglycemic hyperinsulinemic clamp is accompanied by increases in DAG and PKC activity¹³³. Finally we have recently shown in healthy subjects that an increase in the concentration of citrate plus malate in muscle during euglycemic hyperinsulinemic clamps is associated with a simultaneous increase in malonyl CoA concentration and a decrease in fatty acid oxidation⁶⁴.

The results in paper II support data from others by demonstrating that insulin resistance is present in slightly overweight patients with type 2 diabetes^{3,45}. In addition, we demonstrate that insulin resistance in this cohort is associated with increased truncal obesity, impaired physical fitness and an impaired capacity to suppress plasma FFA levels and whole-body fatty acid oxidation. Importantly, for the first time we demonstrate the atypical regulation of muscle malonyl CoA in subjects with type 2 diabetes¹³⁰. Thus, during the high dose insulin clamp the increase in muscle citrate and malate was significantly less in the subjects with type 2 diabetes than in healthy control subjects, but similar increases in the concentration of malonyl CoA were found. Based on these observations it may be speculated that a relative increase in malonyl CoA levels in muscle from type 2 diabetic subjects contribute to a reduced capacity to suppress fatty acid oxidation rates and insulin resistance. Recent data published by Bandyopadhyay et al support this conclusion⁶¹. The authors demonstrate that an increase in muscle malonyl CoA levels in obese insulin-resistant subjects compared with lean control subjects, is correlated with decreased fatty oxidation rates and increased lipogenesis in skeletal muscle.

The synthesis of malonyl CoA is acutely regulated by AMPK which phosphorylates and inhibits the muscle isoform of ACC. Exercise activates AMPK and diminishes ACC- β activity in human muscle^{70,134,135}. In our present study we demonstrate for the first time that exercise training also stimulates the expression and activity of MCD. Thus, by both these effects exercise may decrease the concentration of malonyl CoA. We believe that decreased physical fitness of patients with type 2 diabetes contributes to inappropriately high malonyl CoA levels due to decreased AMPK activity. In agreement with this hypothesis are studies demonstrating that activation of AMPK by AICAR⁸², metformin¹³⁶, rosiglitazone¹³⁷, adiponectin¹³⁸ and leptin¹³⁹ improves insulin sensitivity. Present studies, III and IV suggest that MCD may also be an interesting target for development of novel drugs which increase the enzyme activity and thereby decrease muscle malonyl CoA levels and increase insulin sensitivity.

Increased physical activity is considered to be a cornerstone in the treatment of type 2 diabetes, mainly through its multiple effects on glucose homeostasis, insulin action and clinical features (physical fitness, weight reduction and blood pressure control). Hence, in order to optimize the use of physical training in the treatment of type 2 diabetes we are investigating the impact of well controlled and defined long-term training programs in well characterized cohorts of subjects by age, phenotype and HbA1c. We follow multiple parameters characterizing phenotype, glycemic control, insulin sensitivity, lipids and cardiovascular functions. We also focus specifically on understanding the molecular mechanisms mediating effects of exercise on substrate fluxes and insulin sensitivity. In paper III we have studied healthy, middle-aged subjects before and after a 12-week exercise training program, including combined aerobic and dynamic strength training, three times a week. In paper IV, twelve nearly normal-weight and well-controlled patients with type 2 diabetes participated in the identically designed training program.

Both controls and patients were middle-aged and had similar BMI and physical fitness before entering the study. The results show that long-term exercise training without caloric restriction reduced intra-abdominal fat mass in both controls and patients by 17% respectively 15% and VO_2 max was improved in both groups by 13% and respectively 14%. Interestingly, training had no impact on glycemic control. It is most likely due to the nearly optimal metabolic control in patients participating in the study (HbA1c $5.5 \pm 0.3\%$). In a recent meta-analysis of the effect of structured exercise training on glycemic control in type 2 diabetes the effect tended to be more pronounced in subjects with a high HbA1c level at study inclusion¹⁴⁰. In another, more recent meta-analysis Boulé et al describe a relationship between exercise volume (total weekly energy expenditure) and changes in cardiorespiratory fitness and HbA1c level¹⁴¹. Interventions using higher aerobic exercise intensities produced not only larger improvement in VO_2 max, but also in glycemic control. Thus we cannot rule out the possibility that a more vigorous and intense exercise training program may have led to greater improvements in both VO_2 max and glycemic control in the diabetic subjects. The latter is supported by a study demonstrating that high exercise intensity (45 min of cycling at 75% of VO_2 max two times per week plus an intermittent exercise session one time per week for 2 months), showed the greatest improvement in HbA1c (from 7.9% to 6.4%)¹⁴². Our intention was, however, to study the impact of long-term moderate exercise training in a real-life setting and thus, to avoid interventions at a high intensity which may be hazardous to sedentary people with type 2 diabetes. Furthermore, it has been questioned whether a clear dose response effect of exercise on glycemic control in type 2 diabetes exists⁵².

Exercise training significantly increased insulin sensitivity in patients as reflected by a 77% increase in M-value. Hence, 12 weeks of exercise training, 3 times a week for 50 minutes, is sufficient to increase insulin sensitivity in patients with type 2 diabetes. We have recently confirmed that a similar long-term structured moderate exercise training program significantly improved insulin sensitivity (by 92%) in people with mild type 2 diabetes¹⁴³. Notably, in the present studies no significant effect of training was seen on insulin sensitivity in healthy subjects. There are also other studies on healthy subjects that demonstrate an exercise-induced increase in VO_2 max but had no impact on insulin sensitivity¹⁴⁴⁻¹⁴⁶. In general, the impact of exercise training on insulin sensitivity seems

to depend on age and glycemic control and insulin sensitivity at inclusion of the study as well as the design of the training program ^{144,145}.

An important aim of the present studies was to explore the molecular mechanisms' mediating effects of exercise on muscle metabolism. In this context the most important finding was that training decreased the concentration of malonyl CoA in muscle both in healthy subjects and in patients with type 2 diabetes, which was accompanied by an increase in activity of MCD in both groups, and a significant decrease in DAG in patients. Notably, in these experiments we did not observe an effect of training on the abundance, or phosphorylation of AMPK and ACC. We believe that the activation of muscle AMPK and some of its acute effects, such as phosphorylation of ACC, had dissipated since there was a lag of 24-36 h between the last bout of exercise and muscle biopsy. MCD phosphorylation was not assessed; however, the finding that MCD mRNA in muscle was increased in healthy humans suggests that the change in MCD activity was due, at least in part, to an increase in enzyme abundance. The observation that training also increased PGC-1 α abundance in these experiments is in keeping with this conclusion because PGC-1 α is a coactivator of PPAR α , which activates the transcription of MCD in skeletal and cardiac muscle ¹⁴⁷. We suggest that these changes in PGC-1 α and MCD are effects of prior activation of AMPK by exercise training that persist after other AMPK-mediated events such as ACC phosphorylation and inhibition have dissipated.

Interestingly, as discussed above in this study healthy subjects did not improve insulin sensitivity after training although significant changes were found in VO₂ max, intra-abdominal fat area, malonyl CoA concentrations, MCD activity, and PGC-1 α abundance. There are several explanations for this finding. First, the effect of training on insulin sensitivity is less pronounced in middle-aged and elderly people ¹⁴⁴, who was recruited in our study. Similarly, Short et al ¹⁴⁴ demonstrated that long-term training increased mitochondrial biogenesis and expression of GLUT-4 in muscle in men and women aged 21-87 years but insulin sensitivity was only improved in the group of young subjects. Second, the failure to improve insulin sensitivity in the present study may be related to the training program, which engaged leg muscle more than the rest of the body, suggesting that the enhanced cellular and molecular function found in leg muscle was not sufficient to increase whole-body insulin sensitivity. Third, we studied a group of healthy individuals with normal insulin sensitivity. The latter possibility is strongly supported by present study in insulin resistant patients with type 2 diabetes. They were of similar age, weight and sex but had markedly increased insulin sensitivity after identical training program.

In the above study diabetic patients' training increased postabsorptive activity of muscle MCD and decreased DAG levels. However, in contrast to findings in healthy subjects no change was seen in protein amount or expression of PGC-1 α or in MCD mRNA. It is not clear whether the increased expression of PGC-1 α and MCD was dissipated or did not take place. We also believe that in the group of patients with diabetes, the improvement of insulin sensitivity reflects prior activation of AMPK which has dissipated due to a long lap-time between the last bout of exercise and muscle biopsy. However, it is possible that exercise directly phosphorylated and activated MCD via non-AMPK controlled mechanisms.

Insulin infusion significantly increased malonyl CoA both before and after subjects were trained. The amount and activity of AMPK and ACC were not significantly changed by insulin which supports that an augmented flux of citrate in the muscle mediates the observed increase in malonyl CoA.

Training did not alter postabsorptive fasting plasma FFA concentrations, whole body energy expenditure or fat oxidation rates. This suggests that fasting fat oxidation rates may be unchanged despite the decrease in malonyl CoA levels. There are several explanations to this apparently contradictory finding. First, sensitivity of CPT1 to malonyl is influenced by both hormonal and nutritional factors¹⁴⁸, which were not controlled in the present studies. Secondly, sensitivity of CPT1 to malonyl CoA is increased in trained compared to untrained muscle¹⁴⁹. Third, training increases fat oxidation rates in muscle but this is not detected by whole-body measurements with indirect calorimetry. The latter hypothesis is supported by experiment demonstrating that low intensity endurance training increased fat oxidation rates in resting muscle in healthy non-obese volunteers when measured directly using palmitate tracer, whereas whole body fat oxidation rates, measured by indirect calorimetry were unchanged⁶⁹. Fourth, expression of fatty acid transport proteins, as well as, the fatty acid translocator membrane glycoprotein have been linked to insulin resistance¹⁵⁰⁻¹⁵². It is thus possible that training altered the expression of these proteins, which in turn may have influenced fatty acid oxidation rates in our studies. Finally, subjects were studied 24-36 h after the last bout of exercise when the effect of exercise on fat oxidation in muscle dissipated.

In conclusion, in slightly overweight men with mild type 2 diabetes insulin sensitivity is decreased, which closely correlates with an increase in truncal fat mass and a decrease in physical fitness. We propose that inappropriate high levels of muscle malonyl CoA, in combination with decreased suppression of plasma FFA, constitute an important mechanism behind insulin resistance in patients with type 2 diabetes. In agreement with this hypothesis the long-term moderate-intensity exercise training improved insulin sensitivity in diabetic patients and decreased intra-abdominal fat mass, whereas postabsorptive concentrations of malonyl CoA in muscle decreased. We demonstrate for the first time in humans that training increases MCD activity, in muscle contributing to a decrease in malonyl CoA and DAG levels and facilitating insulin signaling. In contrast, phosphorylation of AMPK or of ACC was not changed which is probably related to a long time-lag before the last bout of exercise and muscle biopsy. In healthy subjects long-term training also increased the abundance of PGC-1 α , a regulator of transcription linked to the mediation of MCD expression by PPAR α . The important question to answer is whether exercise training regulates MCD by phosphorylation, and if so, what kinases and phosphorylases are involved.

6 CONCLUSION

In conclusion, heredity for type 2 diabetes and male sex increased the prevalence of early abnormalities of glucose metabolism. The risk was approximately two-fold more common in men than women irrespective of diabetes heredity. Both maternal and paternal heredity influenced this risk equally in both sexes, although the parental impact on having type 2 diabetes was higher in men than in women. IFG and IGT subjects exhibited higher blood pressure, increased central fat, decreased insulin action and insulin secretion. Notably, IFG had more pronounced defects of both insulin secretion and action than IGT, suggesting different pathogenic backgrounds to the two conditions.

Insulin sensitivity is decreased in slightly overweight subjects with mild type 2 diabetes and this closely correlates with an increase in truncal fat mass, decrease in physical fitness and unexpectedly high levels of malonyl CoA in muscle. We propose that high levels of muscle malonyl CoA, in combination with decreased suppression of plasma FFA, constitute a crucial mechanism behind insulin resistance in type 2 diabetes.

Long-term moderate-intensity exercise training markedly improved insulin sensitivity and decreased intra-abdominal fat area in patients with type 2 diabetes. This study demonstrate for the first time in humans that long-term exercise training increases activity of MCD in muscle, contributing to a decrease in malonyl CoA and DAG levels resulting in improved insulin sensitivity in patients with type 2 diabetes.

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