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Mika Venojärvi

Roles of Exercise Training with Dietary Counselling and Muscle Fibre Composition in the Regulation of Glucose Metabolism in Middle-aged Subjects with Impaired Glucose Tolerance

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EASTERN FINLAND

MIKA VENOJÄRVI

Roles of exercise training with dietary counselling and muscle fibre composition in the regulation of glucose metabolism in middle-aged subjects with impaired glucose tolerance

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ABSTRACT

The prevalence of type 2 diabetes is rapidly increasing worldwide, and yet its primary prevention and treatment are still a challenge. However, there is a great amount of evidence of the risk factors such as obesity and physical inactivity that are the main nongenetic determinants of the disease. Lifestyle interventions, including diet and physical exercise, can result in a reduction of around 50–60% in diabetes incidence and the beneficial effect persists even after the individual lifestyle counselling has stopped. Short-term randomized studies have confirmed that physical training based on endurance and/or resistance exercises can also improve blood glucose control in type 2 diabetics.

The aim of this study was to clarify the role of exercise training with dietary counselling on heat shock proteins, adipokines and glucose metabolism in middle-aged subjects with impaired glucose tolerance. Additionally, in rats we tested the hypothesis that a stronger, non-damaging, metabolic stress may provide greater tissue protection against atrophic and degenerative changes after immobilization.

Both spontaneous physical activity and intensive exercise increased the HSP60 and HSP72 expressions in the lateral gastrocnemius muscle in rats. Similarly, in the subjects with impaired glucose tolerance (IGT), cytoprotection was improved in the skeletal muscle tissue, as shown by increased expression of mitochondrial HSP60 and GRP75 after exercise-diet intervention. Moreover, oxidative stress was reduced as shown by decreased serum levels of uric acid and protein carbonyls in the IGT subjects after exercise-diet intervention.

This study has shown that after the intervention period, the amount of glycogen synthase kinase-3-$\alpha\beta$ (GSK-3-$\alpha\beta$) protein decreased and GSK-ß contents tended to decrease in the IGT subjects who had proportionately more MHC II isoforms in their skeletal muscle. This is a novel finding, suggesting a metabolic pathway that is sensitive to exercise and dietary intervention. Significant weight reduction and improved aerobic capacity was achieved in responders. These improvements led up to restoration of insulin sensitivity and increased circulating adiponectin and decreased leptin levels without changes in skeletal muscle GLUT-4 expressions or cholesterol metabolism in responders. In the non-responders without weight reduction no changes took place.

In conclusion, this study provides new evidences on the vulnerability of the subjects to impaired glucose tolerance and supports promotion of weight reduction and long-term exercise training to improve insulin sensitivity in skeletal muscle.

National Library of Medical Classification: QT 235, QU 55.6, QU 120, WB 541, WE 500, WK 820
Medical Subject Headings: Diet; Exercise; Heat-Shock Proteins; Glucose Intolerance; Glucose/metabolism; Muscle; Skeletal

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TIIVISTELMÄ:

Glukoosiaineenvaihdunnan tasapaino on riippuvainen monista eri tekijöistä, kuten glukoosin kuljetuksesta solun sisään, glykogeenin varastoitumisnopeudesta sekä luustolihaksen solujakaumasta. Tämän lisäksi lämpöshokkiproteiinien (HSP) vähentyneen määrän on havaittu liittyvän diabetekseen sekä veren korkeaan glukoosipitoisuuteen. Myös lisääntynyt vapaiden radikaalien muodostuminen on yhteydessä veren kohonneisiin glukoosiarvoihin.

Tutkimuksella selvitettiin, paraneeko luustolihaksen glukoosiaineenvaihdunta 2-vuotisen intervention aikana sellaisilla keski-ikäisillä henkilöillä, joilla glukoosinsietokyky oli heikentynyt (IGT). Lisäksi selvitettiin, miten liikunta- ja ruokavaliointerventiot vaikuttavat elimistön ja lihaskudoksen antioksidatiiviseen puolustusjärjestelmään, hapetusstressiin sekä lihaksen HSP-proteiinien ilmentymiseen ja adipokiineihin. Tutkimusaineisto koostui liikunta- ja ruokavaliointerventioon osallistuneista, vapaaehtoisista, lihaskudosnäytteen ottoon suostuneista keski-ikäisistä henkilöistä, joilla oli heikentynyt glukoosinsietokyky (n=22). Tämän lisäksi haluttiin selvittää rottakokeilla, miten spontaani harjoittelu tai intensiivisempi juoksumattoharjoittelu vaikuttaa HSP-proteiinien ilmenemiseen immoblisaation jälkeen.

Tutkimus osoitti, että 2-vuotinen interventio paransi luustolihaksen glukoosiaineenvaihduntaa keski-ikäisillä henkilöillä, joiden glukoosin sieto oli heikentynyt. Muutos oli suurempi henkilöillä, joiden luustolihas koostui pääasiallisesti nopeista lihassoluista. Luustolihaksen oksidatiivinen kapasiteetti parani riippumatta tutkittavan lihassolujakaumasta. Liikunta- ja ruokavaliointerventio lisäsivät lihaskudoksen HSP60- ja GRP75-proteiinien määriä ja vähensivät samanaikaisesti seerumin uraattipitoisuutta. Spontaani ja intensiivinen harjoittelu lisäsivät myös rotan kaksoiskantalihaksessa HSP60- ja GRP75-proteiinien määriä ilman, että hapetusstressi kasvoi. Fyysisen aktiivisuuden määrän kasvu ja ruokavalioneuvonnan aikaansaama painon lasku yhdessä paransivat glukoosiaineenvaihduntaa niillä henkilöillä, joiden paino oli merkittävästi laskenut 2-vuotisen intervention aikana. Samalla seerumin leptiinin pitoisuus laski ja adiponektiinin pitoisuus kasvoi.

Yleinen Suomalainen asiasanasto: glukoosi - sietokyky; liikunta; luusto; lihaskudos; proteiinit; ravinto; aineenvaihdunta

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During the years that I carried out my thesis, I was also working at Turku Centre for Biotechnology, at the Population Studies Unit in the Department of Chronic Disease Prevention of the National Institute for Health and Welfare, Turku, and at Turku University of Applied Sciences and carrying out 'NowaStep'-intervention and taking part in two 'Berry projects' (2007–2010). — I am grateful for the all friendship I made at during these years. All my present and past colleagues are also thanked warmly both in Finland and in USA where I performed my researcher training in order to improve my scientific writing skills and to get acquainted with the newest advanced methodology of biochemical and histochemical analyses.

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I dedicate this work to my son, Verneri, and to the memory of my mom and grandma.

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Kuopio, June, 2011

Mika Venojärvi

LIST OF THE ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, which will be referred to by their Roman numerals (I-IV) in the text:

I Venojärvi M, Puhke R, Hämäläinen H, Marniemi J, Rastas M, Rusko H, Nuutila P, Hänninen O, Aunola S. Role of skeletal muscle-fibre type in regulation of glucose metabolism in middle-aged subjects with impaired glucose tolerance during a long-term exercise and dietary intervention. Diabetes, Obesity & Metabolism 7: 745–54, 2005.

II Venojärvi M, Aunola S, Puhke R, Marniemi J, Hämäläinen H, Halonen JP, Lindström J, Rastas M, Hällsten K, Nuutila P, Hänninen O, Atalay M. Exercise training with dietary counselling increases mitochondrial chaperone expression in middle-aged subjects with impaired glucose tolerance. BMC Endocrine Disorders 8: 3. doi: 10.1186/1472-6823-8-3, 2008.

III Venojärvi M, Aunola S, Hämäläinen H, Marniemi J, Halonen J-P, Lindström J, Nuutila P, Atalay M, Hänninen O. Increased aerobic capacity and weight loss improve glycemic control without changes in skeletal muscle GLUT-4 expressions in middle-aged subjects with impaired glucose tolerance. (submitted)

IV Venojärvi M, Kvist M, Jozsa L, Kalimo H, Hänninen O, Atalay M. Skeletal muscle HSP expression in response to immobilization and remobilization. International Journal of Sports Medicine 28: 281–6, 2007.

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In addition, some unpublished data are presented.

Contents

Abbreviations

AdipoR1	Adiponectin receptor 1
AdipoR2	Adiponectin receptor 2
AGE	Advanced glycation end products
Akt	Protein kinase B
AM	Active mobilization
AMP	Adenosine monophosphate
AMPK	5' adenosine monophosphate-activated protein kinase
AP-1	Activator protein-1
ATP	Adenosine-5'-triphosphate
BMI	Body mass index
CAT	Catalase
Cys	Cysteine
CS	Citrate synthase
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DPS	the Finnish Diabetes Prevention Study
FFA	Free fatty acids
FM	Free mobilization
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GLUT	Glucose transporter
GRD	Glutathione reductase
GRP75	Glucose-regulated protein 75
GRP78	Glucose-regulated protein 78
GS	Glycogen synthase
GSH	Reduced glutathione
GSHPx	Glutathione peroxidase
GSK-3	Glycogen synthase kinase-3
GSSG	Oxidized glutathione

GT	Glutamyl transferase (=> γ-GT)
H_2O_2	Hydrogen peroxide
HbA_{1c}	Haemoglobin A1c
HIF-1	Hypoxia-inducible factor-1
HMW	High-molecular weight
HNE	4-hydroxy-nonenal (=> 4-HNE)
HOMA-IR	Homeostasis Model Assessment, insulin resistance
HSP	Heat shock protein
IDDM	Insulin dependent diabetes
IFG	Impaired fasting glucose
IFN-γ	Interferon gamma
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
IL-8	Interleukin-8
IRS	Insulin receptor substrates
kDA	Kilo Dalton
LDH	Lactate dehydrogenase
LDH-1	Lactate dehydrogenase-1
LG	*m. lateral gastrocnemius*
MAPK	Mitogen activated protein kinases
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
MHC	Myosin heavy chain
NIDDM	Non-insulin dependent diabetes
NFAT	Nuclear factor of activated T-cells
NF- κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
NOS	Nitric oxide synthase
NO_2-Tyr	3-nitro-tyrosine
OGTT	Oral glucose tolerance test
ORAC	Oxygen radical absorbing capacity

p53	Protein 53
PC	Protein carbonyls
PCR	Polymerase chain reaction
PDK	3-phosphoinositide-dependent kinases
PI3-kinase	Phosphotidylinositol-3-kinase
PL	*m. plantaris*
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	Standard error
SNO	S-nitrothiols
SOD	Superoxide dismutase
TAC	Total antioxidative capacity
TBARS	Thiobarbituric acid-reactive substances
TNF-α	Tumor necrosis factor alfa
Trx	Thioredoxin
VEGF	Vascular endothelial growth factor
VO_{2max}	Maximal oxygen uptake
XO	Xanthine oxidase

1 INTRODUCTION

Today more than 1.1 billion adults world-wide are overweight (BMI > 25) and 312 million of them are obese (BMI > 30) (Hossain et al., 2007). In Finland, already 56% of adult men and 44% of adult women are overweight and 15% of men and 16% of women are obese (Helakorpi et al., 2008). Obesity is associated with an increased risk of developing insulin resistance and hyperglycaemia leading to the development of type 2 diabetes. Pathogenesis of type 2 diabetes is still uncertain although obesity and physical inactivity are the main non-genetic risk factors and determinants of the disease (Ohlson et al. 1988, Manson et al. 1991).

Insulin resistance, hyperglycaemia and diabetes are associated with an increased oxidative stress and impaired cellular defence systems (Parthiban et al. 1995, Muchova et al. 1999, Atalay et al. 2004). Insulin resistance in skeletal muscle is associated with an elevated adipose tissue mass (Sell et al. 2006). The interplay of adipose tissue and skeletal muscle is an important factor in the pathogenesis of insulin resistance.

Oxidative stress increases production of reactive oxygen species (ROS), overwhelms endogenous antioxidant protection and may result in biomolecular damage (e.g. DNA, lipids and proteins). At lower concentrations, ROS also serve as secondary messengers regulating cellular functions and adaptations. Therefore, a new definition of oxidative stress includes disruption of redox-modulated regulation of cellular functions (Jones 2006, Jones 2008). Oxidative stress may have an important role in the pathophysiology of insulin resistance, hyperglycaemia, as well as, diabetes and its complications through increased oxidative damage, inflammation and apoptosis (Dominques et al. 1998, Hotta et al. 1998 and Atalay and Laaksonen 2002). Increased physical activity and changes in daily dietary habits including weight reduction are the most effective non-pharmacological methods to prevent development of type 2 diabetes. Previous studies have shown that lifestyle changes result in significant weight loss and improved glucose tolerance in middle-aged obese subjects with impaired glucose tolerance (IGT) (Pan et al. 1997, Tuomilehto et al. 2001, Knowler et al. 2002).

Abnormal glucose homeostasis is characterized by the presence of impaired fasting glucose (IFG), impaired glucose tolerance i.e. IGT, or both. Also the increased risk for cardiovascular disease in prediabetes is multifactorial, with etiologies including insulin resistance, hyperglycaemia, dyslipidemia, hypertension, systemic inflammation, and oxidative stress (Hsueh et al. 2010). Strategies to decrease oxidative stress and to modulate stress proteins: expression of heat shock protein (HSP), which is an important component of protein homeostasis and cell survival may have important implications for reducing insulin resistance, improving impaired glucose regulation and increasing the protection against diabetes and its complications. However, combined effects of exercise-diet intervention on the tissue protection, such as expression of HSP, have not been studied before in human subjects with impaired glucose tolerance in the skeletal muscle.

The goal of the present series of studies was to clarify the roles of both long-term exercise training and dietary counselling and of the skeletal muscle fibre type composition in the regulation of glucose metabolism with interaction of heat shock proteins and adipokines in middle-aged obese subjects with IGT. In addition, the hypothesis that a stronger, non-damaging, metabolic stress may provide a greater tissue protection against atrophic and degenerative changes after immobilization was tested in rats.

2 REVIEW OF LITERATURE

2.1 TYPE 2 DIABETES AND ITS MAIN NON-GENETIC RISK FACTORS

Diabetes and its complications are increasing as major causes of mortality and morbidity in the developed countries (Petersen and Shulman 2006). Prevention of diabetes is crucial to lowering the incidence of type 2 diabetes. High risk groups, such as the elderly or those with glucose intolerance have a high risk of developing diabetes. Prediabetes is a state of abnormal glucose homeostasis characterized by impaired fasting glucose, (IFG), impaired glucose tolerance (IGT), or both. The mechanism by which prediabetes predisposes the increased risk for cardiovascular disease is multifactorial, with reasons including insulin resistance, hyperglycaemia, dyslipidemia, hypertension, systemic inflammation, and oxidative stress (Hsueh et al. 2010). Uncontrolled oxidative stress and perturbations in the tissue antioxidant defence network represent a characteristic feature of diabetes among the other important conditions such as dyslipidemia and modification of proteins and lipids (Atalay and Laaksonen 2002, Atalay et al. 2009)

Obesity and physical inactivity are the main non-genetic determinants of the type 2 diabetes (Ohlson et al. 1988, Manson et al. 1991). Overweight (BMI≥25) and obesity (BMI≥30) increase worldwide. The risk of coronary heart disease, ischemic stroke and the type 2 diabetes grows steadily with increasing body mass. It is predicted that in 2015 more than 2.3 billion people worldwide aged 15 years and above will be overweight and more than 700 million will be obese (Chan and Woo 2010). Both obesity and physical inactivity are included in the group of five leading global risk factors for mortality in the world. Only high blood pressure, smoking and high blood glucose are accounted for more mortality in the world. However, in high-income countries solely smoking and high blood pressure caused more mortality than obesity and physical inactivity (Table 1, modified from WHO Global Health Risks 2009).

According to Thyfault and Booth (2011) using human models of physical inactivity (e.g. bed rest and increased sitting time) physical inactivity rabidly impaired metabolic health (e.g. coronary heart disease, metabolic syndrome and type 2 diabetes) which plays a fundamental role in the development of obesity and type 2 diabetes. Ploug et al. (1995) showed that immobilization decreased glucose transport approximately 42% without affecting glucose transporter-1 (GLUT-1) and GLUT-4 protein content in fast-twitch red fibres in rats. In young men, GLUT-4 content in the *vastus lateralis* muscle decreased by approximately 16% after 19 days of bed rest and (Tabata et al. 1999).

Impaired insulin sensitivity of skeletal muscle is an early sign in the pathogenesis of type 2 diabetes and can be observed years before the onset of overt diabetes (Koistinen and Zierath 2002). Skeletal muscle is the main tissue for glucose disposal, accounting for up to 80% of all glucose uptake under insulin-stimulated conditions. Thus, skeletal muscle is a major tissue determining glucose homeostasis (DeFronzo et al. 1981 and Nuutila et al. 1992). However, the acute and chronic regulation of circulating glucose concentrations by insulin is maintained by several organ systems (Figure 1), including skeletal muscle, the liver, adipose tissue, and ß-cells of the endocrine pancreas, the latter being the site of insulin synthesis and secretion (Henriksen 2010).

Table 1 Ranking of selected risk factors (modified from WHO Global Health Risks, 2009)

Table 1: Ranking of selected risk factors: 10 leading risk factor causes of death by income group, 2004

	Risk factor	Deaths (millions)	Percentage of total		Risk factor	Deaths (millions)	Percentage of total
	World				*Low-income countries*[a]		
1	High blood pressure	7.5	12.8	1	Childhood underweight	2.0	7.8
2	Tobacco use	5.1	8.7	2	High blood pressure	2.0	7.5
3	High blood glucose	3.4	5.8	3	Unsafe sex	1.7	6.6
4	Physical inactivity	3.2	5.5	4	Unsafe water, sanitation, hygiene	1.6	6.1
5	Overweight and obesity	2.8	4.8	5	High blood glucose	1.3	4.9
6	High cholesterol	2.6	4.5	6	Indoor smoke from solid fuels	1.3	4.8
7	Unsafe sex	2.4	4.0	7	Tobacco use	1.0	3.9
8	Alcohol use	2.3	3.8	8	Physical inactivity	1.0	3.8
9	Childhood underweight	2.2	3.8	9	Suboptimal breastfeeding	1.0	3.7
10	Indoor smoke from solid fuels	2.0	3.3	10	High cholesterol	0.9	3.4
	Middle-income countries[a]				*High-income countries*[a]		
1	High blood pressure	4.2	17.2	1	Tobacco use	1.5	17.9
2	Tobacco use	2.6	10.8	2	High blood pressure	1.4	16.8
3	Overweight and obesity	1.6	6.7	3	Overweight and obesity	0.7	8.4
4	Physical inactivity	1.6	6.6	4	Physical inactivity	0.6	7.7
5	Alcohol use	1.6	6.4	5	High blood glucose	0.6	7.0
6	High blood glucose	1.5	6.3	6	High cholesterol	0.5	5.8
7	High cholesterol	1.3	5.2	7	Low fruit and vegetable intake	0.2	2.5
8	Low fruit and vegetable intake	0.9	3.9	8	Urban outdoor air pollution	0.2	2.5
9	Indoor smoke from solid fuels	0.7	2.8	9	Alcohol use	0.1	1.6
10	Urban outdoor air pollution	0.7	2.8	10	Occupational risks	0.1	1.1

[a] Countries grouped by gross national income per capita – low income (US$ 825 or less), high income (US$ 10 066 or more).

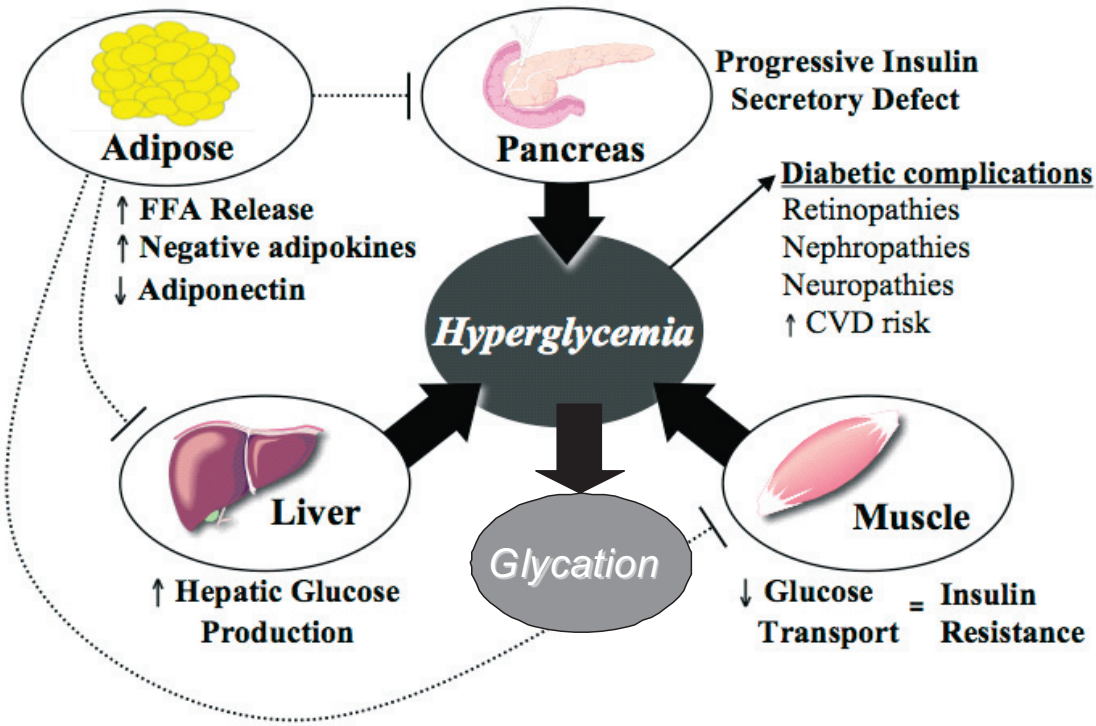

Figure 1. Sites of defects in various organ systems contributing to the development of hyperglycaemia and the related diabetic complications. FFA, free fatty acids; CVD, cardiovascular disease (modified from Henriksen 2010).

2.2 REGULATION OF GLUCOSE DYNAMICS

The acute and chronic regulation of circulating glucose concentrations is coordinated by several organs (Figure 1). Skeletal muscle is the main tissue determining glucose homeostasis and glucose disposal. The glucose dynamics depends on several factors, including glucose transport into the muscles that is influenced by the muscle fibre type composition and the regulation of glycogen synthesis. In hyperglycaemia, glucose oxidation and non-oxidative glucose disposal in skeletal muscle are impaired (Golay et al. 1988 and Thorburn et al. 1990). The major component of the non-oxidative glucose metabolism is glucose stored as glycogen. Particularly insulin resistance in skeletal muscle is associated with an elevated adipose tissue mass (Sell et al. 2006).

Insulin stimulation mediates the transmembrane movement of glucose into the myocytes (Figure2). Insulin binds to the exofacial (outer surface of plasma membrane) insulin receptor α-subunit, thereby activating the tyrosine kinase activity of the membrane spanning ß-subunits. This interaction causes autophosphorylation of the ß-subunits and increases their tyrosine kinase activity. The insulin receptor then phosphorylates insulin receptor substrates (IRS) on tyrosine residues which facilitate the docking of IRS with SH2 domains of the p85 regulatory

subunit of phosphotidylinositol-3-kinase (PI3-kinase) and the activation of the p110 catalytic subunit of this enzyme. PI3-kinase catalyzes the production of phosphoinositide moieties that allosterically activate 3-phosphoinositide-dependent kinases (PDK). PDK1, a serine/threonine kinase, can phosphorylate protein kinase B (Akt) on Thr308, and contribute to Akt activation. These PI3-kinase- and Akt dependent steps are associated with the stimulation of the translocation of GLUT-4, a critical glucose transporter isoform, to the sarcolemmal membrane and the t-tubules, thereby allowing glucose transport to take place by facilitative diffusion (Figure 2). The translocation of GLUT-4 is the major mechanism for insulin-dependent increase in glucose transport activity in mammalian skeletal muscle. (Henriksen 2010). Nevertheless, decreased glucose uptake cannot be explained by decreased expression of the GLUT-4. The decreased function and distribution of GLUT-4 have been suggested to account for the decreased glucose transport in skeletal muscle from subjects with type 2 diabetes (Garvey et al. 1992 and Vogt et al. 1992). However, the GLUT family consists of thirteen members, and GLUT-1, GLUT-4, GLUT-8, GLUT-11 and GLUT-12 are expressed in the human skeletal muscle (Seki et al. 2006 and Zhao and Keating 2007), Seki and co-workers (2006) showed that mRNA content of GLUT-4 was elevated in the exercise-trained subjects compared to sedentary subjects and GLUT-12 was depressed in exercise-trained subjects and there were no difference in GLUT-8 mRNA. Gaster et al (2004) studied protein expression of GLUT-8, GLUT-11 and GLUT-12 in the *vastus lateralis* muscle of obese, type 2 diabetic, sedentary and endurance-trained subjects. They did not find immunoreactivity with their antibody for GLUT-8 or GLUT-12 and concluded that only GLUT-11 is expressed at protein level in adult human skeletal muscle.

Both insulin and exercise (through contraction and hypoxia of myofibrils) stimulate translocation of the glucose transporter protein (GLUT-4) to plasma membrane. These two stimuli act via at least partially independent pathways. Therefore, alternative pathways, additive to insulin-stimulated glucose uptake, could potentially improve glycaemic control in obese insulin-resistant subjects. In addition to insulin stimulation, glucose transport can also be induced by muscle contractions. The key player in this pathway is 5' adenosine monophosphate-activated protein kinase (AMPK). The increased ratio of adenosine monophosphate (AMP) to adenosine-5'-triphosphate (ATP) activates this energy sensor protein. The AMPK is a heterotrimetric protein with catalytic α and regulatory β and γ subunit (Dzamko and Steinberg 2009). The AMPK isoforms $\alpha 1$ and $\alpha 2$ are expressed in skeletal muscle (Winder 2001) and their activities can be increased by moderate intensity exercise but not with low-intensity exercise (at 40%) (Chen et al. 2003).

Glycogen synthesis is impaired in hyperglycaemia like in type 2 diabetes (DeFronzo et al. 1987) because of decreased activity of glycogen synthase (GS), which is the rate-limiting enzyme for this synthesis. Expression of GS protein, however, is normal in the skeletal muscle of type 2 diabetic subjects (Vestergaard et al. 1993). GSK-3 regulates negatively GS (Nikoulina et al. 2000). Moreover, it has been shown that insulin inhibits the action of the glycogen synthase kinase-3 (GSK-3), and the inactivation is associated with phosphorylation of the serine residues Ser21 and Ser9 in GSK-3 isoforms α and β (Nikoulina et al. 2002). The protein expression of the GSK-3 is higher in diabetic subjects than in healthy people.

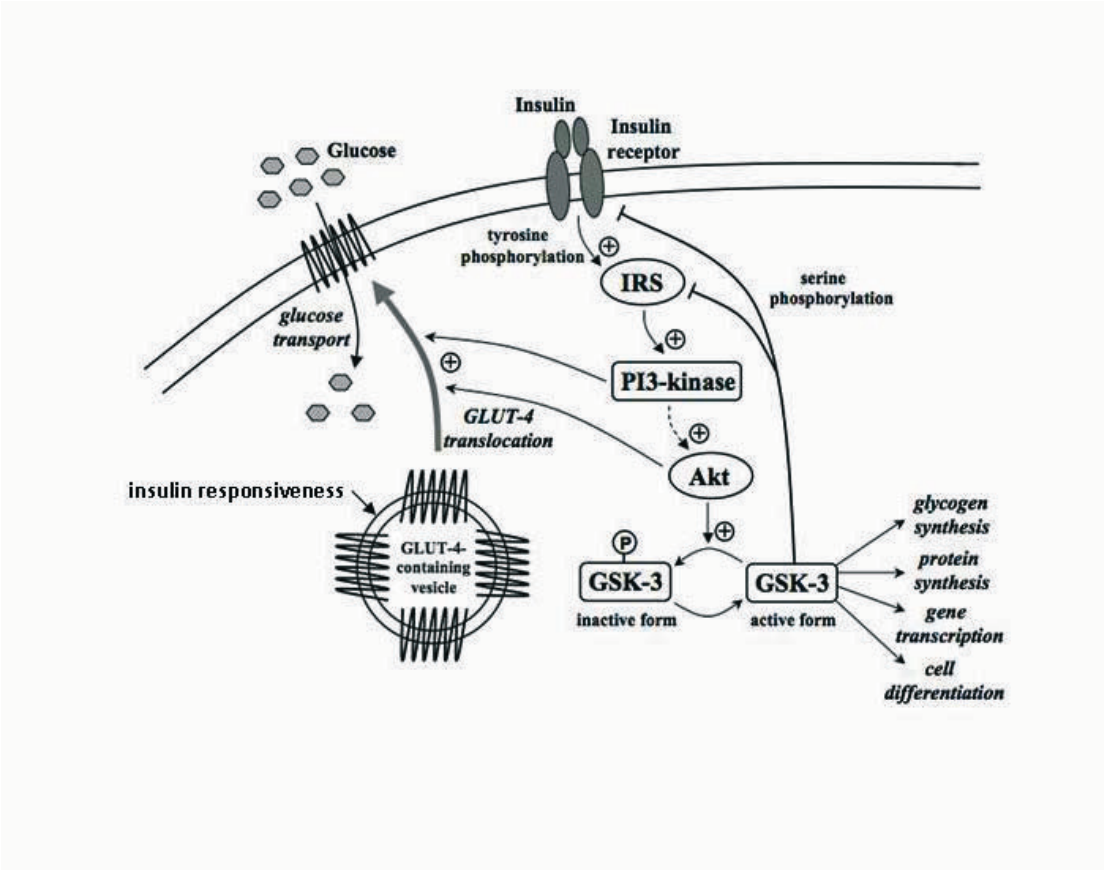

Figure 2. Pathway for regulation by insulin of glycogen synthase kinase-3 phosphorylation and proposed mechanism for the inhibitory effect of glycogen synthase kinase-3 on insulin signalling and glucose transport activity in skeletal muscle. IRS, insulin receptor substrate; PI3-kinase, phosphatidylinositol-3-kinase; GSK-3, glycogen synthase kinase-3, GLUT-4; glucose tranporter-3, Akt; protein kinase B (modified from Henriksen 2010)

2.2.1 Regulation of glycogen synthase kinase-3 in skeletal muscle

Glycogen synthase kinase -3 (GSK-3) is a broadly distributed serine/threonine kinase (Woodgett 2003). GSK-3 is present in two forms: the α-isoform is a 51-53 kDa (kilo Dalton) polypeptide and the β-isoform is a 47- kDa polypeptide (Dolbe and Woodgett 2003). GSK-3 is constitutively active in resting cells and is inhibited by several hormones, such as insulin, the endothelial growth factor, and the platelet-derived growth factor (Nikoulina et al. 2002). GSK-3 is a protein kinase which has the ability to phopshorylate and inactivates glycogen synthase, a rate-limiting enzyme in glycogen synthesis (Dolbe and Woodgett 2003). In addition to its ability to regulate glucose metabolism, GSK-3 mediates the regulation of several cellular processes, including protein synthesis, gene transcription and cell differentiation (Figure 2) (Henriksen 2010).

In type 2 diabetes both basal and insulin stimulated glucose metabolism is impaired in insulin-responsive peripheral tissue like skeletal muscle and liver (Bajaj and Defronzo 2003, Shulman 2004 and Petersen and Shulman 2006). In the skeletal muscle, protein kinase B (Akt) has been identified as the major GSK-3-kinase, and the sites of GSK-3 phosphorylation by Akt have been reported to be the same as those phosphorylated in response to insulin (Cross et al. 1995). Constitutively active GSK-3 can be acutely regulated by Akt-mediated serine phosphorylation. The action is regulated by Akt and by insulin (Henriksen 2010). It has been shown that insulin inhibits the action of the glycogen synthase kinase-3 (GSK-3), and the inactivation is associated with phosphorylation of the serine residues Ser21 and Ser9 in GSK-3 isoforms α and β (Nikoulinna 2002).

Increased expression of GSK-3 protein and activity are poorly controlled in skeletal muscle of type 2 diabetic subjects (Nikoulinna et al. 2000). There are no known disease–associated mutations in the two GSK-3 genes (Hansen et al. 1997), but Pearce and co-workers (2004) have shown that transgenic over-expression of GSK-3-β results in impaired glucose tolerance, elevated plasma insulin and reduced glycogen content in skeletal muscle in male transgenic mice. GSK-3-β is the predominant regulator of glycogen synthase is skeletal muscle, but GSK-3-α is more important regulator of hepatic glucose metabolism and insulin sensitivity than GSK-3-β (MacAulay et al. 2007 and Patel et al. 2008). Inhibitors of GSK-3 have been shown to improve insulin action and glucose metabolism in human skeletal muscle (Nikoulinna et al. 2002). However, these inhibitors do not distinguish these two GSK-3 isoforms (Cohen and Goedert 2004). While both GSK-3-α and GSK-3-β influence insulin signaling, they may do so through a different mechanism (Ciaraldi et al. 2010). However, phoshorylated form of β-isoform is reliably detectable and increased by insulin, and therefore investigation of the role of GSK-3 in insulin resistance of skeletal muscle has focused on β-isoform (Henriksen 2010). In addition to all other factor, increased oxidative stress and disrupted redox regulation affects GSK-3 activity. Oxidative stress can directly and rapidly induce insulin resistance in skeletal muscle which is related in part to reduced insulin-meditated suppression of active form of GSK-3-β *in vitro* (Henriksen 2010).

2.3 HEAT SHOCK PROTEINS AND IMPAIRED GLUCOSE REGULATION

The heat shock proteins (HSPs) are a family of proteins that promote cell survival after a wide variety of environmental stresses. HSPs, originally indentified as heat-inducible gene-products, protect against tissue injury, repairing damaged proteins and promoting healing of the injured tissue (Atalay et al. 2009). HSPs (chaperone function) aid in protein refolding, protect proteins against aggregation and maintain overall integrity of cellular components (Morimoto 1998). HSPs have also less well- characterized roles in inhibition of apoptosis and inflammation and as antioxidants (Geiger and Gupte 2011). The HSP response is a common cellular reaction to external stress and HSPs are synthesized shortly after the organism has been exposed to stress (Liu et al. 2006). The induction of HSPs can be physical, biochemical or pharmacological. Most of physiological and pathophysiological inducers of HSPs take place during strenuous physical exercise including heat shock, ischemia-reperfusion, decreased ATP levels and changes in pH and in osmolarity (Liu and Steinacker 2001). A number of different HSPs have been indentified (Atalay et al. 2009). HSPs are classically classified according their molecular mass (Noble et al. 2008. In the skeletal muscle most prominent HPSs are small HSPs (e.g. alpha B-crystallin), and HSP60, HSP70 and HSP90 (Liu et al. 2006). A number of studies have shown the expression of HSPs to vary depending on the muscle fibre type (Locke et al. 1991, Ornatsky et al. 1995 and Liu and Steinacker 2001).

Diabetes exerts a negative impact on tissue protection through a number of mechanisms including impaired HSP defence. The effect of diabetes on HSP defence is tissue-specific (Atalay et al. 2009). Expression of HSP72 decreased in insulin-sensitive tissues like heart, liver and skeletal muscle in diabetic rats (Atalay et al. 2004) and in liver diabetic monkeys (Kavanagh et al. 2009) but in pancreas of diabetic monkey its expressions is up-regulated and expression of HSP60 is higher in non-insulin-sensitive organ, kidney (Shan et al. 2003).

The most widely studied HSP family is the 70-kDA family, which contains the constitutive HSP73 and inducible HSP72 forms. In hyperglycaemia and in type 2 diabetic subjects, insulin resistance correlates with decreased expression of HSP72 in skeletal muscle (Kurucz et al. 2002). However, HSP72 mRNA levels did not correlate with the fasting glucose or insulin levels in any investigated subgroups (Kurucz et al 2002), but HSP72 mRNA levels correlated glucose disposal rate and oxidative capacity of tissues (Bruce et al. 2003). HSP72 plays a central role in protein synthesis, translocation, folding and assembly/disassembly of multitrimetric protein complexes as molecular chaperones (Bukau and Horwich 1998).This family also includes 75-kDA and 78-kDA proteins which are termed glucose-regulated protein 75 (GRP75) and glucose-regulated protein 78 (GRP78) (Liu et al. 2006).

GRP75 and GRP78 are not specifically induced by heat shock, but by glucose deprivation and calcium influx or agents, which perturb glycolysis (Locke and Noble 1995). GRP75 and HSP60 are located in the mitochondria, where they are involved in the trafficking and processing of nuclear encoded peptides (Kang et al. 1990 and Martin et al. 1992). HSP60 is constitutetively expressed in mitochondria, and stabilizes also pre-existing proteins under stress (Martin et al. 1992). HSP90 is expressed in the cytosol, nucleus and endoplasmic reticulum (Basso et al 2002) and has several physiological roles and it mediates protein kinase B (PKB/AKT) stability and tyrosine kinase receptor maturation (Csermely et al. 1998 and Basso et al. 2002). Højlund et al. (2003) reported increased HSP90 levels in the skeletal muscle of diabetic subjects but they found no correlation between the fasting plasma glucose and increased HSP90 levels. It is interesting observation because HSP90 plays important role in maintenance of protein kinase B activity which is involved in the regulation of both insulin-stimulated glucose transport and glycogen synthesis (Atalay et al. 2009).

In insulin resistant states and diabetes, heat shock factor 1 (HSF-1) is low in insulin sensitive tissues, resulting in low HSP60, HSP 70 and HSP90 levels. (Hooper 2007). HSF-1 activation is reduced in chronic hyperglycaemia in streptozotocin-induced DM monkeys (Kavanagh et al. 2011) and in skeletal muscle in streptozotocin-induced DM rats (Atalay et al. 2004). Low HSPs make organs vulnerable to injury, impair the stress response, accelerate systemic inflammation, raise islet amyloid polypeptide, and increase insulin resistance (Hooper and Hooper 2009). Increased GSK-3 activity (less phosphorylated GSK-3) lowers expression of heat shock factor-1 (HSF-1), a major transcription factor for heat shock protein genes (Hooper 2007, Atalay et al. 2009). Also decreased expression of HSF-1 in insulin resistant tissue could be a result of its inflammatory inhibition (Geiger and Gupte 2011). The antioxidant function of HSPs may have important role in tissue protection against uncontrolled oxidative stress which is one of characteristic features of diabetes (Atalay et al. 2009).

2.3.1 Heat shock proteins and exercise

Exercise is the safest model to study metabolic stress and it's adaptations including HSP induction. It has been well documented that both acute and chronic exercise induces HSP expression (Yamada et al. 2008). Of our interest in addition to physical exercise age and sex (oestrogen levels), HSP response can be influenced by glucose, insulin and antioxidants. The most studied HSP family in response to exercise is HSP70 family in both humans and in different animal models (Liu et al. 2006 and Yamada et al. 2008). Most of the human studies related to HSP70 response to exercise are focused on skeletal muscle (m. *vastus lateralis*) or on lymphocytes (Liu et al. 2006 and Yamada et al. 2006). Intracellular HSP70 has cytoprotective effects, while extracellular HSP70 has whole-body systemic role in antigen presentation and immunity (Yamada el al. 2008). Responses to exercise are much less studied for the other HSPs such as HSP60, GRP75 and HSP90.). It has been well demonstrated that induction of HSPs are dependent on the duration and the intensity of the exercise. In humans, HSP70 induction after acute exercise may require subjects to exercise at lactate threshold or perform one legged exercise (Yamada et al.2008). HSP70 induction is related to the intensity of the training. Vogt et al. (2001) showed that high-intensity group had significantly higher post training HSP72 levels than low-intensity group. Liu's study (1999) showed that intensive 4 weeks rowing training increased skeletal muscle HSP72 significantly. Nevertheless, whether the HSP72 induction is due to metabolic stress or muscle damage cannot be concluded. Much less studied entity is the HSP72 response in sedentary or non-athletic population. In Willoughby's study (2002) 12 weeks training program involving seven subjects with motor-complete spinal cord injury increased also HSP72 in skeletal muscle.

Induction of HSP60 expression in skeletal muscle of healthy humans has been reported after short term exercise program (Khassaf et al.2001, Morton et al. 2006). In animal studies, eight weeks of endurance training increased GRP75 expression in rat skeletal muscle (Atalay et al.2004), and both GRP75 and HSP60 were upregulated in rat skeletal muscles after eighth weeks of endurance training (Mattson et al. 2000). In both Atalay's (2004) and Mattson's (2000) studies the upregulations of GRP75 and HSP60 were associated with increased skeletal muscle citrate synthase activity.

Induction of HSPs following "non-damaging" endurance type activities is thought to be mediated by redox signaling (Morton et al. 2009). Non-damaging exercise protocols may provide a pure methodological approach to study the exercise-induced regulation of HSPs, without causing any inflammatory response (Vasilaki et al. 2006). Damaging exercise models such as resistance training and down hill running have also been used to study stress response of human muscle (Morton et al. 2006). Interpretation of data from damaging exercise is more complicated due to inflammatory response which may have effect on HSPs response (Khassaf et al. 2003).

2.3.2 Factors affecting the HSPs response

HSPs mRNAs are increased both during and immediately after the exercise, as well as, several hours during post-exercise (Morton et al. 2009) and this response has been suggested to be muscle fibre-specific: HSP70 content was increased 80% in *m. vastus lateralis*, and it was specific to type I fibres only (Tupling et al. 2007). In rats, the expression of HSP72 was higher in type I fibres than type II fibres at basal level (Locke et al. 1991). Similarly in healthy and streptozotocin-induced diabetic rats rats HSP72 protein levels were highest in type I red gastrocnemius muscle but induction of 8 weeks of treadmill training was more apparent in type II white vastus lateralis muscle (Atalay et al. 2004).This pattern has not been observed for other HSPs including HSP90, mitochondrial GRP75 and oxidative stress responsive HO-1 (Atalay et al. 2004) Nevertheless, Ornatsky et al. (1995) have shown that highest HSP60 content is found in heart followed by type IIa fibres of *m. gastrocnemius*, type I fibres of *m. soleus* and type IIb fibres of *m. gastrocnemius* in rats. Also GRP75 expression has a very similar pattern as HSP60 in rat muscles (Ornatsky et al. 1995). Therefore, fibre-specific stress response of particular HSPs may be unique. In addition, the intensity, duration and quality (damaging/non-damaging) of exercise, fibre recruitment and study population should be taken into consideration before time-course of synthesis and degradation and fibre specificity of HSPs can be accurately defined (Morton et al. 2009). High individual variations of the stress responses (magnitude and time-course) occur in human skeletal muscles (Morton et al. 2009). Training status (Gonzalez et al. 2000), recent activity levels (Campisi et al. 2003), thermal history (Kregel 2002), energy availability (Febbraio et al. 2002), sex (Paroo et al. 2002) and age (Vasilaki et al. 2002) are possible determinants that affect the baseline levels of HSP or extent of HSP response (Morton et al. 2009).

Very little is known about the effects of weight loss or diet on the expressions of HSPs. Selsby et al. (2005) have however shown that life-long calorie restriction increases both HSP72 and HSP90 levels in rat soleus muscle and decreases protein carbonyls. Also caloric restriction (without malnutrition) has been shown to attenuate the age associated increases in ROS production (López-Torres et al. 2002)

2.4 OXIDATIVE STRESS

Increased oxidative stress as measured by indices of lipid peroxidation and protein oxidation have been shown to be increased in hyperglycaemia and both in insulin dependent and non-insulin dependent diabetes (IDDM and NIDDM, respectively) (Atalay and Laaksonen 2002). In oxidative stress, the increased production of reactive oxygen species (ROS) overwhelms endogenous antioxidant protection and it may result in biomolecular damage, i.e., oxidative damage, inflamation and even apoptosis (Domínguez et al. 1998, Hotta et al. 1998 and Atalay and Laaksonen 2002) Exercise has been shown to up-regulate the muscle antioxidant defence systems (Ji 2008).

Oxidants are produced as a by-product of aerobic metabolism (eg. Janssen-Heiniger et al. 2008). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are well recognized for playing a dual role as both deleterious and beneficial species (Valko 2007). Both aerobic and anaerobic metabolism is accompanied by the production ROS and/or RNS (Janssen-Heiniger et al. 2008). Overproduction of ROS results in oxidative stress that can be an important mediator of damage to cell structures, including lipids and membranes, protein and deoxyribonucleic acid (DNA)(Valko 2007).

Oxidative stress markers are usually measured in studies designed to assess the redox status of response to exercise, nutrition or xenobioticts (Veskoukis et al. 2009) However, the majority of the relevant human studies have utilized blood samples and only a few of them used samples of other tissue types (Veskoukis et al. 2009). In the study of Veskoukis et al. (2009), the aim was to examine whether the blood samples reflect what happens in skeletal muscle tissue. They used samples of the blood, heart, liver and skeletal muscle tissue obtained from the rats and measured contents of xanthine oxidase (XO), thiobarbituric acid-reactive substances (TBARS), protein carbonyls (PC), reduced glutathione (GSH), oxidized glutathione (GSSG), catalase (CAT) and total antioxidative capacity (TAC) at baseline and after exercise and after allopurinol administration in order to get various types of effects on responses of the redox status (Veskoukis et al. 2009). GSSG measured from erythrocytes proved to be the best marker to describe redox status in all examined tissues. Protein carbonyls, GSH and catalase measured from blood samples may adequately reflect the redox status of the heart and skeletal muscle. However, no significant correlations TAC or calculated ratio of GHS/GSSG between blood and any examined tissue were found (Veskoukis et al. 2009).

Biomarkers of oxidative damage associated with diabetes mellitus are e.g. AGE (advanced glycation end products), F_2-isoprostanes (non-enzymatic products of arachidonic acid oxidation), GSH/GSSG ratio (GSH; reduced glutathione and GSSG; oxidized glutathione), HNE (4-hydroxy-nonenal), MDA (malondialdehyde) and NO_2-Tyr (3-nitro-tyrosine) (Valko 2007).

Oxidative stress may have important role in pathophysiology of insulin resistance, diabetes and its complications (Atalay and Laaksonen 2002). In type 2 diabetes, insulin sensitivity is inversely correlated with the levels of plasma ROS and oxidative stress (Paolisso et al.1994). Increased production of ROS has been reported in diabetic subjects, and hyperglycaemia decreases glutathione synthesis and impairs antioxidant defence (Laaksonen et al. 1996, Atalay et al. 1997, Laaksonen et al. 1999 and Atalay and Laaksonen 2002). Decreased uptake of glucose into muscle and adipose tissue leads to chronic extracellular hyperglycaemia resulting tissue damage. Increased oxidative stress has been proposed to be one of the major causes of the hyperglycaemia-induced trigger of diabetic complications like heart diseases, cataract formation, peripheral nerve damage and retinopathy (Valko 2007).

Hyperglycaemia stimulates ROS formation from variety sources like oxidative phosphorylation, glucose auto-oxidation, NADPH oxidase, lipooxygenase, cytochrome P450 monooxygenases, xanthine oxidase (XO) and nitric oxide synthase (NOS). Treatment of non-insulin dependent diabetes subjects with the XO inhibitor allopurinol reduces oxidized lipids in plasma. In addition to ROS, RNS (reactive nitrogen species) have been implicated as sources of nitrosative stress in diabetes (Valko 2007) It is well-known that oxidative stress, in which the increased production of ROS overwhelms endogenous antioxidant production, may result in biomolecule damage. However, at lower concentrations, ROS also serve as secondary messengers, regulating cellular functions and adaptations. A more recent, complementary definition of oxidative stress emphasises a disruption of redox circuits resulting in an imbalance of cell signalling and distraction of redox-control (Jones 2006). In specific, the function and homeostasis of thiol redox circuits are among the most central features of oxidative stress (Jones 2008). In addition the signaling oxidants — which include hydrogen peroxide (H_2O_2), nitric oxide (NO) and S-nitrothiols (SNO) — are produced mainly by NADPH-dependent enzymes (NO synthases and NADPH oxidases) (Janssen-Heiniger et al. 2008). A numerous physiological functions are controlled by redox-responsive signaling pathways (Dröge 2002). These, for example involve: oxidative burst, regulation of vascular tone, redox regulation of cell adhesion and immune responses and a sensor for changes of oxygen concentration (Valko 2007).

Probably the most significant effect of ROS on signaling pathways has been observed in the pathways for mitogen activated protein kinases (MAPK) (Sun and Oberley 1996), involving activation of nuclear transcription factors, including activator protein-1 (AP-1) controlling cell growth and differentiation, hypoxia-inducible factor-1 (HIF-1) regulating many cancer related genes, nuclear factor of activated T-cells (NFAT) that regulates cytokine formation, muscle growth and differentiation, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factors which is associated with inflammatory responses and protein 53 (p53), a guard for the cell-cycle checkpoint(Valko et al. 2007)

The mentioned signaling pathways control the expression of protective genes that repair damaged DNA, power the immune system, arrest proliferation of damaged cells and induce apoptosis (Valko et al. 2007). In addition, physiological ROS signaling also exercise-induced increases in ROS are now accepted as important cellular messengers in signal transduction (Morton et al. 2009). Exercise-induced ROS can activate important transcription factors such as nuclear factor NF-κB AP1 (activator protein 1) and HSF1 (Pattwell and Jackson 2004).

2.4.1 Antioxidative defence

Exposure to free radicals from a variety of sources has led organism to develop several defence mechanisms (Cadenas 1997). The redox states of thiol systems are controlled by thioredoxins (Trx) and glutathione (GSH) and cysteine (Cys) (Jones 2008). Endogenous thiol antioxidants Trx and GSH are potent protein disulfide reductases (Hofmann et al. 2002). Previously, Trx and GSH were considered for separate systems. However, loss of cellular Trx is known to result in elevation of GSH, indicating a link between the Trx and GSH metabolism (Carmel-Harel and Storz 2000). TRx is also a major hydrogen donor for ribonucleotide reductase and has an important role in DNA replication (Holmgren 2000). Furthermore, Trx has been shown to provide protection against autoimmune as well as experimental diabetes (Hotta et al.1998). Disruption of thiol redox circuits leads to aberrant cell signaling and dysfunctional redox control (Jones 2008).

Type 2 diabetes and cardiovascular diseases are linked to oxidative stress and dysfunctional redox control. Oxidized plasma GSH/GSSG has been shown to associate with type 2 diabetes (Samiec et al. 1998). Cys/CySS redox state stimulated monocyte adhesion to endothelial cell, an early proinflammatory step in atherogenesis, by generation H_2O_2, activation NF- κB and transcriptional activation and increased cell surface expression of adhesion molecules (Go and Jones 2005).

Defence mechanisms against ROS/RNS-induced oxidative stress involve preventative and repair mechanisms and physical and antioxidant defences (Valko et al. 2007) Enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT). Non-enzymatic antioxidants include, e.g., ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids and many other antioxidants (Valko et al. 2007). It has been shown that in diabetic patients the plasma concentration of reduced vitamin C is decreased, and also vitamin E is depleted (Valko et al. 2007). Vitamin E has a protective effect mainly by suppressing lipid peroxidation (Valko et al. 2007). The antioxidative function of HSPs could be helpful to protect against diabetic complications (Atalay et al. 2009).

2.5 ADIPOKINES

Low-grade systemic inflammatory state where plasma levels of inflammatory cytokines are increased, is associated with obesity and it is considered a potential mechanism linking obesity to insulin resistance, hyperglycaemia, type 2 diabetes and cardiovascular diseases (Hirai et al. 2010). The first discovery of inflammation in obese subjects was increased TNF-α expression in adipose tissue in an animal study (mices) (Hotamisligil et al. 1995). Nowadays we know that not only TNF-α but an array of inflammatory cytokines are increased in obese tissue (adipose tissue). In obesity increased cytokine expression occurs also in brain, liver and pancreas and possibly in muscle tissue, however, adipose tissue is predominant (Gregor and Hotamisligil 2011).

Adipose tissue is composed of adipocytes embedded in the loose connective tissue meshwork which contains also adipocyte precursors, fibroblast, immune cells and various other cells (Rabe et al. 2008). Adipose tissue is active endocrine organ that releases a large number of bioactive mediators (adipokines) which modulate blood pressure, glucose and lipid metabolism, inflammation and atherosclerosis (Rabe et al. 2008). Adipokines are cytokines, chemokines and hormones secreted by adipose tissue. The adipokines include a large number of cytokines (e.g. interleukin-6 (IL-6) and TNF-α), chemokines (e.g. interleukin-8 (IL-8) and monocyte chemoattractant protein -1 (MCP-1) and hormones (e.g. adiponectin and leptin) (Zhang et al. 2010). Some of the important features of adipokines are as follows: TNF-α (down regulates IRS-1 signaling) and leptin resistance (reduced activation of 5′-AMP activated protein kinase; AMPK) are associated with insulin resistance (Kirwan and Jing 2002, Dyck 2005).

Adipokines have various effects on glucose homeostasis. Adiponectin circulates in plasma as a low-molecular weight trimer, a middle-molecular weight hexamer and high-molecular weight (HMW) 12- to 18-mer (Waki et al. 2003, Wang et al. 2006). HMW adiponectin has been proposed to be the most biologically active form of the hormone (Pajvani et al. 2004). Nevertheless, the results are controversial in comparison to total adiponectin (Rabe et al. 2008). Unlike most other adipokines, plasma adiponectin levels are reduced in animal models of obesity and insulin resistance. In humans, plasma adiponectin correlates negatively with adiposity (Cnop et al 2003, Hanley et al. 2007), insulin resistance (Weyer et al. 2001, Bacha et al. 2004), metabolic syndrome (Gilardini et al. 2006) and type 2 diabetes (Weyer et al. 2001). The effects of adiponectin on glucose metabolism are mediated by two distinct receptors. Adiponectin receptor 1 (AdipoR1) which is expressed ubiquitously and adiponectin receptor 2 (AdipoR2) is expressed most abundantly in liver (Yamauchi et al. 2003). Adiponectin has many metabolic functions. It suppresses hepatic gluconeogenesis; stimulates fatty acid synthesis in liver and skeletal muscle and glucose uptake in skeletal muscle and insulin secretion. It also suppresses IL-6 and TNF-α expression and modulates food intake and energy expenditure (Rabe el al. 2008).

Leptin was indentified first time in 1994 (Friedman and Haalas 1998). Since then it has attracted lots of attention because of its regulatory effects on food intake and energy homeostasis. Leptin also improves peripheral (hepatic and skeletal muscle) insulin sensitivity, stimulates fatty acid oxidation in the liver, pancreas and skeletal muscle and modulates pancreas β-cell function (Rabe et al. 2008). In addition, leptin limits accumulation of triglycerides in liver and skeletal muscle and stimulates adiponectin expressions (Unger 2002).

IL-6 and TNF-α have been most widely studied cytokines produced by adipose tissue (Rabe et al. 2008). Animal studies have shown that the deletion of TNF-α or TNF-α receptors resulted in significantly improved insulin sensitivity both in diet-induced obese mice and in leptin deficient ob/ob mice (Uysal et al. 1997). In humans, adipose tissue TNF-α expression correlates

with BMI, percentage of body fat and hyperinsulinemia (Jellema et al. 2004). Also fasting plasma TNF-α levels have been found to associate with insulin resistance (Hivert et al. 2008).

Interleukin-6 (IL-6) has been considered as a marker of inflammation and an immuno-modulatory cytokine (Febbraio and Pedersen 2002). IL-6 is mainly produced by the immune cells. Also it is secreted by skeletal muscle, and plasma levels of IL-6 may rise up to 100-fold after a strenuous physical exercise (Pedersen et al. 2003). In healthy humans IL-6 stimulates lipolysis and increase fat oxidation (van Hall et al. 2003). IL-6 has been reported to reduce insulin-dependent hepatic glycogen synthesis, glucose uptake in adipocytes, insulin-dependent glycogen synthesis in skeletal muscles and to enhance glucose uptake into myotubes (Rabe et al. 2008), but conflicting results exist regarding the role of IL-6 in insulin resistance (Kim et al. 2004, Inoue et al. 2006). Nevertheless, in obese and insulin resistant state, increased circulating IL-6 levels have been found in humans (Kern et al. 2001).

3 AIMS OF THE STUDY

The current study was designed to assess the possible role of exercise training with dietary counselling on skeletal muscle glucose metabolism, HSP defence and plasma adipokine levels in middle-aged subjects with impaired glucose tolerance. More specifically, the following questions were addressed:

1. To clarify the role of skeletal muscle fibre type in the regulation of glucose metabolism in middle-aged obese subjects with impaired glucose tolerance (IGT) during a 2-year exercise and dietary intervention;
2. to clarify if exercise and dietary intervention improves the antioxidative capacity and HSP defence of different skeletal muscle fibre phenotypes in middle-aged obese subjects with IGT and
3. to clarify the role of adipokines in regulation of glucose metabolism in middle-aged obese subjects with IGT during a long term exercise and dietary intervention based on their response to weight loss.

In addition to human studies, responses of spontaneous physical activity and intensive exercise on skeletal muscle following an immobilisation period were tested in rats.

4 MATERIAL AND METHODS

4.1 PARTICIPANTS AND STUDY PROTOCOLS

The current study is a substudy of the Finnish Diabetes Prevention Study (DPS) which was a multicenter study carried out in 1993–2000 by five participating centers, located in Helsinki, Kuopio, Oulu, Tampere and Turku. The design of the Diabetes Prevention Study has been described in detail elsewhere (Eriksson et al. 1999 and Tuomilehto et al. 2001). Its course was briefly as follows: Overweight subjects (BMI > 25) with IGT, aged 40 to 65 years were eligible for the study. IGT was defined as 2-hour plasma glucose concentration of 7.8–11.0 mmol/l for oral glucose tolerance test (OGTT) with an intake of 75 g glucose in subjects whose fasting plasma glucose concentration was less than 7.8 mmol/l (WHO 1985). The test was repeated for subjects showing abnormal values, and the mean of the two 2-hour plasma glucose values was used as the inclusion criterion.

In Turku, altogether 110 obese subjects with IGT (I–III), based on two oral glucose tolerance tests, were randomized to an intervention (n=55) or a control group (n=55). The participants for this substudy were composed of 22 persons (aged 55.9 ± 7.1 years; 6 female and 16 male subjects) from the intervention group who volunteered to give muscle samples from their *m. vastus lateralis*. Almost all of these 22 subjects were physically inactive before the study, only two subjects exercised to increase their fitness for at least 3 hours/week. Four subjects were not working outside the home, and the work strain of the rest of the subjects was low. Only one subject reported doing heavy manual work and two subjects reported their work to comprise of plenty of walking, carrying and/or lifting.

In order to study role of skeletal muscle fibre types in glucose metabolism (I), the subjects were divided into two categories (IGTslow and IGTfast) on the basis of their baseline myosin heavy chain profile. Depending on the proportion of myosin heavy chain II fibres (MHC II fibres), the subjects belonged either to the IGTslow group (less than 55% of MHC II fibres) or to the IGTfast group. In general, men and women have, on an average, 45% and 55% of MHC I fibres (slow oxidative fibres) (Mahon et al. 1984, Staron et al. 2000). The characteristics of the subjects and their exercise activity during the intervention are shown in Table 2.

Table 2. Baseline characteristics of the two myosin chain profile groups, IGTslow and IGTfast, of subjects with impaired glucose tolerance (IGT) and their exercise activity during the intervention.

Characteristics	**IGTslow**	**IGTfast**
n = (Female/Male)	10 (4/6)	12 (2/10)
Age (years)	58.5 ± 6.1	54.1 ± 7.1
BMI	29.9 ± 2.1	29.9 ± 2.3
Weight (kg)	86.9 ± 8.8	92.0 ± 7.8
Myosin heavy chain profile (%)		
MHC I	46.4 ± 9.0	30.6 ± 3.6
MHC IIa	36.9 ± 11.1	47.1 ± 6.8
MHC IIx	16.7 ± 10.5	22.7 ± 7.4
Exercise frequency (hour/week)		
Strength & power training	1.1 ± 0.6	1.6 ± 1.2
Moderate & heavy aerobic training	1.3 ± 1.2	1.2 ± 0.9
Walking & light aerobic training	2.5 ± 1.7	1.7 ± 2.3
Total amount of training	5.1 ± 2.7	5.1 ± 2.8

Data is given as means ± SD.

In order to investigate the roles of antioxidative capacity and HSP defence in regulation of glucose metabolism (II), the subjects were divided into IGTslow group (less than 55% of MHC II fibres) or to the IGTfast group, depending on the proportion of muscle myosin heavy chain II (MHC II) fibres. The baseline characteristics of the subjects are shown in Table 3.

Table 3. Baseline characteristics of the subjects with impaired glucose tolerance (IGT) divided into the slow and fast fibre type subgroups.

Characteristics	**IGTslow**	**IGTfast**
N = (Female/Male)	10 (4/6)	12 (2/10)
Age, yr	58.5 ± 2.0	54.1 ± 2.0
BMI (kg/m^2)	29.9 ± 0.7	29.9 ± 0.7
Weight, kg	86.9 ± 2.8	92.0 ± 2.2
Myosin heavy chain profile		
MHC I, %	46.4 ± 2.9	30.6 ± 1.0***
MHC IIa, %	36.9 ± 3.5	47.2 ± 2.0**
MHC IIx, %	16.7 ± 3.3	22.2 ± 2.1
Blood chemistry		
Fp-glucose, mmol/l	6.0 ± 0.2	6.0 ± 0.1
2h-glucose, mmol/l	7.8 ± 0.3	7.5 ± 0.5
Fs-insulin, µU/ml	12.9 ± 1.0[a]	19.5 ± 2.7*[b]
2h-insulin,µU/ml	72.0 ± 13.0[a]	117.0 ± 35.0[b]
HbA_{1c}, %	5.7 ± 0.1	5.7 ± 0.1
HOMA-IR	3.5 ± 0.3[a]	5.2 ± 0.7[b]

Data is given as means ± SE; *P < 0.05, **P < 0.01, ***P < 0.001; [a] n = 9, [b] n = 11; Kruskall-Wallis test for differences between the groups.

To study the role of adipokines and some co-factors in regulation of glucose metabolism (III), the subjects were divided into two subgroups, non-responders (n = 9) and responders (n = 13) according to their weight loss during intervention. The subgroup of non-responders was composed of IGT subjects without weight loss or their weight loss was equal to or below 1.3 kg (range 3.0 – 1.3 kg; group change +0.33 ± 0.6 kg) and the subgroup of the responders was composed of IGT subjects achieving at least a weight loss of 2.9 kg (range 2.9 – 14.5 kg; group change –7.7 ± 1.0 kg) during the intervention (Figure 3).

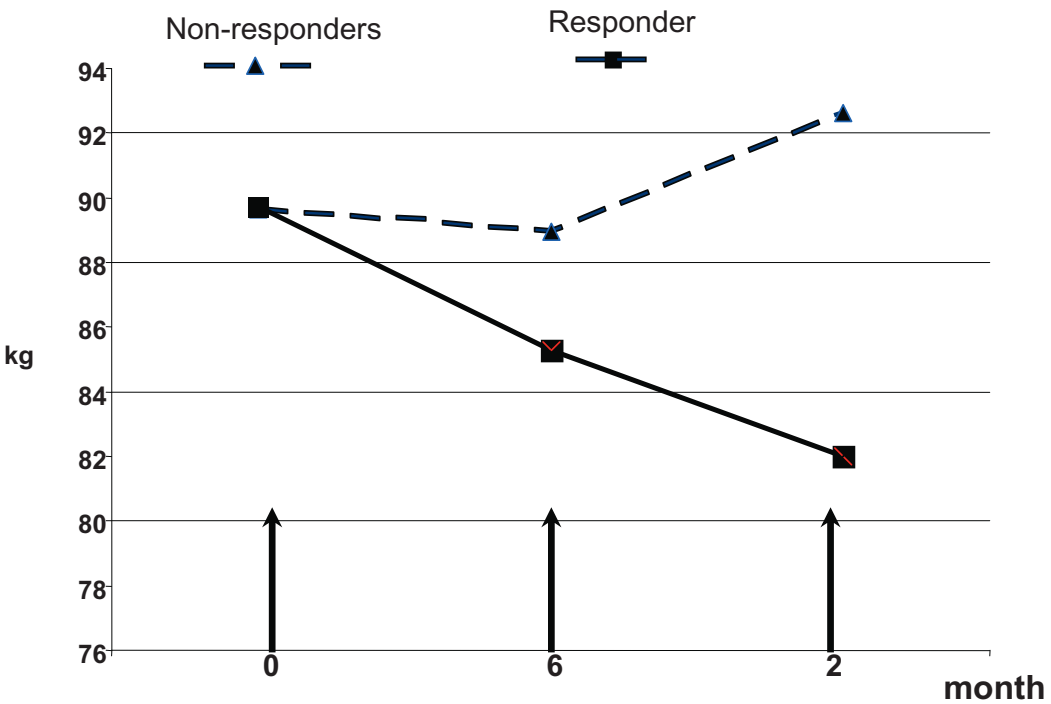

Figure 3. Weight loss during 2-year intervention of IGT subjects.

The characteristics of the subjects are shown in Table 4.

Table 4. Baseline characteristics of the subjects having impaired glucose tolerance (IGT) divided into the non-responders and responders.

Characteristics	**Non-responders**	**Responders**
N = (Female/Male)	9 (2/7)	13 (4/9)
Age (years)	57.1 ± 2.3	55.1 ± 2.0
Height (cm)	174.2 ± 1.5	172.6 ± 2.2
Weight (kg)	89.7 ± 1.8	89.7 ± 2.9
BMI	29.5 ± 0.6	30.1 ± 0.7
WHR	0.96 ± 0.02	0.96 ± 0.02
Aerobic performance capacity		
$VO_{2max,}$ l/min	2.37 ± 0.22	2.26 ± 0.18
$VO_{2max,}$ ml/kg/min	26.7 ± 2.7	26.2 ± 1.9
Muscle fibre type composition		
MHC I	38.0 ± 3.0	37.9 ± 3.3
MHC IIa	41.6 ± 2.7	43.4 ± 3.2
MHC IIx	21.0 ± 2.7	18.7 ± 2.8
Dietary intake		
Energy intake, kcal/day	1823 ± 143	1925 ± 169
Alcohol, E %	2.6 ± 1.3	4.5 ± 1.2
Carbohydrates, E %	44.7 ± 2.0	42.5 ± 1.8
Fat, E %	35.6 ± 1.6	35.1 ± 1.1
Protein, E %	17.1 ± 1.2	17.9 ± 0.7
Dietary fibre, g/day	22.3 ± 2.1	19.7 ± 1.9

Data is given as means ± SE.

The effects of immobilization and remobilization on HSP expression in skeletal muscle were studied with a controlled and randomized intervention trial using rats as experimental animals (IV). Fifty-four, 10–12 week old male Spraugue-Dawley rats (body weight 396 ± 36 g) were used in this study. The rats were fed on standard laboratory chow and water *ad libitum* and were housed in a temperature-controlled room (21 ± 3°C) with a 12:12 h light-dark cycle. The rats were divided into four groups: control group (C, n = 7), immobilization group (IM, n = 7), active mobilization group (AM, n = 24) and free mobilization group (FM, n = 16). The rats in the three experimental groups were immobilized for four weeks as previously described (Jozsa et al. 1988).

4.2 INTERVENTION

The main goals of the intervention were as follows: 1) weight reduction of 5% or more, 2) less than 30% of the daily energy intake from fat, 3) less than 10% of the daily energy intake from saturated fat, 4) fibre intake 15 grams per 1000 kcal or more, and 5) moderately intense physical activity for 30 minutes per day or more. The implementation of the intervention program has been reported previously (Eriksson et al. 1999, Tuomilehto et al. 2001 and Lindström et al. 2003). Briefly, the participants in the intervention group were given detailed and individualized counselling to achieve the set lifestyle goals. They had seven individual counselling sessions with the nutritionist during the first year and every three months thereafter. During the first 6 months, the intervention focused on dietary counselling, in order to allow the subjects to concentrate on changing their eating habits. The subjects were also individually encouraged by the nutritionist to increase their physical activity, and later on, to participate both in regular resistance training at a gym and in aerobic exercise. Thereafter, a supervised exercise training twice a week was added to the intervention programme.

At the beginning, the supervised program consisted of brisk walking together with light gymnastics and stretching exercises, then circuit training without weights, and after a month, basic muscular strength training with weights, equal to 20 to 40% of 1RM (=repetition maximum). Later on, both strength exercises and power exercises with heavier weights (50–70% and 30–50% of 1RM, respectively) were included in order to increase muscle mass and training of the fast twitch fibres. The exercise protocol was designed to recruit both MHC I (cf. type I) and MHC II fibres (cf. type II). At the beginning of the training, the number of circuits was three and later on four, depending on the participant's physical fitness. The number of repetitions in a set was 10–15 at the beginning, and later on it varied with the type of training. The supervised training was progressive and individually designed, consisting mostly of strength and power training, interspersed with spinning exercises and aerobic gymnastic exercises. Power type strength training at a gym was performed as circuit training. In addition, subjects who had previously been involved in recreational sports activities were encouraged to carry on these exercises. All physical activity lasting for 30 min or longer was recorded by the participants. Some subjects had temporary interruptions in their training program due to illness or leg or trunk troubles, lack of motivation, work commitments or some other reason.

Intervention of rats (IV)
Intervention started with four weeks of immobilization which followed an active mobilization period for six days. After immobilization period, the AM group began strenuous training, running in a treadmill 15 degrees uphill twice a day for 15 min at a speed of 12 m/min. After the third session, an additional phase of exhaustive running at a speed of 18 m/min for 5 to 20 minutes was added to the basic programme. Also, the incline of treadmill was increased to 20 degrees on the third day and to 25 degrees on the fifth day and the active mobilization continued for one day more. The FM rats had no active mobilization, but moved freely in their cages immediately after the cast removal.

4.3 ETHICAL APPROVAL

The Ethical Committee of the Hospital District of South-West Finland, Turku, Finland and the Ethical Committee of the Rehabilitation Research Centre of the Social Insurance Institution of Finland approved the protocol of this substudy (I–III) and all subjects gave their written informed consent to participate. In study IV, the experimental procedures were approved by the Ethical Committee for Animal Experiments, University of Turku, Finland.

4.4 BLOOD AND MUSCLE SAMPLES

Blood samples for metabolic indices were taken at the baseline and after 2 years and a maximal exercise test and muscle sampling (with no heavy exercising during the preceding 2 days) were performed at 6 months and after 2 years (I-III). A skeletal muscle biopsy was taken, under rest conditions (no heavy exercising during the preceding 2 days) from the *vastus lateralis* muscle under local anesthesia (lidocaine 10 mg/ml), using the 'semi-open' conchotomy technique (Henriksson 1979). Muscle samples were divided into two separate pieces (approximately 40–60 mg) and immediately frozen in liquid nitrogen and stored at –70°C until analysed. Samples for biochemical analyses were melted in an ice bath, weighed and homogenized in 1:50 (w/v) of 1 M Tris buffer pH adjusted to 7.5 in a manually operated all-glass homogenizer (Aunola et al. 1988).

For muscle sampling in study IV, the rats were killed using carbon dioxide on day 0, 1, 3 or 6 after the cast removal. Two major muscles used in running, i.e. *m. lateral gastrocnemius* (LG) and *m. plantaris* (PL), were dissected, cleared of surrounding fat and connective tissue, and frozen in liquid nitrogen and stored at –75°C until processing and analysis.

4.5 ASSESSMENT OF MAXIMAL OXYGEN UPTAKE (VO_{2MAX}) (II, III)

A 2-min incremental cycle ergometer test, implemented until volitional exhaustion or fatigue of the lower limbs, was employed to measure the maximal oxygen uptake (VO_{2max}).The warm-up loading of three minutes was 30–40 W in women and 40–60 W in men, depending on the age, size and physical fitness of the subjects. Work rate was increased every 2nd minute with equal increments (10–25 W) throughout the test. The increments were individually determined on the basis of the subject's physical fitness so that the maximum work rate would be reached in about 12–15 min (Surakka et al. 2006). Respiratory gas exchange was measured continuously by using a breath-by-breath method. VO_{2max} was recorded as the highest averaged value over 30 s at the work rate maximum (Surakka et al. 2006).

4.6 ASSESSMENT OF DIETARY INTAKE (II, III)

Nutrient intakes were assessed using a dietary analysis program developed by the National Public Health Institute (Ovaskainen et al. 1996). At the baseline and before the 24-month visit, the subjects were asked to complete a three-day food record (Haapa et al. 1985 and Pietinen et al. 1988).

4.7 BLOOD CHEMISTRY AND CELL & MOLECULAR BIOLOGICAL METHODS

All measurements are described more specifically in the original articles (I–IV).

Blood chemistry
Haemoglobin A1c (HbA_{1c}) concentration was assayed by using the latex immunoagglutination inhibition method (I–III).Plasma gamma glutamyl transferase (GT) (III), glucose and 2-hour-glucose (I–III) and serum insulin, 2-hour-insuline (I–III), free fatty acid (III), total cholesterol (III), HDL-cholesterol and triglyceride (III) as previously described in the original papers. LDL-cholesterol calculated with the Friedewald equation (III). Serum uric acid was determined photometrically by the hydroxylamine method (II). Oxygen Radical Absorbing Capacity (ORAC) was used to determine the antioxidant capacity of the samples and was measured by the inhibition of the decrease of the fluorescence of fluorescein (II).

Indices for insulin resistance and insulin sensitivity
Bennett's index was calculated using the formula 1/[ln fasting insulin (μU/ml) x ln fasting glucose (mmol/l)] (McAuley et al. 2001) (III), Homeostasis Model Assessment, insulin resistance (HOMA-IR) was calculated using the formula: HOMA-IR= fasting serum insulin (μU/ml) x fasting plasma glucose (mmol/l)/22.5 (Matthews et al. 1985) (I–III) and McAuley's index was calculated using the formula Mffm/I = exp[2.63–0.28 ln(fasting insulin as μU/ml) – 0.31 x ln(fasting triglyceride as mmol/l)] (McAuley et al. 2001) (III).

Adipokine assays from serum samples
IL-6, leptin, MCP-1 and TNF-α were simultaneously measured with the BioRad Bio-Plex 200 System using the LINCOplex Human Adipokine Panel B according to the manufactures instructions (Millipore, Billerica, MA, USA) (III).

Determination of myosin heavy chain profile
The MHC isoform composition (MHC I, MHC IIa, MHC IIx) in muscle homogenate was determined by sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel electrophoresis (Hämäläinen and Pette 1996) by using a Bio-Rad Protean II Xi vertical slab gel system (I–III).

ELISA assays

Adiponectin was measured using sandwich ELISA assays according to the manufactures instructions (B-Bridge International, Inc Mountain View; CA, USA) (III). Protein carbonyls were measured with a slightly modified method according to Buss et al. (1997) and Oksala et al. (2007) (II).The amount of vascular endothelial growth factor (VEGF) protein in the total lysate was determined using a commercially available ELISA kit (R&D systems) (Sen et al. 2002) (I).

Assessments of muscle tissue enzymes

To find out the effects of the exercise training (I–III), the following enzyme activities were assayed from the original muscle homogenate or after appropriate dilutions: lactate dehydrogenase (LDH, E.C.1.1.1.27) from 25 µl of the 1:21 dilution of the original homogenate (Scandinavian committee on Enzymes 1974) (I); LDH-isoenzyme, LDH-1 (heart type) from 100 µl of the dilution of the homogenate supernatant (Wróblewski and LaDue 1955) (I), using test kits from Sigma Diagnostics (St. Louis, MO, USA; procedure no. 228-20 for total LDH and 229-A for LDH-1) for all these determinations; and citrate synthase (CS, E.C.4.1.3.7) from 50 µl of the homogenate dilution according to Srere (1969) (I–III). All preparation steps were performed at +4°C. Enzyme activities were assayed using an Olli-C analyzer (Kone Oy, Espoo, Finland).

For biochemical assays in study IV, the frozen muscle samples were ground in liquid nitrogen and homogenized in 0.1 M phosphate buffer (pH 7.4) containing a protease inhibitor cocktail. Activities of glutathione peroxidase (GSHPx) and glutathione reductase (GRD) were measured spectrophotometrically from LG muscle homogenates as previously described (Sen et al. 1992 and Atalay et al. 1996). Due to the limited sample size, GRD and GSHPx measurements could not be performed from PL muscle.

Histology

Histological analyses of skeletal muscles by using hematoxylin and eosin staining were performed as previously described (Venojärvi et al. 2004) (IV).

Analyses for protein expressions in muscle tissue

Western blot assays were performed as described previously (Atalay et al. 2004) (I–IV). The blots were incubated overnight at +4°C with the following antibodies and dilutions:

Study I: mouse monoclonal anti-GSK-3-$\alpha\beta$ (Stressgen, Victoria, BC Canada) 1:1000 and sheep polyclonal protein kinase B (Akt-1/PKB) (Upstate biotechnology, Lake Placid NY, USA) 1:500.

Study II: mouse monoclonal anti-HSP60 (Stressgen, Victoria, BC, Canada) 1:500, anti-HSP72 (Stressgen, Victoria, BC, Canada) 1:1000, anti-GRP75 (Stressgen, Victoria, BC, Canada) 1:1000 , anti-HSP-90 (Stressgen, Victoria, BC, Canada) 1:1000 and rabbit polyclonal anti-4-HNE 1:1000 (Alexis Biochemical, San Diego, CA, USA).

Study III: goat polyclonal anti-AMPK-α1 (Santa Cruz, CA, USA).
1:1000 and -AMPK-α2 (Santa Cruz, CA, USA) 1:1000.

Horseradish peroxidase-conjugated immunoglobulins were used as secondary antibodies.

Antibody binding was viewed by using an enhanced chemiluminescence method (NEN Life Science Products, Boston, MA, USA) and quantified by using the image analysis software (NIHImage, MD, USA). The results were normalized according to actin (LabVision/NeoMarkers, Fremont, CA, USA) values. The protein concentration of homogenates was measured by using the BCA method (Pierce, Rockford, IL, USA).

Study IV: mouse monoclonal anti-HSP60 (SPA-806), anti-HSP72 (SPA-810) (Stressgen, Victoria, BC, Canada), rabbit polyclonal anti-4-HNE (210-767-R100) (Alexis Biochemical, San Diego, CA, USA). Heat shock protein expression, content of protein carbonyls and 4-HNE protein adducts were determined using polyacrylamide gel electrophoresis and immunoblotting, as previously described (Atalay et al. 2004).

Real-time PCR

The first-strand cDNA was synthesized from 0.1 μg of total RNA using SuperScript™ II RNase H- Reverse Transcriptase (Invitrogen, Life technologies). Primers and probes (GLUT-4, IFN-γ (interferon-γ), IRS-1 and TNF-α) were designed using Primer Express software (PE Biosystems City, Country) Real-time PCR was performed using the ABI PRISM 7700 Sequence Detector (City, Country). The probes were labelled with FAM at the 5′end as reporter dye and TAMRA at the 3′end as quencher dye. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The data of $\triangle C_t$ values of the gene of interest were normalized to GAPDH mRNA and processed as relative units. The lower value indicates a relative higher expression of the gene of interest. (III).

4.8 STATISTICS

Study I: Data has been reported as means ± standard deviation (SD). Student's paired t- test was used to assess differences within groups (baseline and 2-year follow-up) and Kruskall-Wallis test between groups (IGTslow and IGTfast). For statistical analyses of the Akt-1/PKB and VEGF, the original data was converted into a logarithmic form due to a strong skewness in the distributions of these variables.

Studies II–III: Data have been reported as means ± standard error (SE). Student's paired t test was used to assess differences within groups (baseline and 2-year follow-up) and Kruskall-Wallis test for the difference between the groups. Pearson's correlation coefficients were used to express the associations between the variables.

Study IV: SPSS/PC+ (Chicago, IL) software was used for statistical analyses. Kruskal-Wallis analysis was used assess differences between groups. Normality and homogeneity of distribution were estimated by Kolmogorov-Smirnov Goodness of Fit test and Barlett-Box F test. Results are expressed as means and standard deviations. Because the samples were pooled,

no statistical test was performed for the immunoblotting results. The differences between groups are expressed by multiples of increase in comparison with the control values.

5 RESULTS

The role of skeletal muscle fibre type in the regulation of glucose metabolism in middle-aged obese subjects with IGT (I)

The intervention tended to decrease the GSK-3-β protein content (n.s.; p = 0.086) in the *vastus lateralis* muscle and caused a significant decrease of the GSK-3-αβ protein content ($p < 0.05$) in the IGTfast group (Figure 4) but no changes took place in AKT-1/PKB content in the *vastus lateralis* muscle in either group.

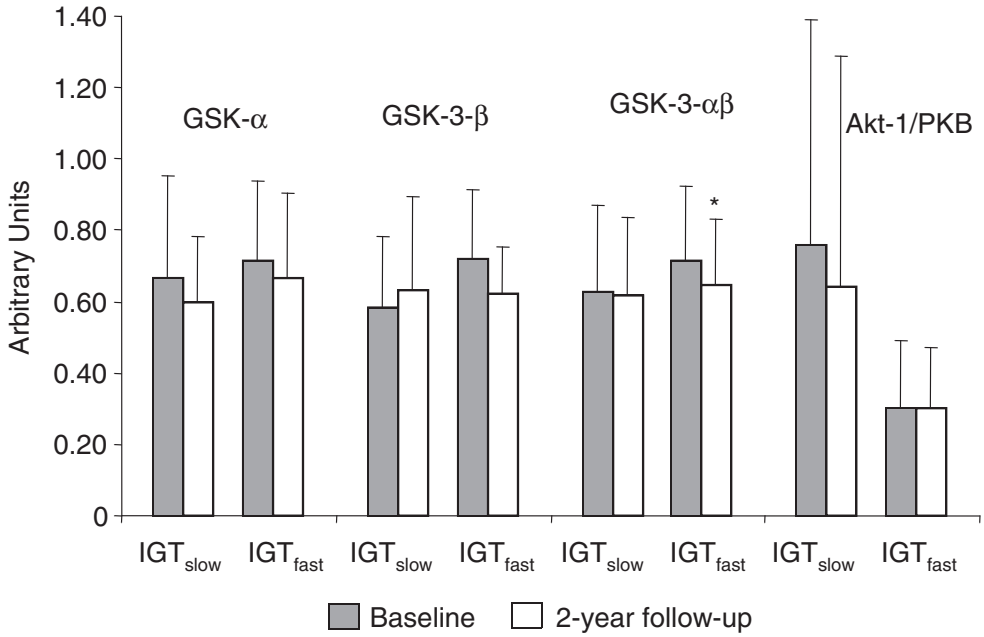

Figure 4. Effects of the 2-year intervention on the protein expression of GSK-3-αβ and GSK-3-αβ and AKT-1/PKB in the *vastus lateralis* muscle of subjects with impaired glucose tolerance (IGT), stratified according to slow and fast fibre types. Data are given as means ± SD. $^{*}p < 0.05$ within groups.

Furthermore, weight reduction during the 2-year follow-up was 5.1 ± 6.2 kg (p = 0.007) in the IGTfast group and 3.6 ± 3.2 kg (p = 0.003) in the IGTslow group. Also during the 2-year intervention, fasting glucose, fasting insulin and HOMA-IR decreased significantly in the IGTfast group, and 2-hour-glucose and HbA_{1c} decreased in both groups. Exercise training increased lactate dehydrogenase (LDH) ($p < 0.01$), lactate dehydrogenase-1 (LDH-1) ($p < 0.05$) and citrate synthase (CS) ($p < 0.05$) activities in the *vastus lateralis* muscle in the IGTslow group, but only increased CS activity ($p < 0.05$) in the IGTfast group.

The role of exercise training with dietary counselling the antioxidative capacity and HSP defence in middle-aged obese subjects with IGT (II)

Maximal oxygen uptake variables (VO_{2max} and VO_{2max}/weight) and the protein content of GRP75 and HSP60 increased significantly, compared with the pre-intervention values (n = 22). In addition, the fasting glucose, 2-h glucose, HbA_{1c}, serum protein carbonyl and uric acid concentrations decreased significantly in the IGT subjects. Body weight decreased by –4.4 kg (4.9%) ($P < 0.001$) (Table 5). In cytoplasmic chaperones HSP72 or HSP90 no changes took place.

Table 5. Indices of glucose metabolism, weight, maximal oxygen uptake and oxidative stress in the subjects with impaired glucose tolerance (IGT), n = 22.

	Baseline	**2-year follow-up**	**Change**	***P* value**
Glucose metabolism				
Fp-glucose, mmol/l	6.0 ± 0.1	5.8 ± 0.1	–0.3 ± 0.1	0.013
2h-glucose, mmol/l	7.7 ± 0.3	6.4 ± 0.4	–1.2 ± 0.5	0.013
HbA_{1c}, %	5.7 ± 0.1	5.4 ± 0.1	–0.4 ± 0.1	<0.001[a]
Weight and fitness				
Weight, kg	89.7 ± 1.8	85.3 ± 2.0	–4.4 ± 1.1	<0.001
BMI, (kg/m^2)	29.9 ± 0.5	28.4 ± 0.5	–1.5 ± 0.4	<0.001
VO_{2max}, l/min	2.28 ± 0.14	2.43 ± 0.13	0.2 ± 0.0	<0.001
VO_{2max}, ml/kg/min	26.1 ± 1.6	28.5 ± 1.5	2.4 ± 0.6	<0.001
Oxidative stress				
GRP75 (muscle), arb.u.	0.95 ± 0.11	1.28 ± 0.16	0.33 ± 0.12	0.014
HSP60 (muscle), arb.u.	0.35 ± 0.05	0.56 ± 0.11	0.21 ± 0.08	0.022
P.carbonyls (serum), arb.u.	0.12 ± 0.00	0.11 ±0.00	–0.00 ± 0.00	0.010[b]
Uric acid, µmol/l	375 ± 12	337 ± 14	–37 ± 9	0.001

Data is given as means ± SE; arb.u. = arbitrary units; [a] n = 21, [b] n = 20.

In a comparison of the IGT subgroups IGTfast and IGTslow, most of the measured markers of oxidative stress and antioxidative capacity were similar in both groups. After a 2-year follow-up only serum protein carbonyls decreased in the IGTfast group ($p = 0.02$) and HSP expression increased in both groups (Figure 5) while GRP75 expression increased only in the IGTslow group (Figure 5).

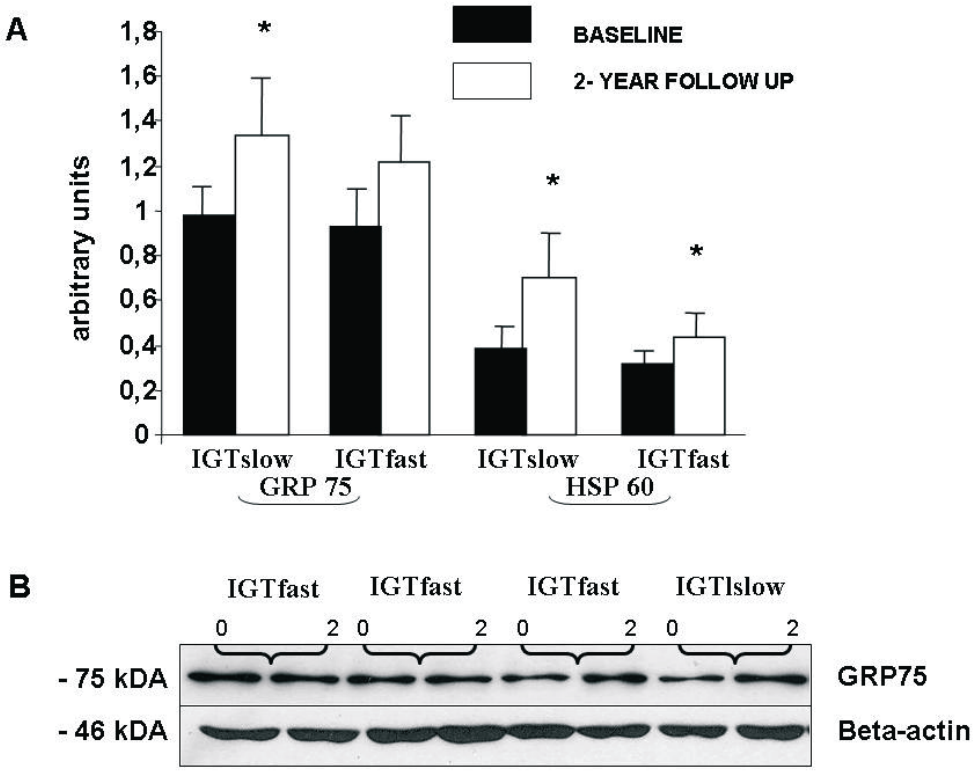

Figure 5. (A) Effects of the 2-year intervention on the protein expression of GRP75 and HSP60 in the *vastus lateralis* muscle of subjects with impaired glucose tolerance (IGT), divided into slow (n = 10) and fast (n = 12) fibre type subgroups. Data is given as means ± SE. *P < 0.05 within groups. (B) Representative Western blots, using anti-GRP75 and beta-actin antibodies, of whole tissue homogenates from skeletal muscle biopsies of four IGT subjects at baseline (0) and after 2-year follow-up (2).

In the IGTslow group the increase of HSP and serum ORAC values were correlated positively with each other (Figure 6) and the increased protein expression of GRP75 correlated positively with improved VO_{2max} value in the IGTfast group and negatively in the IGTslow group (Figure 7).

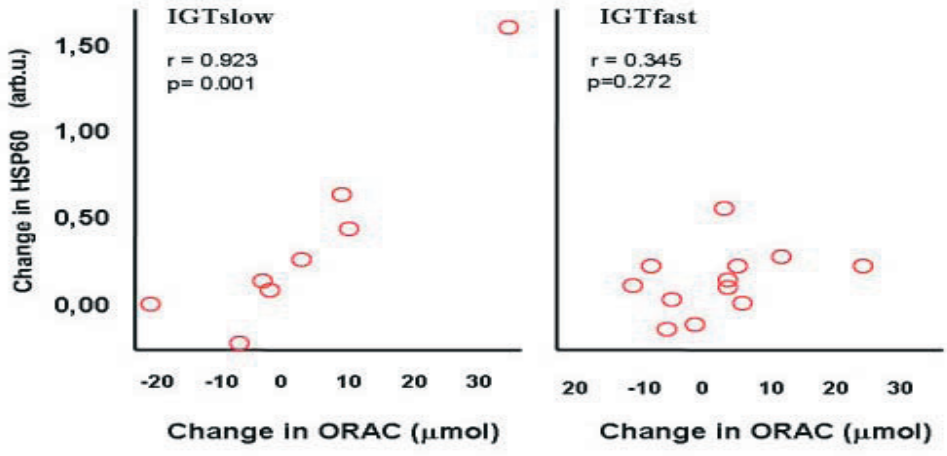

Figure 6. Relationship between the changes of the HSP60 and ORAC values during a 2-year intervention in subjects with impaired glucose tolerance (IGT), divided into slow (n = 8) and fast (n = 12) fibre type subgroups.

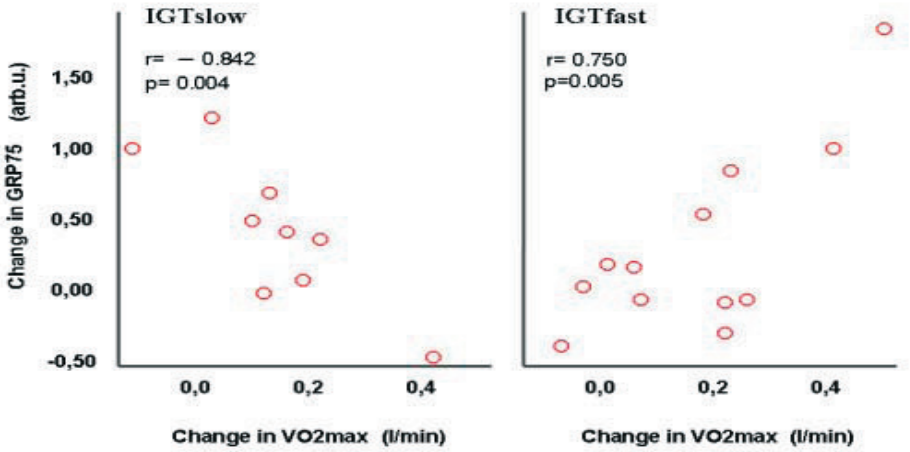

Figure 7. Relationship between the changes of the GRP75 and VO_{2max} values during a 2-year intervention in subjects with impaired glucose tolerance (IGT), divided into slow (n = 9) and fast (n = 12) fibre type subgroups.

Role of adipokines in regulation of glucose metabolism in middle-aged obese subjects with IGT (III)

The exercise-diet intervention increased systemic adiponectin concentrations and decreased the leptin concentration in the responders but not in the non-responders. Leptin concentration increased 15% in the non-responders and decreased 27% in the responders during the 2-year follow-up. The difference between groups was statistically significant ($p < 0.002$). There were no statistically significant differences in the systemic concentrations of IL-6, MCP-1 and TNF-α in either group. The 2-year intervention increased both maximal oxygen uptake values VO_{2max} and VO_{2max}ind ($p < 0.01$ and $p < 0.001$, respectively) and oxidative capacity (CS) ($p < 0.01$) of the muscle tissue in the responders but only VO_{2max} ($p < 0.05$) values in the non-responders. The intervention increased the expression of the GLUT-4 in the non-responders ($p < 0.05$) but not in the responders ($p = 0.19$) (Figure 8).

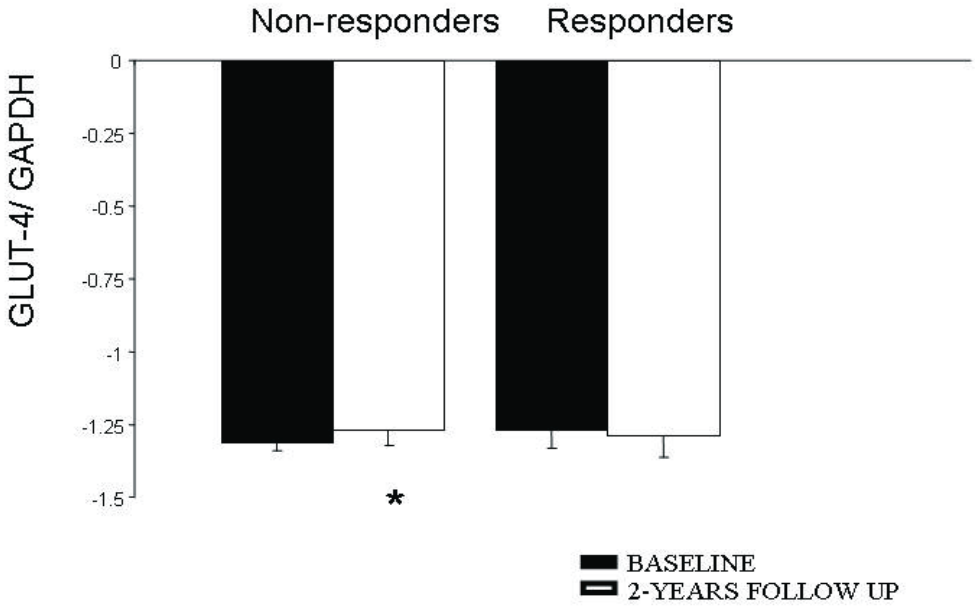

Figure 8. Expressions of GLUT-4 mRNAs in skeletal muscle of IGT subjects.

During the 2-year intervention, serum glucose, 2h-Glucose, insulin, HbA_{1c} and HOMA-IR decreased significantly in the responders, and 2h-insulin tended to decrease (Table 7). Insulin sensitivity (Bennett and McAuley indices) increased in responders ($p < 0.01$) after a 2-years follow up. At this stage, fasting glucose, 2h-insulin values, HOMA-IR were significantly lower in the responders than in the non-responders (Table 6) and Bennett and McAuley indices were significantly higher in responders than non-responders. In the non-responders there were no changes in serum glucose, 2h-Glucose, insulin, 2h-insulin, HbA_{1c} and HOMA-IR values between the baseline and at the 2-year follow- up. (Table 6)

Table 6. Effects of a 2-year exercise-diet intervention on the glucose and lipid metabolism of IGT subjects.

	Non-responders		**Responders**	
	Baseline	**2-year**	**Baseline**	**2-year**
n = (Female/Male)	9 (2/7)	9 (2/7)	13 (4/9)	13 (4/9)
Glucose metabolism				
Fs-Ins μU/ml	16.7 ± 2.7	17.0 ± 3.0	16.4 ± 2.1	11.2 ± 1.3**
2h-InsμU/ml	96 ± 23	81 ± 22	97 ± 33	45 ± 90#
HbA1c %	5.6 ± 0.1	5.4 ± 0.1	5.8 ± 0.1	5.3 ± 0.1***
Fs-Gluc mmol/	6.0 ± 0.1	6.1 ± 0.2	6.1 ± 0.1	5.6 ± 0.1***##
2h-Gluc mmol/l	8.1 ± 0.3	7.0 ± 0.8	7.4 ± 0.4	6.0 ± 0.4*
Bennett index	0.51 ± 0.02	0.52 ± 0.02	0.51 ± 0.02	0.57 ± 0.02**##
HOMA-IR	4.5 ± 0.8	4.6 ± 0.9	4.5 ± 0.6	2.8 ± 0.3**#
McAuley index	5.8 ± 0.3	5.7 ± 0.4	5.6 ± 0.4	6.5 ± 0.3**##
Lipid metabolism				
Cholesterol mmol/l	5.3 ± 0.3	4.9 ± 0.2*	5.6 ± 0.2	5.5 ± 0.3#
LDL mmol/l	3.5 ± 0.3	3.0 ± 0.2**	3.7 ± 0.3	3.5 ± 0.3
HDL mmol/l	1.10 ± 0.10	1.14 ± 0.10	1.14 ± 0.09	1.26 ± 0.09
Triglyceride mmol/l	1.54 ± 0.15	1.76 ± 0.21	1.96 ± 0.24	1.55 ± 0.18**##
FFA mmol/l	0.68 ± 0.07	0.47 ± 0.07	0.69 ± 0.07	0.47 ± 0.04**
Hepatic enzyme				
γ-GT (IU/l)	36.3 ± 3.2	31.4 ± 5.0	40.9 ± 5.1	31.5 ± 4.5**

Data is given as means ± SE; *P < 0.05, ** P < 0.01, *** P < 0.001 within group;
#P < 0.05 between groups, ##P < 0.01 between groups; γ-GT, γ-glutamyltransferase.

Skeletal muscle HSP expression in response to immobilization and remobilization (IV)

This study tested the effects of spontaneous physical activity and strenuous exercise on HSP expression and oxidative stress during recovery from 4 weeks immobilization in the *lateral gastrocnemius* (LG) and *plantaris* (PL) muscles of the rat. Active mobilization (AM) or free mobilization (FM) protocols were used for re-mobilization. HSP72 and HSP60 expressions were up-regulated during the recovery from immobilization, especially in the LG muscle in the AM group (Figure 9). However, markers of oxidative stress, such as protein carbonyls and 4-hydroxynonenal protein adduct, or activities of antioxidant enzymes glutathione peroxidase and glutathione reductase, did not change after the immobilization and subsequent recovery.

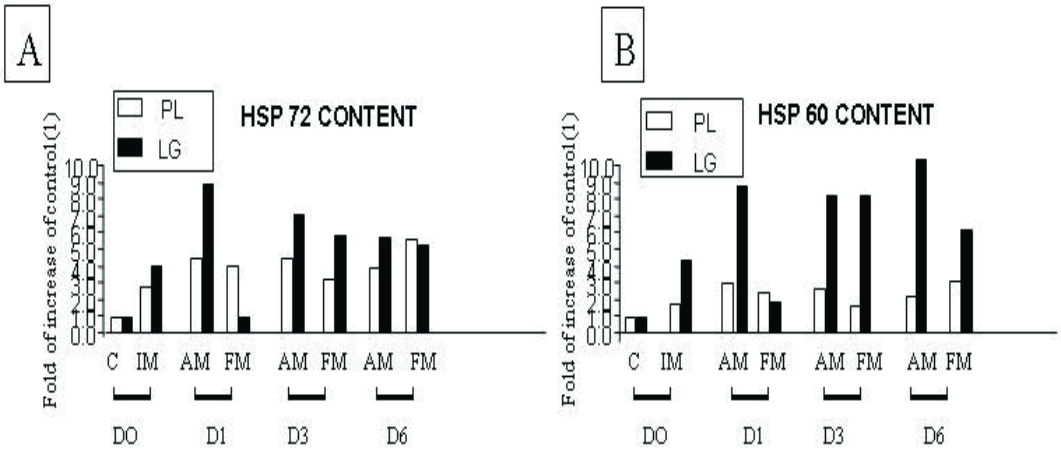

Figure 9. HSP72 (A) and HSP60 (B) levels in PL and LG muscles after immobilization and subsequent intensive exercise or spontaneous physical activity in rats, in comparison with control animals. The differences are expressed as multiples of increase from the control values (C).

6 DISCUSSION

6.1 STUDY DESIGN AND METHODS

This thesis consists of two different study populations. One of the strengths of this study is that the main investigation is a substudy of the Finnish Diabetes Prevention Study (DPS) which was a multicenter study carried out in 1993–2000 by five participating centers, located in Helsinki, Kuopio, Oulu, Tampere and Turku (Tuomilehto 2001). Being a part of the Finnish Diabetes Prevention Study (DPS), offered us an extensive amount of parameters collected during a long and well performed intervention. The other study population (used in study IV) has been used to support the main findings of this particular substudy, which took place in Turku.

However, the design of the substudy had already been performed beforehand concerning 22 persons only from the intervention group who volunteered to give muscle samples from their *m. vastus lateralis*. For this reason we had to make the comparisons within the intervention group and divided the study population on the basis of their baseline myosin heavy chain profile (I, II) or their response on the weight loss (III). The control group would definitely have increased the strength of our findings. Another aspect which would have improved our study would have been additional muscle samplings performed after exercise and/or after insulin stimulus. In this way we could have also investigated insulin signaling pathway e.g. phosphorylation of tyrosine and serine residues and more precisely glucose transport mechanisms.

The main goals of the DPS intervention were as follows: weight reduction, dietary counselling and moderately intense physical activity for 30 minutes per day or more. The study design we used did not allow us to assess individual effects of the exercise or of changes of dietary habits on weight loss or on the improved glucose tolerance. This weakness is a consequence of the fact that our study was limited by the design of the main study, the Diabetes Prevention Study (Tuomilehto et al. 2001), which aimed at investigating the effects of lifestyle changes in preventing type 2 diabetes. Thus, we were not able to focus solely on the effects of exercise or diet on skeletal muscle tissue. For this reason muscle sampling (with no heavy exercising during the preceding 2 days) was performed at 6 months and after 2 years. In skeletal muscle glucose metabolism, the greatest changes due to the dietary counselling were assumed to have occurred within 6 months. While this gives a good view of the changes in the regulation of the skeletal muscle metabolism induced by exercise, it does not allow us to distinguish the effects of either exercise or dietary counselling separately without recruiting proper control groups.

In the basic blood chemistry standardized clinically approved methods have been used. All measurements were carried out in the same laboratory using standard protocols. The inter and intra-assay variability of these measurements is low. All blood chemistry measurements were carried out using previously tested commercial kits and reagents. In all measurements baseline and follow-up samples were equally compared as pairs in the same measurement batch.

6.2 MAIN FINDINGS

6.2.1 Glucose metabolism (I)

One of the main goals of this thesis was to clarify the role of different MHC compositions on skeletal muscle glucose metabolism. We found that the amount of GSK-3-αβ protein decreased and GSK-ß contents tended to decrease in the IGT subjects who had proportionately more of MHC II isoforms in their skeletal muscle. This is a novel finding, offering new clues on the metabolic pathways that respond selectively to exercise and dietary intervention in different types of skeletal muscle fibre. A decreased amount of GSK-3-αβ protein may enhance both insulin signalling (less suppression of IRS-1 phosphorylation) and enhance glycogen storage (Henriksen 2010). It is well-known that both GS activity and the total amount of GSK-3 impair glucose metabolism in type 2 diabetic subjects at basal state, prior to insulin stimulation. We focused on the pathway related to non-oxidative glucose metabolism because the muscle samples were taken at basal state. If we had had the possibility to collect samples after insulin stimulation, a better marker for glucose metabolism; expression of the glucose transporter protein-4 (GLUT-4) could have been measured The glucose metabolism improved both in the IGTslow and IGTfast group during the 2-year exercise and dietary intervention. The change was more prominent in the IGTfast group than in the IGTslow group, associated with the decrease of the GSK-αβ protein expression in skeletal muscle. A previous study showed that muscle protein contents of both α and β isoforms of GSK-3 were higher, up to 30%, in diabetic subjects, in comparison with the lean and weight-matched obese non-diabetic subjects (Nikoulina et al. 2000). Consistent with our results and previous reports the higher the GSK-3 level, the worse the subject's glucose tolerance was (Ciaraldi et al. 2002). This conclusion was supported by the findings of another study of our group in which we compared the expression of the GSK-3 protein between the present sample of IGT subjects and a group of age, gender and BMI matched type 2 diabetic subjects (n=10) (Venojärvi and co-workers, unpublished observation).

6.2.2. Chaperon defence and oxidative stress (II and IV)

The purpose was to clarify whether a 2-year exercise and dietary intervention improves the antioxidative capacity and HSP defence of different skeletal muscle fibre phenotypes in middle-aged obese subjects with impaired glucose tolerance. In addition, in rats, we tested the hypothesis whether a strenuous, non-damaging, metabolic stress may provide greater tissue protection against atrophic and degenerative changes induced by immobilization.

In rats both spontaneous physical activity and intensive exercise increased the HSP60 and HSP72 expressions in the lateral gastrocnemius muscle. Spontaneous physical activity or intensive exercise did not influence lipid peroxidation or protein oxidation in skeletal muscle in immobilized rats. Cytoprotection was improved in the skeletal muscle tissue, observed as increased expressions of mitochondrial HSP60 and GRP75 in the IGT subjects while no response was found in cytoplasmic chaperones HSP72 and HSP90. Both HSP60 and GRP75 expressions increased in the IGTslow subgroup whereas only HSP60 in the IGTfast subgroup. However, skeletal muscle fibre type did not seem to have any significant role in the mitochondrial chaperone expression. The capacity of HSP60 to stabilize mitochondrial proteins, promote mitochondrial protein biosynthesis and to prevent mitochondrial apoptosis seems to be crucial

for its protective function (Gupta and Knowlton 2005). Diabetes has been reported to cause mitochondrial dysfunction and attenuate synthesis of a variety of proteins, including HSP60 in mitochondria (Mokhtar et al. 1993 and Turko and Murad 2003). Although we cannot directly compare the HSP responses of IGT patients in our study with the studies performed in type 2 diabetes patients, it has been well shown that the skeletal muscle insulin resistance and mitochondrial dysfunction are associated with ageing, type 2 diabetes and also in the offspring of type 2 diabetes (Sheerkumar et al 2007). In the present study, the adaptive changes in the expression of GRP75 and HSP60 could support mitochondrial protein import, protein folding and enhance tissue protection in insulin-resistant muscle tissue. These adaptive changes of mitochondrial HSPs and increased oxidative capacity are mainly due to increased contractile activity of skeletal muscle. Although, the 2-year intervention up-regulated mitochondrial HSP expressions in middle-aged subjects with impaired glucose tolerance and these improvements, however, were not correlated directly with enhanced glucose tolerance.

When we compared experimental data collected from rats with that of humans, chronic moderate intensity of exercise coupled with a diet intervention lowered, oxidative stress shown by decreased serum levels of uric acid and protein carbonyls in the IGT subjects. Also serum protein carbonyls decreased only in responders as compared to non-responders whereas uric acid decreased in both groups. The reduced oxidative activity could also be a result of a sedentary lifestyle. No data has been published on correlations between changes in oxidative capacity and insulin sensitivity following exercise in type 2 diabetes (Rabøl et al. 2006). In obese subjects insulin sensitivity is mediated by a reduction in plasma fatty acid mobilization after weight loss and exercise but enhanced oxidative capacity is not affected (Schenk et al. 2009).

6.2.3 Increased aerobic capacity and weight loss improves glycemic control without changes in skeletal muscle GLUT-4 expressions (III)

The main goals of the intervention were to achieve a weight reduction of 5% or more, to obtain healthier dietary habits and to increase physical activity. We divided the study population into two subgroups (non-responders and responders) based on their degree of weight loss. In the responders, there can be at least three possible explanations behind improved glucose control.

Exercise-diet intervention 1) reduced body weight 2) increased oxidative capacity of skeletal muscle 3) reduced level of hepatic lipid content. All these changes may be due to increased physical activity and dietary modification which induced significant weight loss, improved maximal oxygen uptake, increased oxidative capacity of skeletal muscle, and decreased insulin resistance in the responders after a 2-year exercise-diet intervention. Furthermore, the exercise-diet intervention reduced serum leptin and increased serum adiponectin levels in responders which may enhance skeletal muscle fatty acid oxidation. Interestingly, HSP60 response increased in responders but not in non-responders. Although we do not have clear evidence of the effect of an enhanced HSP60 response, we can speculate that higher HSP60 levels may contribute to insulin sensitivity by improving tissue protection and decreasing autoimmunity in people having IGT.

Nevertheless, unlike the responders, non-responders showed no changes in serum glucose, 2h-Glucose, insulin, 2h-insulin, HbA1c and HOMA-IR values between baseline and at a 2-year follow- up. Lack of weight loss and lower training intensity may be responsible for no changes tooking place in the glucose control or in the serum adipokine concentrations in non-responders despite increased GLUT-4 expression in the skeletal muscle. The intervention increased the expression of the GLUT-4 in the non-responders but not in the responders. Similarly the diet

and exercise intervention increased the expression of the GLUT-4 gene in the skeletal muscle only in the non-responders. During exercise AMPK activation occurs through the contraction of fast-twitching muscles leading to an up-regulation of GLUT-4 gene expression (Lim et al. 2009). The increased GLUT-4 gene expressions in non-responders may be due to contractile activity of fast-twitching fibres during strength and power type training, being for longer for non-responders than for responders (2.4 hour per week versus 1.3 hours per week, respectively)

Dietary intake and the total amount of exercise did not differ significantly between responders and non-responders but the relative intensity of the exercise might have been higher in responders (based on increased citrate synthase activity). It can be postulated that a stronger exercise response may have a more favourable interaction with the dietary modification, which includes increased fibre intake and decreased fat intake, and ultimately exerts its favourable effects on weight and loss of glucose homeostasis. Therefore, sustained weight loss by dietary modification and a combination of aerobic, strength and power types of exercises are powerful expedients behind improved glucose control in responders.

7 FUTURE ASPECTS

The present study raised a number of questions which still remain open. In the future, studies should recruit larger populations and with respective controls so that the effects of increased exercise and changes in dietary habits can be distinguished from each other. Investigation of the long-term effects and the role of skeletal muscle fibre type, the period and type of exercise and the mechanisms of improving the skeletal muscle glucose metabolism by diet or exercise, or by combining diet and exercise, would give valuable knowledge for further recommendations to improve the skeletal muscle metabolism in IGT subjects.

In future studies another important perspective is to focus research on the interplay between the adipose, liver and muscle tissue for preventing diabetes and improving glucose control of type 2 diabetic persons. Also, exercise effects on incretins should be studied in more detail.
One factor associated with reduced insulin action is enhanced activity of the serine/threonine kinase glycogen synthase kinase-3 (GSK-3) in skeletal muscle, the liver, and adipose tissue. The evidence to date supports an important role of GSK-3 dysfunction in the multifactorial etiology of insulin resistance in skeletal muscle. GSK-3 remains an important target for the interventions designed to improve insulin action in obesity-associated insulin resistance and type 2 diabetes.

8 CONCLUSIONS

The most important findings from the present studies were:

The amount of glycogen synthase kinase-3- αβ (GSK-3-αβ) protein decreased and GSK-ß contents tended to decrease in the IGT subjects who had proportionately more MHC II isoforms in their skeletal muscle. This is a novel finding, suggesting a metabolic pathway that is sensitive to exercise and dietary intervention.

After a 2 year dietary and exercise intervention period, oxidative stress was reduced, as shown by decreased serum levels of uric acid and protein carbonyls in the IGT subjects. In addition cytoprotection was improved in the skeletal muscle tissue, observed as increased expression of mitochondrial HSP60 and GRP75 in the IGT subjects whereas no response was found in cytoplasmic chaperones HSP72 and HSP90. In addition, in the animal experiments both spontaneous physical activity and intensive exercise increased the HSP60 and HSP72 expressions in the lateral gastrocnemius muscle in rats. Additionally more intense exercise may provide quicker adaption to atrophic and degenerative changes during the early mobilization of immobilized extremity.

The responders to the dietary and exercise intervention achieved two important goals: significant weight reduction and improved aerobic capacity. In other words the increased physical activity and dietary modification induced significant weight loss, improved maximal oxygen uptake, increased oxidative capacity of skeletal muscle, reduced serum leptin and increased serum adiponectin concentrations. Also it improved glucose control without affecting cholesterol metabolism in responders and decreased insulin resistance in the responders after 2-year exercise-diet intervention without changes in skeletal muscle GLUT-4 expressions.

9 REFERENCES

Atalay M, Laaksonen DE. Diabetes, oxidative stress and physical exercise. J Sport Sci Med. 2002 1:1-14.

Atalay M, Laaksonen DE, Niskanen L, Uusitupa M, Hänninen O, Sen CK. Altered antioxidant enzyme defences in insulin-dependent diabetic men with increased resting and exercise-induced oxidative stress. Acta Physiol Scand 1997 161:195-201

Atalay M, Oksala NK, Laaksonen DE, Khanna S, Nakao C, Lappalainen J, et al. Exercise training modulates heat shock protein response in diabetic rats. J Appl Physiol 2004 97:605-611.

Atalay M, Oksala N, Lappalainen J, Laaksonen DE, Sen CK, Roy S. Heat shock proteins in diabetes and wound healing. Curr Protein Pept Sci 2009 10:85-95.

Atalay M, Seene T, Hänninen O, Sen CK. Skeletal muscle and heart antioxidant defences in response to sprint training. Acta Physiol Scand 1996 158:129-134.

Aunola S, Marniemi J, Alanen E, Mäntylä M, Saraste M, Rusko H. Muscle metabolic profile and oxygen transport capacity as determinants of aerobic and anaerobic thresholds. Eur J Appl Physiol Occup Physiol 1988 57:726-734.

Bacha F, Saad R, Gungor N, Arslanian SA. Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and beta-cell function. Diabetes Care 2004 27:547-552.

Bajaj M, DeFronzo RA. Metabolic and molecular basis of insulin resistance. J Nucl Cardiol 2003 10:311-323.

Basso AD, Solit DB, Chiosis G, Giri B, Tsichlis P, Rosen N. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. J Biol Chem 2002 277:39858-39866.

Bruce CR, Carey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. Diabetes 2003 52:2338-2345.

Bukau B, Horwich AL. The Hsp70 and Hsp60 chaperone machines. Cell 1998 92:351-366.

Buss H, Chan TP, Sluis KB, Domigan NM, Winterbourn CC. Protein carbonyl measurement by a sensitive ELISA method. Free Radic Biol Med 1997 23:361-366.

Cadenas E. Basic mechanisms of antioxidant activity. Biofactors 1997 6:391-397.

Campisi J, Leem TH, Greenwood BN, Hansen MK, Moraska A, Higgins K, et al. Habitual physical activity facilitates stress-induced HSP72 induction in brain, peripheral, and immune tissues. Am J Physiol Regul Integr Comp Physiol 2003 284:R520-530.

Carmel-Harel O, Storz G. Roles of the glutathione- and thioredoxin-dependent reduction systems in the Escherichia coli and saccharomyces cerevisiae responses to oxidative stress. Annu Rev Microbiol 2000 54:439-461.

Chan RS, Woo J. Prevention of overweight and obesity: how effective is the current public health approach. Int J Environ Res Public Health 2010 7:765-783.

Chen ZP, Stephens TJ, Murthy S, Canny BJ, Hargreaves M, Witters LA, et al. Effect of exercise intensity on skeletal muscle AMPK signaling in humans. Diabetes 2003 52:2205-2212.

Ciaraldi TP, Carter L, Mudaliar S, Henry RR. GSK-3beta and control of glucose metabolism and insulin action in human skeletal muscle. Mol Cell Endocrinol 2010 315:153-158.

Ciaraldi TP, Nikoulina SE, Henry RR. Role of glycogen synthase kinase-3 in skeletal muscle insulin resistance in Type 2 diabetes. J Diabetes Complications 2002 16:69-71.

Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003 46:459-469.

Cohen P, Goedert M. GSK3 inhibitors: development and therapeutic potential. Nat Rev Drug Discov 2004 3:479-487.

Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995 378(6559):785-789.

Csermely P, Schnaider T, Sőti C, Prohászka Z, Nardai G. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacol Ther 1998 79:129-168.

DeFronzo RA, Jacot E, Jéquier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. Diabetes 1981 30:1000-1007.

DeFronzo RA, Sherwin RS, Kraemer N. Effect of physical training on insulin action in obesity. Diabetes 1987 36:1379-1385.

Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. J Cell Sci 2003 116(Pt 7):1175-1186.

Domínguez C, Ruiz E, Gussinye M, Carrascosa A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. Diabetes Care 1998 21:1736-1742.

Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002 82:47-95.

Dyck DJ. Leptin sensitivity in skeletal muscle is modulated by diet and exercise. Exerc Sport Sci Rev 2005 33:189-194.

Dzamko NL, Steinberg GR. AMPK-dependent hormonal regulation of whole-body energy metabolism. Acta Physiol (Oxf) 2009 196:115-127.

Eriksson J, Lindström J, Valle T, Aunola S, Hämäläinen H, Ilanne-Parikka P, et al. Prevention of Type II diabetes in subjects with impaired glucose tolerance: the Diabetes Prevention Study (DPS) in Finland. Study design and 1-year interim report on the feasibility of the lifestyle intervention programme. Diabetologia 1999 42:793-801.

Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 2002 16:1335-1347.

Febbraio MA, Steensberg A, Walsh R, Koukoulas I, van Hall G, Saltin B, et al. Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. J Physiol 2002 538:911-917.

Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998 395:763-770.

Garvey WT, Maianu L, Hancock JA, Golichowski AM, Baron A. Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. Diabetes 1992 41:465-475.

Gaster M, Handberg A, Schürmann A, Joost HG, Beck-Nielsen H, Schrøder HD. GLUT11, but not GLUT8 or GLUT12, is expressed in human skeletal muscle in a fibre type-specific pattern. Pflügers Arch 2004 448:105-113.

Geiger PC, Gupte AA. Heat shock proteins are important mediators of skeletal muscle insulin sensitivity. Exerc Sport Sci Rev 2011 39:34-42.

Gilardini L, McTernan PG, Girola A, da Silva NF, Alberti L, Kumar S, et al. Adiponectin is a candidate marker of metabolic syndrome in obese children and adolescents. Atherosclerosis 2006 189:401-407.

Go YM, Jones DP. Intracellular proatherogenic events and cell adhesion modulated by extracellular thiol/disulfide redox state. Circulation 2005 111:2973-2980.

Golay A, DeFronzo RA, Ferrannini E, Simonson DC, Thorin D, Acheson K, et al. Oxidative and non-oxidative glucose metabolism in non-obese type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1988 3:585-591.

González B, Hernando R, Manso R. Stress proteins of 70 kDa in chronically exercised skeletal muscle. Pflügers Arch 2000 440:42-49.

Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011 29:415-445.

Gupta S, Knowlton AA. HSP60, Bax, apoptosis and the heart. J Cell Mol Med 2005 9:51-58.

Haapa E, Toponen T, Pietinen P, Räsänen L. Annoskuvakirja. Helsinki: Painokaari Oy, 1985.

Hämäläinen N, Pette D. Slow-to-fast transitions in myosin expression of rat soleus muscle by phasic high-frequency stimulation. FEBS Lett 1996 399:220-222.

Hanley AJ, Bowden D, Wagenknecht LE, Balasubramanyam A, Langfeld C, Saad MF, et al. Associations of adiponectin with body fat distribution and insulin sensitivity in nondiabetic Hispanics and African-Americans. J Clin Endocrinol Metab 2007 92:2665-2671.

Hansen L, Arden KC, Rasmussen SB, Viars CS, Vestergaard H, Hansen T, et al. Chromosomal mapping and mutational analysis of the coding region of the glycogen synthase kinase-3alpha and beta isoforms in patients with NIDDM. Diabetologia 1997 40:940-946.

Helakorpi S., Paavola M., Prättälä R., Uutela A. Suomalaisen aikuisväestön terveyskäyttäytyminen ja terveys, kevät 2008. [Health behaviour and health among the Finnish adult population, Spring 2008]. Terveyden ja hyvinvoinnin laitos. Raportti 2/2009. [Report of National Institute for Health and Welfare 2/2009].

Henriksen EJ. Dysregulation of glycogen synthase kinase-3 in skeletal muscle and the etiology of insulin resistance and type 2 diabetes. Curr Diabetes Rev 2010 6:285-293.

Henriksson KG. "Semi-open" muscle biopsy technique. A simple outpatient procedure. Acta Neurol Scand 1979 59:317-323.

Hirai S, Takahashi N, Goto T, Lin S, Uemura T, Yu R, et al. Functional food targeting the regulation of obesity-induced inflammatory responses and pathologies. Mediators Inflamm 2010 2010:367838 (doi: 10.1155/2010/367838).

Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB Sr, Wilson PW, et al. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. J Clin Endocrinol Metab 2008 93:3165-3172.

Hofmann B, Hecht HJ, Flohe L. Peroxiredoxins. Biol Chem 2002 383:347-364.

Højlund K, Wrzesinski K, Larsen PM, Fey SJ, Roepstorff P, Handberg A, et al. Proteome analysis reveals phosphorylation of ATP synthase beta -subunit in human skeletal muscle and proteins with potential roles in type 2 diabetes. J Biol Chem 2003 278:10436-10442.

Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. Antioxid Redox Signal 2000 2:811-820.

Hooper PL. Insulin signaling, GSK-3, heat shock proteins and the natural history of type 2 diabetes mellitus: a hypothesis. Metab Syndr Relat Disord 2007 5:220-230.

Hooper PL, Hooper PL. Inflammation, heat shock proteins, and type 2 diabetes. Cell Stress Chaperones 2009 14:113-115.

Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world – a growing challenge. N Engl J Med 2007 356:213-215.

Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995 95:2409-2415.

Hotta M, Tashiro F, Ikegami H, Niwa H, Ogihara T, Yodoi J, et al. Pancreatic beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. J Exp Med 1998 188:1445-1451.

Hsueh WA, Orloski L, Wyne K. Prediabetes: the importance of early identification and intervention. Postgrad Med 2010 122:129-143.

Inoue H, Ogawa W, Asakawa A, Okamoto Y, Nishizawa A, Matsumoto M, et al. Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. Cell Metab 2006 3:267-275.

Janssen-Heininger YM, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, et al. Redox-based regulation of signal transduction: principles, pitfalls, and promises. Free Radic Biol Med 2008 45:1-17.

Jellema A, Plat J, Mensink RP. Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. Eur J Clin Invest 2004 34:766-773.

Ji LL. Modulation of skeletal muscle antioxidant defense by exercise: role of redox signaling. Free Radic Biol Med 2008 44:142-152.

Jones DP. Redefining oxidative stress. Antioxid Redox Signal 2006 8:1865-1879.

Jones DP. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol 2008 295:C849-868.

Jozsa L, Thoring J, Järvinen M, Kannus P, Lehto M, Kvist M. Quantitative alterations in intramuscular connective tissue following immobilization: an experimental study in the rat calf muscles. Exp Mol Pathol 1988 49:267-278.

Kang PJ, Ostermann J, Shilling J, Neupert W, Craig EA, Pfanner N. Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. Nature 1990 348:137-143.

Kavanagh K, Flynn DM, Jenkins KA, Zhang L, Wagner JD. Restoring HSP70 deficiencies improves glucose tolerance in diabetic monkeys. Am J Physiol Endocrinol Metab 2011 300:E894-901.

Kavanagh K, Zhang L, Wagner JD. Tissue-specific regulation and expression of heat shock proteins in type 2 diabetic monkeys. Cell Stress Chaperones 2009 14:291-299.

Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 2001 280:E745-751.

Khassaf M, Child RB, McArdle A, Brodie DA, Esanu C, Jackson MJ. Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. J Appl Physiol 2001 90:1031-1035.

Khassaf M, McArdle A, Esanu C, Vasilaki A, McArdle F, Griffiths RD, et al. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. J Physiol 2003 549:645-652.

Kim HJ, Higashimori T, Park SY, Choi H, Dong J, Kim YJ, et al. Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo. Diabetes 2004 53:1060-1067.

Kirwan JP, Jing M. Modulation of insulin signaling in human skeletal muscle in response to exercise. Exerc Sport Sci Rev 2002 30:85-90.

Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002 346:393-403.

Koistinen HA, Zierath JR. Regulation of glucose transport in human skeletal muscle. Ann Med 2002 34:410-418.

Kregel KC. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 2002 92:2177-2186.

Kurucz I, Morva A, Vaag A, Eriksson KF, Huang X, Groop L, et al. Decreased expression of heat shock protein 72 in skeletal muscle of patients with type 2 diabetes correlates with insulin resistance. Diabetes 2002 51:1102-1109.

Laaksonen DE, Atalay M, Niskanen L, Uusitupa M, Hänninen O, Sen CK. Increased resting and exercise-induced oxidative stress in young IDDM men. Diabetes Care 1996 19:569-574.

Laaksonen DE, Atalay M, Niskanen L, Uusitupa M, Hänninen O, Sen CK. Blood glutathione homeostasis as a determinant of resting and exercise-induced oxidative stress in young men. Redox Rep 1999 4:53-59.

Lindström J, Louheranta A, Mannelin M, Rastas M, Salminen V, Eriksson J, et al. The Finnish Diabetes Prevention Study (DPS): Lifestyle intervention and 3-year results on diet and physical activity. Diabetes Care 2003 26:3230-3236.

Liu Y, Gampert L, Nething K, Steinacker JM. Response and function of skeletal muscle heat shock protein 70. Front Biosci 2006 11:2802-2827.

Liu Y, Mayr S, Opitz-Gress A, Zeller C, Lormes W, Baur S, et al. Human skeletal muscle HSP70 response to training in highly trained rowers. J Appl Physiol 1999 86:101-104.

Liu Y, Steinacker JM. Changes in skeletal muscle heat shock proteins: pathological significance. Front Biosci 2001 6:D12-25.

Locke M, Noble EG. Stress proteins: the exercise response. Can J Appl Physiol 1995 20:155-167.

Locke M, Noble EG, Atkinson BG. Inducible isoform of HSP70 is constitutively expressed in a muscle fiber type specific pattern. Am J Physiol 1991 261:C774-779.

López-Torres M, Gredilla R, Sanz A, Barja G. Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. Free Radic Biol Med 2002 32:882-889.

MacAulay K, Doble BW, Patel S, Hansotia T, Sinclair EM, Drucker DJ, et al. Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. Cell Metab 2007 6:329-337.

Mahon M, Toman A, Willan PL, Bagnall KM. Variability of histochemical and morphometric data from needle biopsy specimens of human quadriceps femoris muscle. J Neurol Sci 1984 63:85-100.

Manson JE, Rimm EB, Stampfer MJ, Colditz GA, Willett WC, Krolewski AS, et al. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet 1991 338:774-778.

Martin J, Horwich AL, Hartl FU. Prevention of protein denaturation under heat stress by the chaperonin Hsp60. Science 1992 258:995-998.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985 28:412-419.

Mattson JP, Ross CR, Kilgore JL, Musch TI. Induction of mitochondrial stress proteins following treadmill running. Med Sci Sports Exerc 2000 32:365-369.

McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, et al. Diagnosing insulin resistance in the general population. Diabetes Care 2001 24:460-464.

Mokhtar N, Lavoie JP, Rousseau-Migneron S, Nadeau A. Physical training reverses defect in mitochondrial energy production in heart of chronically diabetic rats. Diabetes 1993 42:682-687.

Morimoto RI. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev 1998 12:3788-3796.

Morton JP, Kayani AC, McArdle A, Drust B. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. Sports Med 2009 39:643-662.

Morton JP, MacLaren DP, Cable NT, Bongers T, Griffiths RD, Campbell IT, et al. Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. J Appl Physiol 2006 101:176-182.

Muchova J, Liptakova A, Orszaghova Z, Garaiova I, Tison P, Carsky J, et al. Antioxidant systems in polymorphonuclear leucocytes of Type 2 diabetes mellitus. Diabet Med 1999 16:74-78.

Nikoulina SE, Ciaraldi TP, Mudaliar S, Carter L, Johnson K, Henry RR. Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. Diabetes 2002 51:2190-2198.

Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. Diabetes 2000 49:263-271.

Noble EG, Milne KJ, Melling CW. Heat shock proteins and exercise: a primer. Appl Physiol Nutr Metab 2008 33:1050-1065.

Nuutila P, Koivisto VA, Knuuti J, Ruotsalainen U, Teräs M, Haaparanta M, et al. Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. J Clin Invest 1992 89:1767-1774.

Ohlson LO, Larsson B, Björntorp P, Eriksson H, Svärdsudd K, Welin L, et al. Risk factors for type 2 (non-insulin-dependent) diabetes mellitus. Thirteen and one-half years of follow-up of the participants in a study of Swedish men born in 1913. Diabetologia 1988 31:798-805.

Oksala, N.K., Paimela H, Alhava E, Atalay M. Heat shock preconditioning induces protein carbonylation and alters antioxidant protection in superficially injured guinea pig gastric mucosa in vitro 2007 Dig. Dis Sci. 52:1897-1905.

Ornatsky OI, Connor MK, Hood DA. Expression of stress proteins and mitochondrial chaperonins in chronically stimulated skeletal muscle. Biochem J 1995 311:119-123.

Ovaskainen ML, Valsta LM, Lauronen J. The compilation of food analysis values as a database for dietary studies – The Finnish experience. Food Chemistry 1996 57**:**133-136.

Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 2004 279:12152-12162.

Pan XR, Li GW, Hu YH, Wang JX, Yang WY, An ZX, et al. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. Diabetes Care 1997 20:537-544.

Paolisso G, D'Amore A, Volpe C, Balbi V, Saccomanno F, Galzerano D, et al. Evidence for a relationship between oxidative stress and insulin action in non-insulin-dependent (type II) diabetic patients. Metabolism 1994 43:1426-1429.

Paroo Z, Dipchand ES, Noble EG. Estrogen attenuates postexercise HSP70 expression in skeletal muscle. Am J Physiol Cell Physiol 2002 282:C245-251.

Parthiban A, Vijayalingam S, Shanmugasundaram KR, Mohan R. Oxidative stress and the development of diabetic complications – antioxidants and lipid peroxidation in erythrocytes and cell membrane. Cell Biol Int 1995 19:987-993.

Patel S, Doble BW, MacAulay K, Sinclair EM, Drucker DJ, Woodgett JR. Tissue-specific role of glycogen synthase kinase 3beta in glucose homeostasis and insulin action. Mol Cell Biol 2008 28:6314-6328.

Pattwell DM, Jackson MJ. Contraction-induced oxidants as mediators of adaptation and damage in skeletal muscle. Exerc Sport Sci Rev 2004 32:14-18.

Pearce NJ, Arch JR, Clapham JC, Coghlan MP, Corcoran SL, Lister CA, et al. Development of glucose intolerance in male transgenic mice overexpressing human glycogen synthase kinase-3beta on a muscle-specific promoter. Metabolism 2004 53:1322-1330.

Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: is IL-6 a candidate? J Muscle Res Cell Motil 2003 24:113-119.

Petersen KF, Shulman GI. New insights into the pathogenesis of insulin resistance in humans using magnetic resonance spectroscopy. Obesity (Silver Spring) 2006 14 Suppl 1:34S-40S.

Pietinen P, Hartman AM, Haapa E, Räsänen L, Haapakoski J, Palmgren J, Albanes D, Virtamo J, Huttunen JK. Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. Am J Epidemiol 1988 128:655-666.

Ploug T, Ohkuwa T, Handberg A, Vissing J, Galbo H. Effect of immobilization on glucose transport and glucose transporter expression in rat skeletal muscle. Am J Physiol 1995 268:E980-986.

Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Mol Med 2008 14:741-751.

Rabøl R, Boushel R, Dela F. Mitochondrial oxidative function and type 2 diabetes. Appl Physiol Nutr Metab 2006 31:675-683.

Samiec PS, Drews-Botsch C, Flagg EW, Kurtz JC, Sternberg P Jr, Reed RL, et al. Glutathione in human plasma: decline in association with aging, age-related macular degeneration, and diabetes. Free Radic Biol Med 1998 24:699-704.

Scandinavian Committee on Enzymes. Scandinavian standardizations of enzyme determination: recommemded methods for the determination of four enzymes in blood. Scan J Clin Lab Invest 1974 33: 287-306.

Schenk S, Harber MP, Shrivastava CR, Burant CF, Horowitz JF. Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity. J Physiol 2009 587:4949-4961.

Seki Y, Berggren JR, Houmard JA, Charron MJ. Glucose transporter expression in skeletal muscle of endurance-trained individuals. Med Sci Sports Exerc 2006 38:1088-1092.

Sell H, Dietze-Schroeder D, Eckel J. The adipocyte-myocyte axis in insulin resistance. Trends Endocrinol Metab 2006 17:416-422.

Selsby JT, Judge AR, Yimlamai T, Leeuwenburgh C, Dodd SL. Life long calorie restriction increases heat shock proteins and proteasome activity in soleus muscles of Fisher 344 rats. Exp Gerontol 2005 40:37-42.

Sen CK, Khanna S, Venojärvi M, Trikha P, Ellison EC, Hunt TK, et al. Copper-induced vascular endothelial growth factor expression and wound healing. Am J Physiol Heart Circ Physiol 2002 282:H1821-1827.

Sen CK, Marin E, Kretzschmar M, Hänninen O. Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization. J Appl Physiol 1992 73:1265-1272.

Shan YX, Yang TL, Mestril R, Wang PH. Hsp10 and Hsp60 suppress ubiquitination of insulin-like growth factor-1 receptor and augment insulin-like growth factor-1 receptor signaling in cardiac muscle: implications on decreased myocardial protection in diabetic cardiomyopathy. J Biol Chem 2003 278:45492-45498.

Shulman GI. Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy. Physiology (Bethesda) 2004 19:183-190.

Sreekumar R, Nair KS. Skeletal muscle mitochondrial dysfunction & diabetes. Indian J Med Res 2007 125:399-410.

Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, et al. Fiber type composition of the vastus lateralis muscle of young men and women. J Histochem Cytochem 2000 48:623-629.

Sun Y, Oberley LW. Redox regulation of transcriptional activators. Free Radic Biol Med 1996 21:335-348.

Surakka J, Alanen E, Aunola S, Karppi SL, Pekkarinen H. Effects of external light loading in power-type strength training on muscle power of the lower extremities in middle-aged subjects. Int J Sports Med 2006 27:448-455.

Tabata I, Suzuki Y, Fukunaga T, Yokozeki T, Akima H, Funato K. Resistance training affects GLUT-4 content in skeletal muscle of humans after 19 days of head-down bed rest. J Appl Physiol 1999 86:909-914.

Thorburn AW, Gumbiner B, Bulacan F, Wallace P, Henry RR. Intracellular glucose oxidation and glycogen synthase activity are reduced in non-insulin-dependent (type II) diabetes independent of impaired glucose uptake. J Clin Invest 1990 85:522-529.

Thyfault JP, Booth FW. Lack of regular physical exercise or too much inactivity. Curr Opin Clin Nutr Metab Care 2011 Apr 23. (Epub ahead of print).

Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001 344:1343-1350.

Tupling AR, Bombardier E, Stewart RD, Vigna C, Aqui AE. Muscle fiber type-specific response of Hsp70 expression in human quadriceps following acute isometric exercise. J Appl Physiol 2007 103:2105-2111.

Turko IV, Murad F. Quantitative protein profiling in heart mitochondria from diabetic rats. J Biol Chem 2003 278:35844-35849.

Unger RH. Lipotoxic diseases. Annu Rev Med 2002 53:319-336.

Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997 389:610-614.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007 39:44-84.

van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab 2003 88:3005-3010.

Vasilaki A, Jackson MJ, McArdle A. Attenuated HSP70 response in skeletal muscle of aged rats following contractile activity. Muscle Nerve 2002 25:902-905.

Vasilaki A, McArdle F, Iwanejko LM, McArdle A. Adaptive responses of mouse skeletal muscle to contractile activity: the effect of age. Mech Ageing Dev 2006 127:830-839.

Venojärvi M, Kvist M, Atalay M, Jozsa L, Kalimo H. Recovery from immobilisation: responses of fast-twitch muscle fibres to spontaneous and intensive exercise in rat calf muscles. Pathophysiology 2004 11:17-22.

Veskoukis AS, Nikolaidis MG, Kyparos A, Kouretas D. Blood reflects tissue oxidative stress depending on biomarker and tissue studied. Free Radic Biol Med 2009 47:1371-1374.

Vestergaard H, Lund S, Larsen FS, Bjerrum OJ, Pedersen O. Glycogen synthase and phosphofructokinase protein and mRNA levels in skeletal muscle from insulin-resistant patients with non-insulin-dependent diabetes mellitus. J Clin Invest 1993 91:2342-2350.

Vogt B, Muhlbacher C, Carrascosa J, Obermaier-Kusser B, Seffer E, Mushack J, et al. Subcellular distribution of GLUT 4 in the skeletal muscle of lean type 2 (non-insulin-dependent) diabetic patients in the basal state. Diabetologia 1992 35:456-463.

Vogt M, Puntschart A, Geiser J, Zuleger C, Billeter R, Hoppeler H. Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. J Appl Physiol 2001 91:173-182.

Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem 2003 278:40352-40363.

Wang Y, Lam KS, Chan L, Chan KW, Lam JB, Lam MC, et al. Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. J Biol Chem 2006 281:16391-16400.

Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001 86:1930-1935.

World Health Organization. Diabetes Mellitus. Report of a WHO Study Group. Technical Report Series No 727. Geneva: WHO 1985.

World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. Report of a WHO Study Group. Geneva: WHO 2009.

Willoughby DS, Priest JW, Nelson M. Expression of the stress proteins, ubiquitin, heat shock protein 72, and myofibrillar protein content after 12 weeks of leg cycling in persons with spinal cord injury. Arch Phys Med Rehabil 2002 83:649-654.

Winder WW. Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. J Appl Physiol 2001 91:1017-1028.

Woodgett JR. Physiological roles of glycogen synthase kinase-3: potential as a therapeutic target for diabetes and other disorders. Curr Drug Targets Immune Endocr Metabol Disord 2003 3:281-290.

Wróblewski F, LaDue JS. Lactic dehydrogenase activity in blood. Proc Soc Exp Biol Med 1955 90: 210-213.

Yamada P, Amorim F, Moseley P, Schneider S. Heat shock protein 72 response to exercise in humans. Sports Med 2008 38:715-733.

Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003 423:762-769.

Zhang H, Zhang C. Adipose "talks" to distant organs to regulate insulin sensitivity and vascular function. Obesity (Silver Spring) 2010 18:2071-2076.

Zhao FQ, Keating AF. Functional properties and genomics of glucose transporters. Curr Genomics 2007 8:113-128.

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Roles of Exercise Training with Dietary Counselling and Muscle Fibre Composition in the Regulation of Glucose Metabolism in Middle-aged Subjects with Impaired Glucose Tolerance

The aim of this study was to clarify the role of exercise training with dietary counselling on heat shock proteins, adipokines and glucose metabolism in middle-aged subjects with impaired glucose tolerance. Additionally, in rats we tested the hypothesis that a stronger, non-damaging, metabolic stress may provide greater tissue protection against atrophic and degenerative changes after immobilization. This thesis provides new evidences on the vulnerability of the subjects to impaired glucose tolerance and supports promotion of weight reduction and long-term exercise training to improve insulin sensitivity in skeletal muscle.

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