TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 861

MEDICA - ODONTOLOGICA

EFFECTS OF WEIGHT LOSS ON ADIPOSE TISSUE, LIVER AND HEART METABOLISM IN HUMANS

Studies with PET, MRI and MRS

by

Antti Viljanen

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2009

From Turku PET Centre, the Department of Medicine, and the Department of Clinical Physiology and Nuclear Medicine, University of Turku, Turku, Finland

Research school membership: Turku Graduate School of Clinical Sciences

Supervised by

Professor Pirjo Nuutila, MD, PhD
Turku PET Centre and the Department of Medicine
University of Turku
Turku, Finland

and

Docent Patricia Iozzo, MD, PhD
PET centre, Institute of Clinical Physiology
CNR, National Research Council
Pisa, Italy

and

Professor Olli T. Raitakari, MD, PhD
Department of Clinical Physiology
Turku University Hospital
Turku, Finland

Reviewed by

Docent Katriina Kukkonen-Harjula, MD, PhD
UKK Institute for Health Promotion Research
Tampere, Finland

and

Docent Jukka Westerbacka, MD, PhD
Department of Medicine
Helsinki University Hospital
Helsinki, Finland

Dissertation Opponent

Professor Markku Savolainen, MD, PhD
Department of Medicine
University of Oulu
Oulu, Finland

ISBN 978-951-29- 3977-0 (PRINT)
ISBN 978-951-29- 3978-7 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2009

To my family

ABSTRACT

Antti Viljanen

EFFECTS OF WEIGHT LOSS ON ADIPOSE TISSUE, LIVER AND HEART METABOLISM IN HUMANS
Studies with PET, MRI and MRS
Turku PET Centre, the Department of Medicine, and the Deparment of Clinical Physiology and Nuclear Medicine, University of Turku, Turku, Finland
Annales Universitatis Turkuensis
Painosalama OY, Turku, Finland 2009

Obesity has become the leading cause of many chronic diseases, such as type 2 diabetes and cardiovascular diseases. The prevalence of obesity is high in developed countries and it is also a major cause of the use of health services. Ectopic fat accumulation in organs may lead to metabolic disturbances, such as insulin resistance. Weight loss with very-low-energy diet is known to be safe and efficient. Weight loss improves whole body insulin sensitivity, but its effects on tissue and organ level in vivo are not well known. The aims of the studies were to investigate possible changes of weight loss in glucose and fatty acid uptake and perfusion and fat distribution at tissue and organ level using positron emission tomography and magnetic resonance imaging and spectroscopy in 34 healthy obese subjects.

The results showed that whole-body insulin sensitivity increased after weight loss with very-low-energy diet and this is associated with improved skeletal muscle insulin-stimulated glucose uptake, but not with adipose tissue, liver or heart glucose uptake. Liver insulin resistance decreased after weight loss. Liver and heart free fatty acid uptakes decreased concomitantly with liver and heart triglyceride content. Adipose tissue and myocardial perfusion decreased.

In conclusion, enhanced skeletal muscle glucose uptake leads to increase in whole-body insulin sensitivity when glucose uptake is preserved in other organs studied. These findings suggest that lipid accumulation found in the liver and the heart in obese subjects without co-morbidities is in part reversible by reduced free fatty acid uptake after weight loss. Reduced lipid accumulation in organs may improve metabolic disturbances, e.g. decrease liver insulin resistance.

Keywords: Obesity, weight loss, very-low-energy diet, adipose tissue metabolism, liver metabolism, heart metabolism, positron emission tomography

TIIVISTELMÄ

Antti Viljanen

LAIHDUTUKSEN VAIKUTUKSET RASVAKUDOKSEN, MAKSAN JA SYDÄMEN AINEENVAIHDUNTAAN IHMISILLÄ
Tutkimuksia positroniemissiotomografialla, magneettikuvauksella ja magneettispektroskopialla

Valtakunnallinen PET-keskus, Sisätautioppi, sekä Kliinisen fysiologian ja isotooppilääketieteen oppiaine, Turun yliopisto, Turku
Annales Universitatis Turkuensis
Painosalama OY, Turku, 2009

Lihavuudesta on tullut tärkein pitkäaikaisten sairauksien, kuten tyypin 2 diabeteksen ja sydän- ja verisuonisairauksien aiheuttaja. Lihavuuden esiintyvyys on suuri teollisuusmaissa ja lihavuus on merkittävä sairaanhoitokulujen aiheuttaja. Rasvan kertyminen elimiin voi aiheuttaa aineenvaihdunnan häiriöitä, kuten insuliiniresistenssia. Laihduttaminen erittäin niukkaenergiaisella ruokavaliolla on todettu turvalliseksi ja tehokkaaksi. Laihdutus parantaa koko kehon insuliiniherkkyyttä. Muutoksista yksittäisten elinten ja kudosten tasolla on vain vähän tietoa. Näiden väitöskirjatutkimusten tarkoituksena oli selvittää laihdutuksen vaikutuksia glukoosin ja rasvahapon soluunottoon, verenkierron määrän ja rasvan jakautumiseen kudosten ja elinten tasolla 34 terveellä lihavalla koehenkilöllä. Menetelmänä käytettiin positroniemissiotomografiaa ja magneettikuvausta sekä magneettispektroskopiaa.

Tulokset osoittivat, että koko kehon insuliiniherkkyyden paraneminen erittäin niukkaenergiaisella ruokavaliolla oli yhteydessä luurankolihaksen parantuneeseen glukoosin soluunottoon, mutta se ei ollut yhteydessä rasvakudoksen, maksan tai sydänlihaksen glukoosin soluunottoon. Maksan insuliiniresistenssi väheni laihduttamisella. Maksan ja sydänlihaksen rasvahapon soluunotto väheni yhdessä maksan ja sydänlihaksen rasvapitoisuuden kanssa. Rasvakudoksen ja sydänlihaksen verenvirtaus väheni.

Johtopäätöksenä todetaan, että luurankolihaksen parantuva glukoosin soluunotto johtaa koko kehon insuliiniherkkyyden parantumiseen, samalla kun muissa tutkituissa kudoksissa glukoosin soluunotto ei muutu. Tutkimustulokset viittaavat siihen, että lihavuuteen liittyvä rasvan kertyminen maksaan ja sydänlihakseen on palautuvaa, ja johtuu osittain rasvahapon soluunoton vähenemisestä laihduttamisen myötä. Vähentynyt rasvan kertyminen elimiin voi parantaa aineenvaihdunnan häiriöitä, kuten vähentää maksan insuliiniresistenssia.

Avainsanat: Lihavuus, laihdutus, erittäin niukkaenergiainen ruokavalio, rasvakudoksen aineenvaihdunta, maksan aineenvaihdunta, sydämen aineenvaihdunta, positroniemissiotomografia

TABLE OF CONTENTS

ABBREVIATIONS

ALAT	alanine aminotransferase
BMI	body mass index
EGIR	European group for the study of insulin resistance
FABP	fatty acid binding protein
FFA	free fatty acids
[^{18}F]FDG	[^{18}F]-2-fluoro-2-deoxyglucose
[^{18}F]FTHA	[^{18}F]-fluoro-6-thia-heptadecanoic acid
GLUT	glucose-specific transport molecyle
GT	gamma-glutamyl trasferase
HbA_{1c}	glycosylated hemoglobin A_{1c}
HDL	high density lipoprotein
IL	interleukin
Ki	influx rate constant
LDL	low density lipoprotein
min	minute
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
OGIS	oral glucose sensitivity index
PET	positron emission tomography
ROI	region of interest
sec	seconds
SE	standard error of the mean

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, which are referred to in the text by the corresponding Roman numerals.

I Viljanen A.P.M., Lautamäki R., Järvisalo M., Parkkola R., Huupponen R., Lehtimäki T., Rönnemaa T., Raitakari O.T., Iozzo P., Nuutila P. Effects of Weight Loss on Visceral and Abdominal Subcutaneous Adipose Tissue Blood Flow and Insulin-mediated Glucose Uptake in Healthy Obese Subjects. Annals of Medicine 2009;41(2):152-60.

II Viljanen A.P.M., Iozzo P., Borra R., Kankaanpää M., Karmi A., Lautamäki R., Järvisalo M., Parkkola R., Rönnemaa T., Guiducci L., Lehtimäki T., Raitakari O.T., Mari A., Nuutila P. Effect of Weight Loss on Liver Free Fatty Acid Uptake and Hepatic Insulin Resistance. Journal of Clinical Endocrinology and Metabolism 2009 Jan;94(1):50-5.

III Viljanen A.P.M., Karmi A., Borra R, Pärkkä J.P., Lepomäki V., Parkkola R., Lautamäki R., Järvisalo M., Taittonen M., Rönnemaa T., Iozzo P., Knuuti J., Nuutila P., Raitakari O.T. Effect of Caloric Restriction on Myocardial Fatty Acid Uptake, Left Ventricular Mass and Cardiac Work in Obese Adults. Accepted for publication in American Journal of Cardiology 2009.

1 INTRODUCTION

Overweight and obesity has become a global burden. Excess fat accumulation in the human body is closely associated with decreased whole-body insulin sensitivity (Ferrannini *et al.*, 1997; Arner, 1997). In obesity, decrease in whole-body insulin sensitivity is mainly caused by skeletal muscle insulin resistance. Weight loss improves whole-body insulin sensitivity, but its effects at tissue and organ level in vivo are not well known.

Abdominal obesity is strongly associated with insulin resistance (Despres *et al.*, 1989; Goodpaster *et al.*, 1997; Rönnemaa *et al.*, 1997) and increased waist circumference is a clear risk factor for the development of diabetes and cardiovascular diseases. Visceral adipose tissue is suggested to be the key to the harmful effect of upper body obesity due to its vascular drainage to the liver (Björntorp, 1990), thus giving a direct route for adipose tissue -derived adipokines to affect liver metabolism (Ruan & Lodish, 2004), and hence glucose metabolism and systemic inflammation.

Obesity is commonly associated with an accumulation of fat in the liver (Ludwig *et al.*, 1980; Powell *et al.*, 1990), and liver adiposity correlates with insulin resistance in whole-body (Marchesini *et al.*, 1999; Sanyal *et al.*, 2001) and hepatic insulin resistance (Seppälä-Lindroos *et al.*, 2002; Bugianesi *et al.*, 2005). Low-energy diets have been shown to reduce hepatic steatosis, (Drenick *et al.*, 1970; Andersen *et al.*, 1991), and improve insulin sensitivity (Goodpaster *et al.*, 1999). Liver fat content can be measured non-invasively with magnetic resonance spectroscopy (Szczepaniak *et al.*, 1999). Positron emission tomography has proved to be a superior tool for the direct assessment of liver glucose and free fatty acids (FFA) metabolism non-invasively (Iozzo *et al.*, 2007; Iozzo *et al.*, 2003c).

In animal models, obesity and insulin resistance increase the availability and myocardial uptake of FFA. Weight loss studies in humans concerning myocardial fatty acid and glucose metabolism are few probably due to the limited number of techniques available to measure myocardial substrate metabolism *in vivo*. In a study by Peterson et al. (Peterson *et al.*, 2004), myocardial fatty acid uptake measured with positron emission tomography tended to be increased in obese compared to lean subjects, but no differences were detected in myocardial glucose uptake or blood flow.

In the present studies, obese subjects without co-morbidies were selected as the study group. These studies were designed to investigate the effects of weight loss on adipose tissue, liver and heart metabolism at tissue level. This was possible with the use of positron emission tomography (PET) imaging technique. A short-lived, and a low radiation exposure positron-emitting radioisotope is attached to a glucose or fatty acid molecule, which can be traced in the blood and tissue. Regions of interest can be drawn to isolated organs and tissue molecule uptake can quantified with mathematical modelling. [^{18}F]-2-fluoro-2-deoxy-D-glucose ([^{18}F]FDG) and 14(R,S)-[^{18}F]fluoro-6-thia-heptadecanoid acid ([^{18}F]FTHA) were used as tracers to study glucose and FFA metabolism, respectively, and [^{15}O]H_2O was used to study blood flow. In addition,

advanced techniques such as magnetic resonance imaging, magnetic resonance spectroscopy and euglycemic clamping were combined with the positron emission tomography.

2 REVIEW OF THE LITERATURE

2.1. Obesity

2.1.1. Prevalence of obesity

Cardiovascular diseases are the leading cause of chronic noncontagious disease mortality but obesity and diabetes are showing worrying trends in mortality, not only because they already affect a large proportion of the population, but also because they have started to appear earlier in life (Jackson-Leach & Lobstein, 2006). Overweight (body mass index over 25 kg/m^2) and obesity (body mass index over 30 kg/m^2) are increasing substantially (Popkin, 2002) and the number of people with diabetes will increase more than 2.5-fold, from 84 million in 1995 to 228 million in 2025 (World Health Organisation, 2001) in the developing world. On the other hand, lifestyle changes leading to weight reduction decreases the risk for diabetes (Tuomilehto *et al.*, 2001). Prevalence of obesity in the middle-aged in Europe is 15-20 % (Björntorp, 1997). Prevalence of obesity in Finland is 19-20 % and prevalence of overweight is 48 % in males and 33 % in females in the age group of 25-64 years (Lahti-Koski *et al.*, 2002).

2.1.2. Classification of obesity

Obesity is defined as excess body fat mass. The body mass index, calculated as the ratio of body weight and squared height (kg/m^2) is a good correlate of body adipose composition (Revicki & Israel, 1986; Garrow & Webster, 1985). The upper limit for the normal body mass index is 25 kg/m^2, because the risk of certain diseases is elevated at a higher body mass index value (World Health Organisation, 2000). The term "overweight" is used when the body mass index is 25 – 29.9 kg/m^2, and the term "obesity" when the body mass index is 30 kg/m^2 or higher. The body mass index does not distinguish between those who have enlarged muscle mass and those who have large fat mass. This difference can be found with a proportion of fat mass measurement, for example, with bioelectrical impedance analysis (Abu *et al.*, 1988), skin-fold thickness (Durnin & Rahaman, 1967) or with dual-energy X-ray absorptiometry (Lukaski, 1993). Also abdominal obesity, i.e. the amount of intra-abdominal, visceral adipose tissue is used in the classification of severity of overweight. Waist circumference is associated with area of visceral fat (Pouliot *et al.*, 1994) and it is suggested to be a better predictor of cardiovascular diseases than body mass index (Zhu *et al.*, 2002). Waist circumference over 90 cm in men and over 80 cm in women is related to a small risk to above mentioned diseases, and values over 100 cm in men and over 90 cm in women are related to a high risk of diseases (Han *et al.*, 1995; Lemieux *et al.*, 1996; Tanko *et al.*, 2005). Fat composition in the body increases with age and is higher in women (Schutz *et al.*, 2002; Jackson *et al.*, 2002).

2.1.3. Mortality and morbidity in obesity

All-cause mortality is increased when the body mass index is over 30 kg/m^2 (Troiano *et al.*, 1996; McGee, 2005) and both weight level and weight change in adult-age have independent effects on total mortality (Mikkelsen *et al.*, 1999). On the other hand, obesity-related excess mortality declines with age (Bender *et al.*, 1999). Obesity increases risk of chronic noncontagious diseases such as hypertension (Jousilahti *et al.*, 1995), type 2 diabetes (Colditz *et al.*, 1990; Everhart *et al.*, 1992; Ohlson *et al.*, 1985; Moore *et al.*, 2000; Wannamethee & Shaper, 1999; French *et al.*, 1997; Colditz & Coakley, 1997; Chan *et al.*, 1994), several types of cancer (Bergström *et al.*, 2001), coronary artery disease (Silventoinen *et al.*, 2003; Wilson *et al.*, 2002; Kip *et al.*, 2004; Alexander, 2001; Shirai, 2004), and liver steatosis (Wanless & Lentz, 1990; Bellentani *et al.*, 2000), and morbidity is related to the severity of obesity.

2.1.4. Obesity and chronic diseases

Risk factors for chronic diseases such as diabetes and cardiovascular diseases are well established: obesity and physical inactivity for diabetes and atherosclerosis (Jousilahti *et al.*, 1996; Wannamethee *et al.*, 1998; Davey-Smith G. *et al.*, 2000), high blood pressure for coronary heart disease (MacMahon *et al.*, 1990;Kannel, 1996) and high serum cholesterol for coronary heart disease (Hu *et al.*, 2000). Hyperinsulinemia seems to be an independent risk factor for coronary heart disease (Pyörälä *et al.*, 1998; Despres *et al.*, 1996). The clustering of risk factors has been described as the metabolic syndrome including central obesity, hypertension, elevated plasma triglyceride and low high density lipoprotein (HDL) cholesterol, elevated fasting glucose and hyperinsulinemia (Reaven, 1988; DeFronzo & Ferrannini, 1991) with varying exact definitions (Zimmet *et al.*, 2005).

2.1.5. Obesity and insulin resistance

Insulin resistance is the condition where normal insulin levels are inadequate to produce normal insulin response in tissue. Prevalence of insulin resistance increases with body mass index (Ferrannini *et al.*, 1997). In the European Group for the Study of Insulin Resistance (EGIR) database, insulin resistance was measured with the euglycemic clamp technique (DeFronzo *et al.*, 1979). In subjects with a body mass index 30-35 kg/m^2, prevalence of insulin resistance was 34 %, and in subjects with a body mass index over 35 kg/m^2, prevalence was 41 % in the EGIR database (Ferrannini *et al.*, 1997). Insulin resistance in obesity includes resistance to insulin-stimulated glucose utilization and resistance to insulin-induced inhibition of hepatic glucose output (Bonadonna *et al.*, 1990). Also increased lipid oxidation and reduced glucose oxidation are found in insulin resistance (Ferrannini *et al.*, 1987). Hyperinsulinemia is common in obesity and it closely follows the degree of obesity (Ferrannini *et al.*, 1997).

Whole body insulin resistance measured with euglycemic hyperinsulinemic clamp is predominantly localized to skeletal muscle (Bonadonna *et al.*, 1990; Natali *et al.*, 1991; Capaldo *et al.*, 1991). There are various mechanisms that can contribute to insulin resistance. Insulin signaling defects are found in obesity (Kolterman *et al.*, 1980). Studies of morbidly obese human skeletal muscle biopsies showed defects in insulin signaling (Goodyear *et al.*, 1995), and depletion of glucose transporters (Dohm *et al.*, 1988). In animal studies, muscle-specific insulin receptor knockout mice have increased fat mass and plasma FFA, but normal glucose tolerance (Bruning *et al.*, 1998). Liver-specific insulin receptor knockout mice have defects early in the insulin signaling cascade, such as in insulin receptor substrate protein phosphorylation, and they are severely insulin-resistant and glucose-intolerant, because insulin cannot suppress hepatic glucose production (Michael *et al.*, 2000). Fat-specific insulin receptor knockout mice have adipose tissue selective insulin resistance and low fat mass with normal whole-body glucose metabolism (Bluher *et al.*, 2002). Skeletal muscle blood flow is suggested to play a role in insulin resistance (Baron *et al.*, 1991) since insulin´s effect stimulating blood flow is decreased in insulin resistant obese humans (Laakso *et al.*, 1992). On the other hand, insulin resistance can not be overcome by normalizing blood flow in skeletal muscle (Laine *et al.*, 1998b). Fat accumulation in insulin sensitive tissues, such as skeletal muscle (Jacob *et al.*, 1999; Perseghin *et al.*, 1999; Greco *et al.*, 2002) and liver (Marchesini *et al.*, 1999; Sanyal *et al.*, 2001; Westerbacka *et al.*, 2004) is related to insulin resistance. Skeletal muscle fat content and insulin resistance are shown to concomitantly increase with lipid infusion and concomitantly decrease by lowering plasma FFA in healthy humans (Boden *et al.*, 2001).

2.2. Adipose tissue and obesity

2.2.1. Adipose tissue anatomy

Adipocytes are formed from mesodermal multi-potent stemcells. Mature adipocytes are incapable of mitosis, and new adipocytes can be develope at any time. In mature adipocyte, 90 % of cell volume is lipids, mainly trilycerides (Leeson & Leeson, 1976). Of adipose tissue, 80 % is fat and 15 % is water, with the remaining 5 % being connective tissue proteins, lymphoid cells and blood vessels. Intra-abdominal fat consists of retroperitoneal and intraperitoneal fat. Intraperitoneal fat is called visceral fat and it consists of mesenterical and omental fat.

The largest fat depot is subcutaneous fat (Mårin *et al.*, 1992). On the avarage, subcutaneous fat mass is approximately 50 % in men and 70 % in women of the total adipose tissue mass (Hattori *et al.*, 1991). Visceral fat is approximately 18 % in men (Ross *et al.*, 1992) and 6 % in women (Ross *et al.*, 1993) of the total adipose tissue mass and visceral adipose tissue mass increases with age (Goodman-Gruen & Barrett-Connor, 1996). Adipocyte density is higher in visceral fat and visceral adipocytes are smaller than in subcutaneous fat (Björntorp, 1996). Genetic factors affect fat

distribution; for example abdominal obesity phenotype markers are found (Perusse *et al.*, 2001).

Positive energy balance leads to overweight and obesity. Sedentary lifestyle is a major cause of positive energy balance. Genes and environment predispose to obesity. Low physical activity can be accompanied with high energy meals and high energy drinks. Fat cells enlarge during the development of obesity and it is thought that enlargement triggers the formation of new fat cells, also gene expression changes in adipocytes (Dahlman & Arner, 2007). In subcutaneous adipose tissue, the adipocyte size of 0.8 µg is suggested to be the upper limit after which new cells develope (Sjöström & Björntorp, 1974). Fat cell size is almost the same in subcutaneous and visceral adipose tissue in overweight subjects (Mårin *et al.*, 1992; Fried *et al.*, 1998). Both these adipose tissue depots increase concomitantly in obesity, and in obese men, subcutaneous fat mass can increase to 80 % of total adipose mass (Mårin *et al.*, 1992).

2.2.2. Adipose tissue and insulin resistance

Abdominal obesity is strongly associated with insulin resistance (Despres *et al.*, 1989; Goodpaster *et al.*, 1997; Rönnemaa *et al.*, 1997). When the delivery of nutritients excess oxidative and storage capacities of tissues, metabolites that inhibit insulin signaling can be formed (Kraegen *et al.*, 2001; Unger, 2003; Lelliott & Vidal-Puig, 2004). The sphingolipid ceramide is one metabolite that could induct insulin resistance (Summers, 2006). Visceral adipose tissue has a direct drainage to the liver via the portal vein offering a direct route for adipose tissue -derived adipokines to affect liver metabolism (Björntorp, 1990; Ruan & Lodish, 2004).

When subjects with similar abdominal subcutaneous adipose tissue depots, but different visceral depots were compared, subjects with larger visceral adipose tissue depots had higher plasma glucose values during oral glucose test and lower glucose disposal rates (Ross *et al.*, 2002). In addition, when comparing subjects with matched visceral adipose tissue depots and different abdominal subcutaneous adipose tissue depots, no statistically different results were found for these glucose values. Ross et al. concluded that visceral adipose tissue has a role in insulin action, independent of subcutaneous adipose tissue, although the importance of visceral fat has been questioned by others (Abate *et al.*, 1995; Goodpaster *et al.*, 1997). On the other hand, subcutaneous adipose tissue is responsible for the majority of FFA delivered into the circulation in humans in the fasting state (Jensen, 2006), and FFA are associated as a marker with insulin resistance.

2.2.3. Adipose tissue FFA and glucose metabolism and blood flow

Fatty acids are used as building blocks in the human body. Fatty-acid-derived phospholipids and glycolipids are components of biological membranes. Fatty acid derivates also act as intracellular messengers and hormones. Fatty acids are stored in the form of triacylglycerol, i.e. triglycerides mainly in adipocytes, but also other

organs including liver, heart and skeletal muscle. During fast, insulin levels fall and permit the release of FFA and glycerol (Frayn *et al.*, 1989; Shulman *et al.*, 1997). Arterial plasma FFA concentration closely correlates with abdominal subcutaneous FFA release (Frayn *et al.*, 1989).

FFA are transported in the plasma in a nonesterified form attached to albumin, or bound covalently to triglycerides, which are transported in chylomicrons and very-low-density lipoproteins. FFA are hydrolyzed from circulating chylomicrons or very-low-density lipoproteins by lipoprotein lipase, which locates in the capillary endothelium. FFA are taken up by the tissue passively and by facilitated transport (Berk & Stump, 1999) and lipoprotein lipase expression is the rate-limiting step in this uptake process (Preiss-Landl *et al.*, 2002). Insulin stimulates lipoprotein lipase in adipose tissue (Farese, Jr. *et al.*, 1991). FFA are re-esterified to triglycerides in adipocytes if glycerol 3-phosphate is abundant. Glucose level inside the cell can affect FFA release via glycerol 3-phosphate supply (Lönnroth & Smith, 1992). Insulin promotes energy storaging by stimulating triglyceride synthesis, i.e. lipogenesis, and inhibiting lipolysis in adipocytes (Hagström-Toft *et al.*, 1992).

Glucose is taken up by adipose tissue by mass effect and by facilitated diffusion with glucose-specific transport molecyles, GLUT4 and GLUT1. GLUT4 is insulin-dependent and GLUT1 is more insulin-independent (Kahn, 1992). Insulin stimulates GLUT4 translocation (Sun *et al.*, 1994). Glucose is phosphorylated by hexokinase (Katzen *et al.*, 1970) to glucose-6-phosphate, which can be stored as glycogen or further converted to pyruvate. Pyruvate is oxidized to acetyl coenzyme-A, which is used in the mitochondrion to produce adenosine triphosphate. Glucose-6-phosphate can be converted to glycerol 3-phosphate, which can be further converted to triglycerides.

Insulin stimulates adipose tissue glucose uptake (Green & Newsholme, 1979), and insulin-stimulated adipose tissue glucose uptake is impaired in obesity (Virtanen *et al.*, 2002). Adipose tissue blood flow is a regulator of adipose tissue metabolism (Frayn *et al.*, 1997) and affects regional fatty acid storage after meals (Romanski *et al.*, 2000). Visceral adipose tissue has a dense circulation (West *et al.*, 1989), which leads to a higher blood flow than in subcutaneous adipose tissue (Enevoldsen *et al.*, 2000; Virtanen *et al.*, 2002). Postprandial adipose tissue blood flow is associated with insulin sensitivity, possibly via symphathetic activation (Karpe *et al.*, 2002), because insulin-induced vasodilatation does not change subcutaneous adipose tissue blood flow (Stallknecht *et al.*, 2000).

2.2.4. Adipocytes and inflammation

Adiponectin is a collagen-like circulating protein, which is secreted by adipocytes (Scherer *et al.*, 1995). Circulating adiponectin concentration is associated with insulin sensitivity (Tschritter *et al.*, 2003) and it is decreased in obesity (Weyer *et al.*, 2001). Recombinant adiponectin infusion liver fat in mice (Xu *et al.*, 2003). The ob gene product, leptin, is mainly synthesized in adipose tissue depots (Zhang *et al.*, 1994).

Leptin affects energy balance by inhibiting the activity of neurons, that contain neuropeptin-y, these neurons regulate appetite (Himms-Hagen, 1999). Leptin also regulates the immune function (Lord *et al.*, 1998; Farooqi *et al.*, 2002) providing a link between adipokines and cytokines, e.g. interleukin (IL)-6, which is partly synthesized from adipose tissue (Mohamed-Ali *et al.*, 1997). Low-grade systemic inflammation that can be measured with c-reactive protein, tumor necrosis-factor-alpha, IL-6 and leptin is found in obesity, and this elevation of inflammatory cytokines is associated with cardiovascular risk (Das, 2001). Adipose tissue is a complex network of adipocytes, stromal cells, vascular cells and macrophages. The network allows different adipokines and cytokines to act also locally as autocrine and paracrine signals.

2.3. Liver

2.3.1. Liver anatomy

The liver is located in the abdominal cavity in the right hypochondrium. The avarage liver weight is 1.4-1.6 kg in men and 1.2-1.4 kg in women. The liver consists of four lobes which are divided into the lobules 1.0-2.5 mm in measure. Blood supply comes from the hepatic artery and the portal vein in the relation of 20% / 80% of liver perfusion (Hall *et al.*, 1996) and portal vein drains the gastrointestinal tract and most of the visceral organs including the visceral adipose tissue depot. Of cardiac output, 25-30 % comes to the liver (Hall *et al.*, 1996). The liver controls e.g. glucose homeostatis and lipid and amino acid turnover.

2.3.2. FFA and glucose metabolism in liver

Glucose transport is facilitated with GLUT2, and distribution of GLUT2 in the liver shows zonation (Ogawa *et al.*, 1996). A rate-limiting step of glucose uptake is its phosphorylation by hexokinases (Wilson, 2003), mainly glucokinase. Glucose is first phosphorylated to glucose-6-phosphate. This can then enter glycogen synthesis, glycolysis, and the pentose phosphate pathway, or be hydrolyzed back to glucose. Glucose-6-phosphate can be produced from different circulating precursors, including lactate, glycerol and pyruvate via gluconeogenesis.

Liver FFA are oxidized during fasting and incorporated to triglycerides postprandially. FFA oxidation-derived acytyl-CoA can be used in gluconeogenesis, which is stimulated by FFA (Blair *et al.*, 1999; Chen *et al.*, 1999; Williamson *et al.*, 1966). FFA-induced increase in gluconeogenesis is compensated by a reduction in glucogenolysis, so hepatic glucose production is not changed, this is called hepatic autoregulation (Chu *et al.*, 2002; Clore *et al.*, 1991; Roden *et al.*, 2000). Hepatic autoregulation can be interrupted in insulin resistance or insulin deficiency. Also, elevation of FFA can interrupt hepatic autoregulation by lowering insulin-stimulated glycogen synthesis (Kim *et al.*, 2001), and insulin-mediated suppression of glycogenolysis (Boden *et al.*, 2002) leading to an increase in hepatic glucose

production. Elevation of FFA decreases hepatic glucose uptake and whole-body glucose uptake in vivo (Iozzo *et al.*, 2004a).

2.3.3. Liver and obesity

Obesity is commonly associated with an accumulation of fat in the liver (Ludwig *et al.*, 1980; Powell *et al.*, 1990), and liver steatosis correlates with insulin resistance in whole-body (Marchesini *et al.*, 1999; Sanyal *et al.*, 2001) and hepatic insulin resistance (Bugianesi *et al.*, 2005). Increased flux in FFA via the portal vein from the visceral fat to the liver in obesity is proposed to be one of the mechanisms causing accumulation of fat in the liver (Björntorp, 1992)**.**

Obesity-related liver disorders are collectively named as non-alcoholic fatty liver diseases (NAFLD). NAFLD is defined as fat accumulation in the liver exceeding 5 % to 10 % by weight and non-alcoholic as 20 g a day of ethanol, which is roughly two doses a day (Neuschwander-Tetri & Caldwell, 2003). The clinical spectrum of NAFLD varies from simple, reversible liver steatosis to a non-alcoholic steatohepatitis (NASH) (Kleiner *et al.*, 2005; Bondini *et al.*, 2007; Yeh & Brunt, 2007), which may lead to hepatic injury, such as fibrosis and cirrhosis (Brunt, 2001; Farrell & Larter, 2006; Charlton *et al.*, 2001), and to need for liver transplantation (Burke & Lucey, 2004).

Hepatic steatosis is developed after excess energy intake leading to an increase in circulating fatty acids and increase in adipocyte fatty acid uptake (Bradbury & Berk, 2004; Berk & Stump, 1999). Adipocyte fatty acid uptake initially increases passively, and after up-regulation of facilitated transport leading to an increased triglyceride mass (Berk, 2008). Also a single or a combination of other mechanisms can lead to an accumulation of triglycerides in the liver, including 1) increase in fatty acid synthesis, 2) increase in triglyceride synthesis by de-novo lipogenesis, 3) increase in lipoprotein triglyceride uptake via low-density lipoprotein receptor and re-assembly, 4) decrease in Apolipoprotein B_{100} synthesis, 5) decrease in triglyceride mobilization, 6) decrease in very-low-density lipoprotein assembly, 7) decrease in very-low-density lipoprotein secretion, or 8) decrease in fatty acid β-oxidation (Bradbury & Berk, 2004; Chirieac *et al.*, 2000; Diehl, 2005; Donnelly *et al.*, 2005; Rinella *et al.*, 2008; Minehira *et al.*, 2008). Excess triglycerides may lead to oxidative stress, cell apoptosis, inflammation and cytokine cascades in hepatocytes, and further fibrosis and cirrhosis of the liver (McCullough, 2006; Merriman *et al.*, 2006; Mantena *et al.*, 2008; Malhi & Gores, 2008; Jou *et al.*, 2008). Several genes are upregulated in NAFLD e.g. ceramide related genes (Westerbacka *et al.*, 2007; Greco *et al.*, 2008)

2.4. Heart

2.4.1. Heart anatomy and blood flow

The myocardium consists of right and left halves, both containing an atrium and a ventricle. The size of the heart is close to that of a persons´ fist, and it is associated

with a lean body mass (Whalley *et al.*, 1999; Whalley *et al.*, 2004). The weight of the heart muscle is 200-350 grams. Epicardial coronary arteries supply blood from the aorta to the myocardium. At rest, normal blood flow of the heart is approximately 5 % of cardiac output. Of the delivered oxygen, 70-80 % is extracted in the myocardium at rest compared to 25 % of other tissues. Capillary density in the myocardium is about four times greater than in skeletal muscle (Schremmer & Dhainaut, 1990; Opie, 1998). Blood flow in the heart can increase three- to five-fold under stimulated physiological conditions, e.g. under adenosine infusion, measured with positron emission tomography (Dayanikli *et al.*, 1994; Uren *et al.*, 1994; Pitkänen *et al.*, 1998). Blood flow can be regulated with local metabolic control (Berne, 1964; Muller *et al.*, 1996; Opie, 1998), with autoregulation (Chilian & Layne, 1990; Narishige *et al.*, 1993; Smith & Canty, 1993) and with neurogenic mechanisms (Chilian, 1991; Opie, 1998). Insulin enhances adenosine-stimulated myocardial blood flow dose-dependently (Sundell *et al.*, 2002) while myocardial vasoreactivity and insulin response are reduced in obesity (Sundell *et al.*, 2002).

2.4.2. FFA and glucose metabolism in heart

The normal myocardium uses FFA and glucose as an energy source (Opie LH, 1991). Additional energy sources include lactate, pyruvate and ketone bodies. Both FFA and glucose are broken down to acetate, which is bound to coenzyme-A to form acetyl-CoA. Acetyl-CoA enters the citrate cycle (Opie, 1998; Depre *et al.*, 1999) in a mitochondrial cytochrome chain to form adenosine triphosphate (ATP) as energy. ATP breakdown produces energy for myocardial cell contraction and ion gradient formation. Transmembrane gradient of glucose and facilitated diffusion via glucose transporters GLUT4 and GLUT1 are responsible for glucose delivery into myocardial cells (Sun *et al.*, 1994). Glucose can enter glycogen formation or be processed to pyruvate via the glycolytic pathway (Stanley *et al.*, 1997) and further to acetyl coenzyme-A. Glucose 6-phosphate is converted to fructose 1,6-phosphate by phosphofructokinase. Phosphofructokinase activity increases in hypoxia, leading to increased breakdown of fructose 1,6-phosphate into two pyruvate molecules and four adenosine tri-phosphates. Pyruvate forms lactate in anaerobic conditions and undergoes oxidative decarboxylation, forming acetyl coenzyme-A in aerobic conditions (Stanley *et al.*, 1997).

In the fasting state, serum FFA concentrations are high and FFA oxidation is the main energy source for the myocardium (Opie LH, 1991). High FFA levels inhibit glucose utilization in the myocardium (Randle *et al.*, 1963; Neely & Morgan, 1974; Wisneski *et al.*, 1985; Nuutila *et al.*, 1992). After a high carbohydrate meal, blood glucose and insulin levels increase and insulin changes the myocardial energy source from FFA to glucose by suppressing lipolysis (Ferrannini *et al.*, 1993; Knuuti *et al.*, 1995). Glucose possibly inhibits fatty acid oxidation by malonyl-CoA, which is formed from acetyl-CoA. Increases in glucose and insulin lead to increased formation of acetyl-CoA and citrate, which stimulate the formation of malony-CoA (Ruderman *et al.*, 1999). Malonyl-CoA is an inhibitor of carnitine palmitoyl transferase I (CPTI), which is

thought to be a rate-limiting step of fatty acid β-oxidation (Lopaschuk *et al.*, 1994; Taegtmeyer, 1994).

Fatty acid binding proteins (FABP) facilitate fatty acid uptake by binding fatty acid and maintaining the gradient across the cell membrane (Schaap *et al.*, 1998). Fatty acids are transformed to fatty acyl-CoA inside the cell, and acyl-CoA undergoes β-oxidation, or it is esterified to triglyceride or structural lipids (Neely & Morgan, 1974; Liedtke, 1981). Of FFA, 80 % undergoes rapid oxidation in the fasting state (Wisneski *et al.*, 1987; Nellis *et al.*, 1992; Bergmann *et al.*, 1996). Long chain fatty acyl-CoA is further metabolized to acetyl-CoA, which enters the citrate cycle. Endogenous fatty acids, i.e. triglycerides are also used in the oxidation process, in an isolated rat heart this accounted for 12-20 % of total oxidation (Saddik & Lopaschuk, 1991).

2.4.3. Heart and obesity, heart functions and triglyceride accumulation

In animal models, obesity and insulin resistance increase the availability and myocardial uptake of FFA. Obesity and elevated FFA and triglycerides can result in lipotoxicity, i.e. accumulation of triglycerides and ceramides to the myocardium, which is associated with contractile dysfunction and cell apoptosis (Finck *et al.*, 2003; McGarry & Dobbins, 1999; Schaffer, 2003; Unger, 2002; Unger, 2003; Yagyu *et al.*, 2003; Zhou *et al.*, 2000). These changes are linked with increased cardiac work and oxygen consumption that may lead to impaired left ventricular contractility (Mazumder *et al.*, 2004; Aasum *et al.*, 2003; Unger, 2002). In obese rats and high-fat-fed rabbits, accumulation of triglycerides in cardiomyocytes impairs left ventricular function and promotes fibrosis and apoptosis (Zhou *et al.*, 2000; Carroll & Tyagi, 2005). We have previously shown that also in humans triglyceride content in the heart is increased in obesity (Kankaanpää *et al.*, 2006). In the study by Peterson et al. (Peterson *et al.*, 2004), myocardial fatty acid uptake measured with positron emission tomography tended to be increased in obese subjects compared to lean ones, but no differences were detected in myocardial glucose uptake or blood flow. In a recent study by Hammer et al. (Hammer *et al.*, 2008), a 16-week very-low-energy diet decreased myocardial triglyceride content and improved diastolic function in patients with type 2 diabetes.

2.5. Treatment of obesity

2.5.1. Weight loss and chronic diseases

Weight reduction enhances insulin sensivity and metabolic profile by lowering blood pressure, plasma insulin and glucose levels and improving the lipid profile. Incidence and prevalence of hypertension decreases after weight loss (Stamler *et al.*, 1989; Stevens *et al.*, 2001; Horvath *et al.*, 2008). In subjects with impaired glucose tolerance, a moderate weight loss decreases the incidence of type 2 diabetes (Tuomilehto *et al.*, 2001; Knowler *et al.*, 2002). Weight loss reduces the need for

diabetes medication (Pascale *et al.*, 1995) and enhances glucose tolerance in patients with type 2 diabetes (Norris *et al.*, 2004). Weight loss decreases serum triglycerides and increases plasma HDL cholesterol (Yu-Poth *et al.*, 1999; Stefanick *et al.*, 1998; Metz *et al.*, 2000). HDL cholesterol first decreases during active weight loss and increases gradually to a higher level then before when weight is stabilized (Dattilo & Kris-Etherton, 1992; Noakes & Clifton, 2000). Coronary artery disease symptoms decrease and weight loss after heart infarction decreases one-year mortality (Ornish *et al.*, 1990; Singh *et al.*, 1992). Weight loss decreases the mortality rate in obese subjects with type 2 diabetes or obesity-related co-morbodity in observational cohort studies; in simple obesity, this has not been studied (Williamson *et al.*, 1995; Williamson *et al.*, 2000).

2.5.2. Adipocytes in weight loss

Weight loss decreases mainly fat mass. Adipocytes reduce in size and in number. Apoptosis of mature adipocytes and pre-adipocytes has been found; omental adipocytes are more prone to apoptosis than subcutaneous adipocytes (Niesler *et al.*, 1998). Further, visceral adipose tissue is more prone to decrease in mass than subcutaneous adipose tissue after short-term energy restriction (Jones & Edwards, 1999).

2.5.3. Energy-restricted diets in weight loss

Energy restriction is an effective method to loose weight (Ayyad & Andersen, 2000;Avenell *et al.*, 2006). A low-energy diet consists of 1200 – 1500 kcal/day and it reduces 4-12 kg (3-11 %) of body weight in 4 - 36 months (Karvetti & Hakala, 1992; Wood *et al.*, 1991; Katzel *et al.*, 1995; Avenell *et al.*, 2004; Avenell *et al.*, 2006). A very-low-energy diet consist of less than 800 kcal/day and it reduces 15-21 kg of body weight in 8-16 weeks (Gilden & Wadden, 2006); very-low-energy diets have proven to be safe (Ryttig *et al.*, 1997; Amatruda *et al.*, 1988). In patients with pre-diabetes, incidence of diabetes decreases after energy restriction (Norris *et al.*, 2005). In patients with type 2 diabetes, glucose levels are lower and medication-free time is longer when using a very-low-energy diet compared to a low-energy diet (Wing *et al.*, 1991; Wing *et al.*, 1994a; Wing *et al.*, 1994b). Very-low-energy diets are designed for the patients with BMI over 30 kg/m^2 and they increase risk of gallstones, hair loss, headache, fatigue, dizziness, cold intolerance, volume depletion, muscle cramps, and constipation, thou these side effects are usually mild (Moloney, 2000; Gilden & Wadden, 2006).

2.5.4. Medical and surgical weight loss

There are currently two licensed pharmacological products in Finland for weight loss, orlistat and sibutramine. Orlistat inhibits gastrointestinal lipases and reduces absorption of ingested fat by about 30 % (Zhi *et al.*, 1994). The difference between

orlistat and placebo in weight loss is approximately 3 kg (Padwal *et al.*, 2003). Sibutramine is a noradrenaline and serotonine reuptake inhibitor that affects food intake (Stock, 1997). The difference between sibutramine and placebo in weight loss is approximately 4 kg (Padwal *et al.*, 2003). Gastric banding, gastroplasty, gastric bypass and sleeve gastrectomy are operative methods to treat severely obese subjects (Marceau *et al.*, 1998; Sjöström, 2000; Colquitt *et al.*, 2009): the latter two methods are mainly used. Weight loss is 24 kg to over 45 kg after these operative procedures (Marceau *et al.*, 1998; Colquitt *et al.*, 2009). In Swedes Obese Subjects –study, a reduction in the incidence of hypertension and diabetes (Sjöström *et al.*, 1999) and mortality (Sjöström, 2008) was reported after weight loss surgery.

2.6. Positron emission tomography in the assessment of tissue metabolism and perfusion

Positron emission tomography is based on the use of short-lived (2 min – 2 hours) radioactive isotopes, which are used as labels of natural substrates. This computerized imaging technique enables noninvasive and quantitative measurement of physiological and biochemical processes in humans *in vivo*. Positrons are emitted from the nucleus of the isotope, and annihilation with electrons produces two gamma quants. These gamma quants radiate in opposite directions and can be detected by the positron emission tomogpraphy scanner as a co-incident event (Saha *et al.*, 1992). The scanner is a ring detector and forms cross sectional images of the accumulation of the tracer in the scanned region.

2.6.1. Assessment of glucose uptake

Positron emission tomography combined with [^{18}F]FDG and the model of Sokoloff (Sokoloff *et al.*, 1977) have been validated and used to measure regional glucose uptake in the adipose tissue (Virtanen *et al.*, 2001), in the liver (Iozzo *et al.*, 2004a; Iozzo *et al.*, 2007), and in the heart and skeletal muscle (Nuutila *et al.*, 1992). [^{18}F]FDG is transported to heart cell and skeletal muscle cell and phosphorylated. In contrast to glucose, it cannot be further metabolised and it remains trapped in the cytosol (Sokoloff *et al.*, 1977; Ratib *et al.*, 1982). With [^{18}F]FDG it is possible to study glucose transport and phosphorylation. In previous studies, obese insulin-resistant subjects had lower insulin-stimulated glucose uptake in adipose tissue compared with lean subjects (Virtanen *et al.*, 2002). Skeletal muscle insulin-stimulated glucose uptake is lower in the obese (Hällsten *et al.*, 2003) and in subjects with type 2 diabetes (Utriainen *et al.*, 1998) than in normal, lean subjects. Hepatic glucose uptake is reduced in type 2 diabetes (Iozzo *et al.*, 2003b), but insulin-stimulated glucose uptake in the heart is normal in subjects with uncomplicated type 2 diabetes (Utriainen *et al.*, 1998). Weight loss improves whole-body glucose uptake, i.e. insulin sensitivity (Ferrannini & Camastra, 1998), but the effect of weight loss on insulin-stimulated glucose uptake in adipose tissue, skeletal muscle, liver or heart is not known.

2.6.2. Assessment of FFA uptake

[^{18}F]FTHA (DeGrado *et al.*, 1991) and positron emission tomogaphy is used to measure FFA uptake in the liver (Iozzo *et al.*, 2003c) and in the heart and skeletal muscle (Mäki *et al.*, 1998). [^{18}F]FTHA is a metabolically trapped tracer (DeGrado *et al.*, 1991): 89 % of [^{18}F]FTHA taken up by the heart enters mitochondria, and the rate of radioactivity accumulation of [^{18}F]FTHA reflects the β-oxidation in the heart (Takala *et al.*, 2002). 36 % of [^{18}F]FTHA accumulated in skeletal muscle enters mitochondria, suggesting that in skeletal muscle [^{18}F]FTHA traces FFA uptake but not specifically FFA beta-oxidation (Takala *et al.*, 2002). By using simple graphical analysis (Patlak & Blasberg, 1985), the FFA uptake rates in the tissue can be calculated. Ability of the liver to extract FFA is impaired in subjects with impaired glucose tolerance (Iozzo *et al.*, 2004b). FFA uptake is impaired in skeletal muscle, but not in heart muscle in subjects with impaired glucose tolerance (Turpeinen *et al.*, 1999). There are no previous studies about the effects of weight loss on liver and heart FFA uptake.

2.6.3. Assessment of perfusion

Positron emission tomography and [^{15}O]-labeled water provide non-invasive and quantitative measurements of blood flow in the in adipose tissue (Virtanen *et al.*, 2001; Virtanen *et al.*, 2002; Viljanen *et al.*, 2005) even in deep, visceral adipose tissue depots (Virtanen *et al.*, 2002) which are not accessible by catheterization. Blood flow measurement with [^{15}O]-labelled water is used in the heart (Laine *et al.*, 1998a) with arterial input function of the left ventricle time activity curve (Iida *et al.*, 1992). In a previous study of subcutaneous and visceral adipose tissue metabolism and perfusion in vivo in humans, it was found that obese insulin-resistant subjects had a lower blood flow in both visceral and abdominal subcutaneous fat as compared with lean subjects (Virtanen *et al.*, 2002). Visceral adipose tissue blood flow is higher than in subcutaneous adipose tissue (Virtanen *et al.*, 2002). Basal blood flow in the heart does not differ in obese and lean subjects, but insulin-induced enhancement of myocardial blood flow is blunted in obesity (Sundell *et al.*, 2002). Effects of weight loss on adipose tissue and heart perfusion are not known.

3 AIMS OF THE STUDY

1. To study the effects of weight loss on whole-body, skeletal muscle and adipose tissue metabolism and blood flow simultaneously in obese subjects without co-morbidies. (I)

2. To compare the effects of weight loss on liver metabolism and liver fat content in obese subjects without co-morbidies. (II)

3. To evaluate the effects of weight loss on heart metabolism, function and heart fat content and mass in obese subjects without co-morbidies. (III)

4 SUBJECTS AND STUDY DESIGN

4.1. Subjects

The study included 34 subjects who were recruited from an occupational health service clinic and by announcement in a local newspaper. Inclusion and exclusion criteria are presented below. One subject withdrew her consent during the dieting period. Study subject characteristics are presented in **Table 1**. They were healthy, as judged by medical history and physical examination. Subjects selected to the studies did not use any medication and no medication was started during the studies. All subjects were non-smokers.

Inclusion criteria for the study
1) Age 20-64 years

2) Body mass index (BMI) > 27 kg/m^2

3) Motivation for weight reduction

4) Weight < 140 kg according to imaging technical issues

Exclusion criteria for the study
1) Abuse of alcohol, (> 3 doses (one dose 12 g of ethanol) a day in men and > 2 doses a day in women) or abuse of medication

2) Age < 20 or > 64 years

3) Anemia (hemoglobin < 100 g/l in men and < 90 g/l in women)

4) Anorexia, bulimia or other eating disorder in history

5) Any previous or present hepatic disease (GT > 100 U/l, ALAT > 3 x upper limit of the reference range, U/l) or renal disease (S-creatine > 130 µmol/l)

6) Blood pressure > 160/100 mmHg or medication for hypertension

7) Congestive heart failure

8) Diagnosed coronary heart disease

9) Gallstones in history

10) Gout in history

11) Medication for dyslipidemia

12) Mental disorder in history

13) Metal object in the body as a contraindication for MRI studies

14) Type 1 or 2 diabetes

Screening laboratory tests included a standard oral glucose tolerance test to exclude diabetes with the limit of 7.0 mmol/L at baseline and 11.1 mmol/L at 2 hours. Weight was measured with a calibrated scale in light hospital clothing, height was measured with a height rule. Waist circumference was measured with a tape horizontally at a level midway between lower ribs and iliac crest after gentle exhale and with one finger between the tape and subject´s body. Hip circumference was measured as the maximal circumference over the buttocks. Blood pressure was measured repeatedly in a sitting position from the right arm.

Table 1. Study subjects; data are mean ± SE.

Value	Study I	Studies II and III
Number of subjects	16	33 (including all 16 from study I)
Male/female	4/12	10/23
Age, years (range)	45 (28 – 56)	44 (28 – 57)
Weight, kg	95.7 ± 3.3	98.2 ± 2.1
BMI, kg/m^2	33.3 ± 1.2	33.7 ± 0.7
Visceral fat mass, kg	1.6 ± 0.2	2.1 ± 0.1
Waist, cm	106 ± 2.02	105 ± 1.46
Hip circumference, cm	115 ± 1.9	114 ± 1.3
Waist to hip ratio	0.917 ± 0.014	0.923 ± 0.011
Systolic blood pressure, mmHg	135 ± 4.3	140 ± 2.9
Diastolic blood pressure, mmHg	91 ± 2.7	93 ± 1.7
Glucose, mmol/L	5.5 ± 0.1	5.8 ± 0.1
HbA_{1C}, %	5.6 ± 0.1	5.6 ± 0.1
Insulin, pmol/l	49.4 ± 9.4	62.7 ± 6.8
C-peptide, nmol/l	0.64 ± 0.06	0.74 ± 0.04
FFA, mmol/L	0.87 ± 0.06	0.74 ± 0.04
Total cholesterol, mmol/L	4.5 ± 0.2	4.7 ± 0.1
HDL-cholesterol, mmol/L	1.4 ± 0.1	1.4 ± 0.1
Triglycerides, mmol/L	0.9 ± 0.1	1.1 ± 0.1
LDL-cholesterol, mmol/L	2.7 ± 0.2	2.8 ± 0.1

4.2. Study design

The study design is presented in **Figure 1**. Because of radiation dose limits, the same subjects were not studied with all radiotracers. Maximal calculated radition dose of the study was 12.8 mSv, which equals one diagnostic abdominal CT-scan. Of 34 subjects, 16 consecutive subjects were measured with [^{15}O]H_2O and [^{18}F]FDG in hyperinsulinemic euglucemic clamp (I, II ,III) (**Figure 2**) and 18 consecutive subjects were measured with [^{18}F]FTHA (II, III) (**Figure 3**). One subject withdrew from the study in the [^{18}F]FTHA protocol. Thus, 33 subjects were finally included in the analyses.

After inclusion, the subjects participated in the first positron emission tomography, and magnetic resonance spectroscopy and imaging study. Thereafter, they started a very-low-energy diet. All daily meals were replaced by a very-low-energy diet for a period of six weeks (Nutrifast, Leiras Finland, Novartis Medical Nutrition AB, Sweden, 2.3 MJ (550 kcal), 4.5 g fat, 59 g protein, and 72 g carbohydrate per day). Written instructions with calculated energy tables were given to eat 1.0 MJ (250 kcal), vegetables preferred, to obtain total energy of 3.3 MJ (800 kcal) daily, and to drink 2-3 L water or zero-energy drinks daily. Changes in physical activity were not allowed during the diet. After the six week diet, a one-week recovery period with instructions to eat 6.6 – 8.4 MJ (1600-2000 kcal) daily was allowed to reverse the catabolic state. The assessments were repeated after this recovery period. All imaging studies were done after overnight fast and any kind of strenuous physical activity and alcohol were prohibited for the 48 hours before the studies.

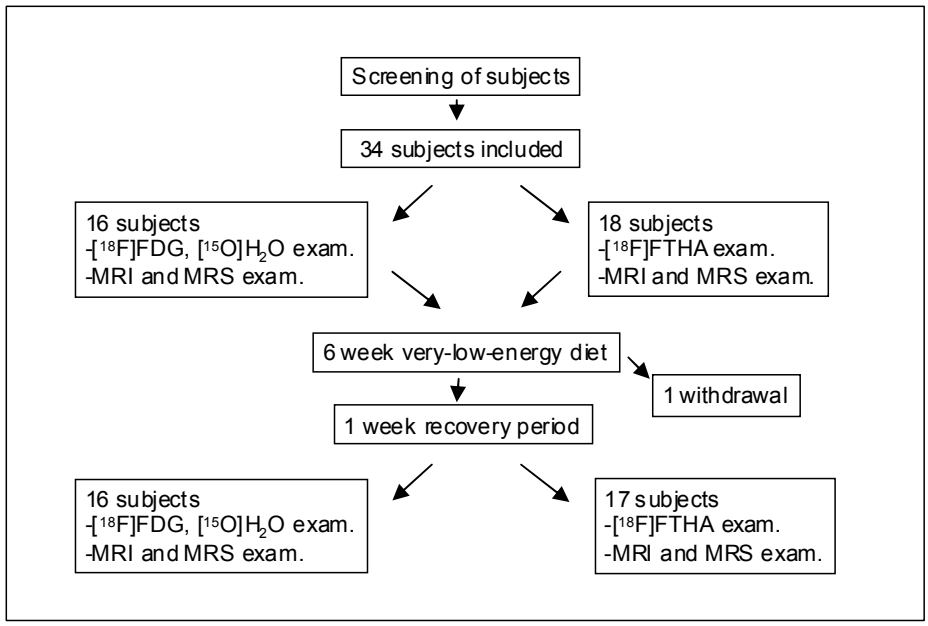

Figure 1. [^{18}F]FTHA = [^{18}F]-fluoro-6-thia-heptadecanoic acid, MRI = magnetic resonance imaging, MRS = magnetic resonance spectroscopy. [^{18}F]FDG = [^{18}F]-2-fluoro-2-deoxyglucose.

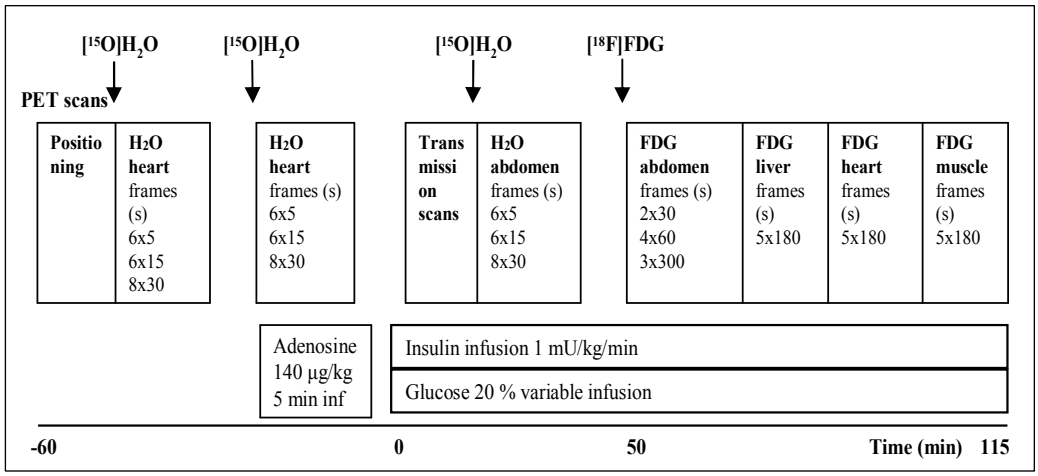

Figure 2. FDG and clamp study protocol (I).

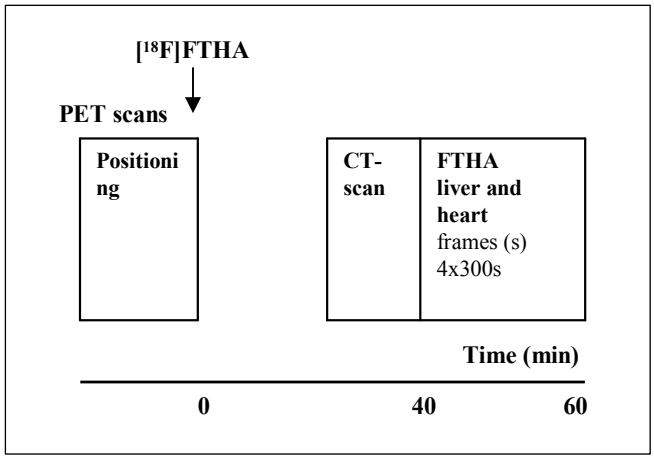

Figure 3. FTHA study protocol (II, III).

4.3. Ethics

Written informed consent was obtained after the purpose and potential risks of the study had been explained to the subjects. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland and conducted according to the principles of the Declaration of Helsinki.

5 METHODS

5.1. Image acquisition and processing (I-III)

For production of [^{15}O] ($t_{½}$ = 123 sec), a low-energy deuteron accelerator Cyclone 3 (Ion Beam Application Inc., Louvain-la-Neuve, Belgium) was used. [^{15}O]H_2O was produced using the dialysis technique in a continuously working water module (Sipilä et al., 2001). Sterility and pyrogenity tests were done to verify the purity of the product. The radiochemical purity of the [^{15}O] was approximately 97 %. For production of [^{18}F]FDG ($t_{½}$ = 109 min), an automatic apparatus with a modification of the method of Hamacher (Hamacher *et al.*, 1986) was used. Radiochemical purity of the [^{18}F]FDG was over 99 %. For production of [^{18}F]FTHA ($t_{½}$ = 109 min), a nucleophilic radionucleation of benzyl 14 (R,S)-tosyloxy-6-thia-heptadecanoate (DeGrado *et al.*, 1991) was used. Radiochemical purity of the [^{18}F]FTHA was over 98 %.

An eight-ring ECAT 931/08-tomograph was used for image acquisition in the [^{15}O]H_2O and [^{18}F]FDG studies (I-III) (Siemens/CTI, Knoxville, TN, USA). A 5-min transmission scan was performed with a removable ring source containing ^{68}Ge before the emission scan to correct for tissue attenuation of gamma photons. In [^{18}F]FTHA studies (II, III), an integrated PET/CT (GE Discovery^{TM} STE System, General Electric Medical Systems, Milwaukee, WI, USA) was used for image acquisition.

For heart blood flow studies (III), [^{15}O]H_2O was injected intravenously, and a dynamic scan was performed for 6 min, using 6 x 5-, 6 x 15-, and 8 x 30-sec frames. Imaging was performed at rest and 60 sec after the adenosine infusion (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$). After the euglycemic clamp was started and [^{15}O]H_2O was injected intravenously in the abdominal adipose tissue blood flow study (I), a dynamic scan was performed for 6 min, using 6 x 5, 6 x 15, and 8 x 30 sec frames. [^{18}F]FDG was injected intravenously 55 min after the start of the euglycemic clamp and dynamic scans were performed, using 2 x 30, 4 x 60, and 3 x 300 sec frames in adipose tissue and 5 x 180 sec frames in liver and heart and leg skeletal muscle (I-III). [^{18}F]FTHA was injected intravenously, and after 40 minutes, a dynamic scan was performed using 4 x 300 sec frames in the fasting liver and heart (II, III). Arterialized blood samples were collected throughout the imaging studies to measure whole-body and plasma radioactivity. All data were corrected for dead time, decay and photon attenuation, and reconstructed (Alenius & Ruotsalainen, 1997).

5.2. Measurements of tissue glucose uptake (I-III)

Plasma and tissue [^{18}F]FDG time-activity curves were analyzed graphically (Patlak & Blasberg, 1985) to quantify the fractional rate of tracer uptake (Ki). Graphical analysis of [^{18}F]FDG kinetics was used (Sokoloff *et al.*, 1977). Plasma radioactivity was measured with an automatic gamma counter (Wizard 1480 3; Wallac, Turku, Finland). In adipose tissue, glucose uptake was measured by drawing regions of interest (ROIs) on

MRI-PET fusion images avoiding tissue borders. Adipose tissue and skeletal muscle glucose uptakes were calculated as previously described with lumped constant values of 1.14 and 1.2, respectively (Peltoniemi *et al.*, 2000; Virtanen *et al.*, 2001). In the liver, a lumped constant 1.0 was used in the liver glucose uptake analysis, as previously validated (Iozzo *et al.*, 2007; Peltoniemi *et al.*, 2000). The rate of glucose uptake was obtained by multiplying Ki by the plasma glucose concentration (Iozzo *et al.*, 2007). Myocardial regions of interest were drawn on four subsequent mid-heart cross-sectional planes covering the anterior, lateral, and septal walls of the left ventricle. Myocardial glucose uptake (μmol/100g tissue/min) was calculated as previously described (Stolen *et al.*, 2003) with a lumped constant value of 1.0 (Ng *et al.*, 1998).

5.3. Hyperinsulinemic euglycemic clamp and whole-body glucose uptake

For determination of the rate of whole-body glucose uptake, the euglycemic hyperinsulinemin clamp technique was used (DeFronzo *et al.*, 1979). Serum insulin was increased for 120 min using a primed-continuous (1 mU · kg^{-1} · min^{-1}) infusion of insulin (Actrapid; Novo Nordisk A/S, Bagsvaerd, Denmark). Normoglycemia was based on plasma glucose measurements performed every 5 min from arterialized blood, and whole-body glucose uptake was calculated from the glucose infusion rate during the last 60 min when steady state was achieved, and expressed per kg of body weight (μmol·min^{-1}·kg^{-1}).

5.4. Measurements of tissue FFA uptake (II, III)

The fractional uptake constant of [^{18}F]FTHA Ki was calculated according to the graphical analysis of Patlak and Blasberg (Patlak & Blasberg, 1985). Tissue FFA uptake was calculated by multiplying the Ki with the mean serum FFA concentration during the corresponding PET scan, and liver total FFA uptake was calculated by multiplying tissue FFA uptake with the liver volume. In the heart, [^{18}F]FTHA plasma and tissue time-activity curves were analyzed as previously described (Mäki *et al.*, 1998). The non-metabolized fraction of [^{18}F]FTHA was used to correct the plasma input function (Mäki *et al.*, 1998).

5.5. Measurements of tissue blood flow (I, III)

In adipose tissue, the same ROIs were drawn on the (^{18}F)FDG and (^{15}O)H_2O images of cross-sectional slices from identical planes. Abdominal subcutaneous and visceral adipose tissue blood flow was measured as previously described (Virtanen *et al.*, 2002). The autoradiographic method and a 250 sec integration time were applied to calculate [^{15}O]H_2O blood flow pixel by pixel. The adipose tissue glucose extraction rate was calculated by dividing glucose uptake by blood flow. Regions of interest were drawn in four representative transaxial slices in each study as previously described (Laine *et al.*, 1998a). The regions of interest outlined in the baseline images were copied to the images obtained after adenosine administration. Values of regional

myocardial blood flow (expressed in mL/gram of tissue per minute) were calculated according to the previously published method using the single compartment model (Iida *et al.*, 1995). The arterial input function was obtained from the left ventricle time activity curve using a previously validated method (Iida *et al.*, 1992), in which corrections were made for the limited recovery of the left ventricle regions of interest and the spillover from the myocardial signals. Myocardial glucose extraction rate was calculated by dividing myocardial glucose uptake by myocardial blood flow at rest. The coronary flow reserve values were calculated by dividing adenosine-stimulated blood flow by basal blood flow. The rate pressure product was defined as the product of systolic blood pressure and heart rate.

5.6. MR imaging (I-III) and 1H MR spectroscopy (II, III)

A 1.5 T MR imager (Gyroscan Intera CV Nova Dual, Philips Medical Systems, Best, the Netherlands) was used for MRI and MRS. A single 10 mm thick T1-weighted fast field echo image was obtained at the level of the intervertebral disc L2/L3 for analysis of abdominal adipose tissue mass as previously described (Abate *et al.*, 1997). Fat volume was converted into fat weight using an adipose tissue specific gravity of 0.9196 kg/L. Anatomic T1-weighted fast field echo images with 18 slices and slice thickness of 10 mm covering the area of the upper abdomen were also obtained.

Patients were instructed to fast for 12 hours prior to the 1H MRS studies. In addition to the 1H MRS measurement of hepatic triglyceride content, MRI was performed to obtain an anatomical reference for PET. Axial T1-weighted dual fast field echo images (TE 2.3 and 4.6 ms, TR 120 ms, slice thickness 10 mm without gap) covering the area of the liver were acquired during standardized breath-hold intervals. A single voxel with a volume of 27 cm^3 was positioned in the liver outside the area of the great vessels. To ensure similar voxel placement before and after the intervention, the voxel location was recorded in each patient. A PRESS 1H MRS sequence was used with the following parameters: TR = 3000 ms, TE = 25 ms with data acquired during breath-hold intervals. 1H MRS findings of the liver have been validated in both animal and human studies (Garbow *et al.*, 2004; Szczepaniak *et al.*, 1999). Using a local workstation, liver margins were outlined manually on each individual image. Total liver volume was calculated by multiplying the measured surface areas of each slice by the slice thickness, as previously described (Lewis *et al.*, 2006).

Myocardial triglyceride content was measured by proton magnetic resonance spectroscopy as previously described (Kankaanpää *et al.*, 2006). In the current study, spectra were analysed with the user-independent LCModel software (Provencher, 1993). A blinded objective quality check of the analysis of each individual spectrum (acquired during a single breath hold) was performed before final analysis of the summed spectra for each visit. The triglyceride and water amplitudes were corrected for different T2 decay and molar concentrations of ^1H nuclei in triglyceride and water (Nikolaidis *et al.*, 2006; Szczepaniak *et al.*, 1999). Myocardial triglyceride content was defined as triglyceride in relation to the total weight of myocardial tissue (Thomsen *et al.*, 1994; Clarke & Mosher, 1952).

Left ventricular function and dimensions of the heart were measured from continuous short axis slices using the balanced turbo field echo sequence. Ten to fourteen slices were acquired during serial breath holds and vectorcardiographic gating to cover the left ventricle completely from apex to atrium. Slice thickness was 8 mm without gap between slices. Imaging parameters consisted of a repetition time of 3.4 msec, echo time of 1.7 msec, flip angle of 60º and matrix of 256 x 256. Image analysis was performed by using Philips post-processing software (ViewForum R5.1; Philips Medical Systems). Cine loops were reviewed to identify end-diastolic and end-systolic frames. Epicardial and endocardial contours were outlined manually (Alfakih *et al.*, 2004; Koskenvuo *et al.*, 2007). Papillary muscles were separately outlined and included in the myocardium. End-diastolic volume and end-systolic volume were calculated, and cardiac output and ejection fraction were computed from these. Myocardial left ventricle mass was calculated from diastolic images. Stroke volume was calculated as cardiac output/heart rate. Cardiac work was calculated as mean arterial pressure x cardiac output x 1.36 (Peterson *et al.*, 2004).

5.7. Oral glucose tolerance test (II)

An oral glucose tolerance test was done after an overnight fast within three days of the imaging studies. A commercial liquid product of 75 g of glucose was given orally and venous blood sampling was done at -15, 0, 15, 30, 45, 60, 90 and 120 min. The oral glucose insulin sensitivity (OGIS) index was calculated from the oral glucose tolerance test 0, 90 and 120 min insulin and glucose values as previously described (Mari *et al.*, 2001). Endogenous glucose production was calculated from plasma (^{18}F)FDG kinetics as previously validated (Iozzo *et al.*, 2006).

5.8. Hepatic insulin resistance and fractional extraction (II)

Hepatic insulin resistance was calculated as a product of endogenous glucose production and insulin levels (Matsuda & DeFronzo, 1999). Insulin clearance was calculated from the oral glucose tolerance test as mean insulin secretion divided by mean insulin concentration during the oral glucose tolerance test. Insulin clearance from the euglycemic clamp was calculated as the insulin infusion divided by the steady-state insulin concentration during the clamp. Hepatic insulin fractional extraction was calculated as 1-(clamp insulin clearance)/(oral glucose tolerance test insulin clearance).

5.9. Biochemical analyses (I-III)

Arterialized plasma glucose was determined in duplicate by the glucose oxidase method (Analox GM9 Analyzer; Analox Instruments, London, UK). Glycosylated hemoglobin A_{1C} (HbA_{1c}) was determined by high performance liquid chromatography (Variant II, Bio-Rad, CA, USA). Serum insulin was determined by time-resolved immunofluorometric assay (AutoDELFIA, PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). Serum C-peptide was determined by time-resolved

immunofluorometric assay, (AutoDELFIA, PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland). Plasma cholesterol was determined by photometric, enzymatic (cholesterol esterase, cholesterol oxidase) assay (Modular P800, Roche Diagnostics GmbH, Mannheim, Germany). Plasma high density lipoprotein cholesterol and triglycerides were determined by direct photometric enzymatic (PEG-modified cholesterol esterase and cholesterol oxidase with dextran sulphate) assay (Modular P800, Roche Diagnostics GmbH, Mannheim, Germany). Serum low density lipoprotein cholesterol was estimated using the Friedewald equation. Serum FFA were determined by photometric enzymatic assay (NEFA C, ACS-ACOD, Wako Chemicals GmbH, Neuss, Germany, Modular P800, Roche Diagnostics GmbH, Mannheim, Germany). Serum high-sensitivity C-reactive protein was analyzed with the sandwich immunoassay method using an Innotrac Aio1 immunoanalyzer (Innotrac Diagnostic, Turku, Finland). Radioimmunoassay was used for determination of serum leptin (Human Leptin RIA kit, Linco Research) and total adiponectin (Human Adiponectin RIA kit, Linco Research, St. Charles, Missouri, USA) determinations. The Luminex 200 and the Luminex XYP^{TM} platform came from the Luminex Corporation (Luminex, Austin, TX). The software, Bio-Plex^{TM} Manager version 4.1 came from Bio-Rad (Bio-Rad Laboratories AB, Sundbyberg, Sweden). The array reader was calibrated using the Bio-Plex Calibration kit (kit cat no. 171-203060, Bio-Rad Laboratories AB, Sundbyberg, Sweden). The calibration curves for each analyte were calculated using the Bio-Plex 4.1 software. Serum concentrations of cytokines were analysed in duplicate using Bio-Plex Human Cytokine Assays (containing IL-4, IL-10, INFγ and TNFα), and Bio-Plex cytokine reagent Kits (Cytokine Reagent Kit, cat no. 171-304000) as recommended by the manufacturer (Bio-Rad Laboratories AB, Sundbyberg, Sweden). IL-6 concentration was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's instructions (Pelikine Compact human IL-6 ELISA kit, CLB, Amsterdam, the Netherlands). The optical density of the samples was determined using a Multiscan Ascent spectrophotometer (Thermo Labsystems, Helsinki, Finland). The detection limit for the IL-6 assay was 0.6 pg/mL (range 0–51.2 pg/mL) (Lehtimäki *et al.*, 2005).

5.10. Statistical analyses (I-III)

Results are given as mean ± SE, unless stated otherwise. Effects of treatment were examined by comparing pre- and post-treatment values with each other using non-parametric Wilcoxon signed rank test. Univariate associations between the study variables were analyzed by calculating the Pearson´s or Spearman's correlation coefficients when appropriate. All statistical analyses were performed using the SAS statistical analysis system, version 9.1 (SAS Institute Inc., Cary, NC, USA).

6 RESULTS

Baseline characteristics did not differ between FDG examination (n=16) and FTHA examination (n=17) and other than PET data was pooled when applicable to the studies II and III. All subjects lost weight with the avarage of 11.2 kg (range 6.6 – 19.4 kg). One adverse effect was reported to the ethical committee: one subject had constipation and this probable predisposed to vitreous ablation and retinal hole which was treated accordingly with no permanent impairment.

Effects of weight loss on anthropometrics and some laboratory parameters are shown in **Table 2**. Significant decrease in weight, BMI, and waist circumference were found along with a reduction of total cholesterol, high density and low density lipoprotein cholesterol values and triglycerides. Fasting plasma glucose decreased with HbA_{1C}, with a borderline decrement in serum insulin values. Serum FFA levels did not change.

Table 2. Characteristics of 33 subjects at baseline and after six-week diet, (studies II, III). Data are mean ± SE and fasting. P-values compare pre/post treatment values using non-parametric Wilcoxon signed rank test.

Value	Baseline	After weight loss	P value
Weight, kg	98.2 ± 2.1	87.0 ± 2.0	<0.0001
BMI, kg/m^2	33.7 ± 0.7	29.9 ± 0.7	<0.0001
Waist, cm	105 ± 1.5	94 ± 1.4	<0.0001
Insulin, pmol/L	62.7 ± 6.8	55.3 ± 7.7	0.065
Glucose, mmol/L	5.8 ± 0.1	5.6 ± 0.1	0.036
HbA_{1C}, %	5.6 ± 0.1	5.4 ± 0.1	<0.0001
FFA, mmol/L	0.74 ± 0.04	0.71 ± 0.05	0.21
Gamma-GT, U/L	27.1 ± 3.2	19.1 ± 2.2	<0.0001
Total cholesterol, mmol/L	4.7 ± 0.1	3.7 ± 0.1	<0.0001
HDL cholesterol, mmol/L	1.4 ± 0.1	1.3 ± 0.1	<0.001
Triglycerides, mmol/L	1.1 ± 0.1	0.9 ± 0.1	<0.001
LDL cholesterol, mmol/L	2.8 ± 0.1	2.1 ± 0.1	<0.0001

6.1. Effects of weight loss on skeletal muscle and whole-body glucose uptake

Skeletal muscle glucose uptake increased by 35 % in parallel with whole body glucose uptake (**Table 3).** Skeletal muscle glucose uptake correlated strongly with whole-body insulin sensitivity before and after weight loss ($r = 0.94$ and $r = 0.91$, respectively; $p<0.0001$ for both).

6.2. Effects of weight loss on adipose tissue metabolism

Subjects lost weight with the avarage of 11.1 kg (range 6.6 – 19.4 kg). in study I (**Table 3)**. Fasting plasma glucose was unchanged. Both abdominal subcutaneous and

visceral adipose tissue masses decreased significantly. Abdominal subcutaneous adipose tissue masses correlated inversely at baseline and after weight loss with insulin-stimulated glucose uptake in the same tissue ($r = -0.74$ and -0.57, respectively; $p<0.03$ for both). Abdominal subcutaneous adipose tissue masses also correlated inversely with whole-body insulin sensitivity before and after weight loss ($r = -0.60$ and -0.68, respectively; $p<0.02$ for both). Visceral adipose tissue mass correlated inversely with insulin-stimulated glucose uptake in the same tissue ($r = -0.56$, $p<0.03$), and with whole-body insulin sensitivity at baseline ($r = -0.60$, $p<0.02$).

Table 3. Effects of weight loss on 16 study subjects (study I). Data are mean ± SE. P-values compare pre/post treatment values using non-parametric Wilcoxon signed rank test.

Value	Baseline	After weight loss	P-value
Weight, kg	95.7 ± 3.3	84.6 ± 2.9	<0.0001
BMI, kg/m^2	33.3 ± 1.1	29.5 ±1.0	<0.0001
Systolic blood pressure, mmHg	135 ± 4.3	124 ± 4.4	<0.001
Diastolic blood pressure, mmHg	91 ± 2.7	82 ± 2.6	<0.002
Rate pressure product	8597 ± 386	7436 ± 432	<0.01
fP-glucose, mmol/L	5.5 ± 0.1	5.4 ± 0.1	0.62
Abdominal subcutaneous fat mass, kg	6.7 ± 0.8	5.6 ± 0.7	<0.001
Visceral fat mass, kg	1.6 ± 0.2	1.2 ± 0.1	<0.001
Abdominal subcutaneous glucose extraction, µmol/ml	0.63 ± 0.08	0.68 ± 0.06	0.25
Visceral glucose extraction, µmol/ml	0.52 ± 0.06	0.57 ± 0.07	0.30
Skeletal muscle glucose uptake, µmol/min/100ml tissue	4.8 ± 0.7	6.5 ± 0.8	<0.002
Whole-body insulin sensitivity, µmol/kg/min	24.2 ± 2.5	31.9 ± 3.9	<0.01

6.2.1. Adipose tissue blood flow and glucose uptake

Visceral blood flow and insulin-stimulated glucose uptake were significantly higher, as compared with corresponding values in abdominal subcutaneous adipose tissue before and after the intervention ($p<0.01$ for all). Weight reduction did not change visceral or abdominal subcutaneous insulin-stimulated glucose uptake or blood flow when expressed per gram of mass. When the results were calculated for the whole adipose tissue depots, glucose uptake values did not change, whereas adipose tissue blood flow values decreased significantly in both visceral and abdominal subcutaneous depots (**Figure 4**).

In univariate analysis, there was no association between adipose tissue blood flow and insulin-stimulated glucose uptake in either of the compartments. Glucose extraction, as calculated from independently measured glucose uptake and blood flow values did not change in these measured areas.

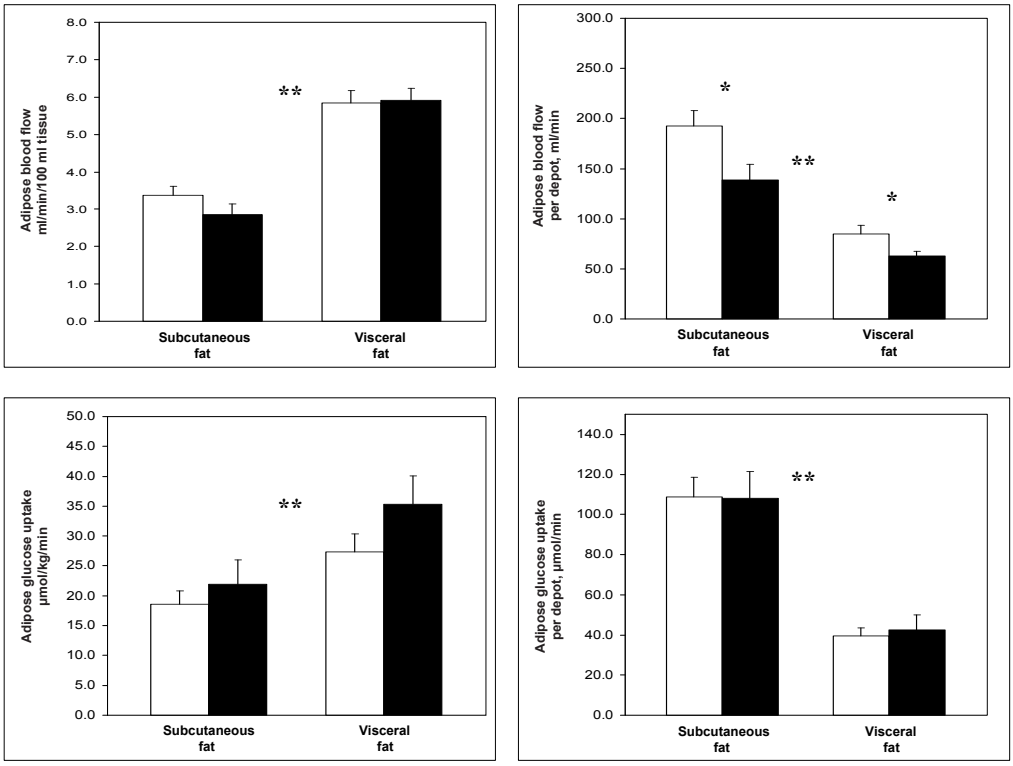

Figure 4. Blood flow and glucose uptake in adipose tissue, n=16, (study I). White bar represents before and black bar after the weight loss, data are mean ± SE. * $p < 0.05$ compared baseline, ** $p < 0.05$ compared between abdominal subcutaneal and visceral adipose tissue both at baseline and after weight loss.

6.2.2. Adipokines and cytokines

Of the measured adipokines and cytokines, only leptin and IL-6 concentrations decreased after weight loss (**Table 4)**. The high-sensitivity C-reactive protein as a marker of inflammation decreased.

Table 4. Effects of weight loss on adipokines and cytokines and high sensitivity C-reactive protein, n=16, (study I). Data are mean ± SE. P-values compare pre/post treatment values using non-parametric Wilcoxon signed rank test.

Value	Baseline	After weight loss	P-value
Leptin, ng/ml	29.2 ± 4.3	14.6 ± 2.8	<0.001
Adiponectin, µg/ml	11.3 ± 1.1	11.9 ± 1.0	0.59
Tumor necrosis factor-α, pg/ml	1.0 ± 0.4	0.8 ± 0.3	0.21
IL-4, pg/ml	1.9 ± 0.6	1.6 ± 0.4	0.55
IL-6, pg/ml	2.7 ± 0.4	1.9 ± 0.2	<0.03
IL-10, pg/ml	2.6 ± 0.9	2.6 ± 0.6	0.50
Interferon gamma, pg/ml	17.1 ± 5.5	18.6 ± 6.7	0.70
C-reactive protein, mg/l	2.9 ± 2.2	1.4 ± 1.4	<0.001

Leptin correlated with abdominal subcutaneous adipose tissue masses before and after weight loss (r = 0.58 and 0.73, respectively; p<0.02 for both). Inverse correlations were found between leptin and abdominal subcutaneous adipose tissue blood flow (r = -0.75, p<0.001) and insulin-stimulated glucose uptake (r = -0.54, p<0.03) at baseline. These associations were weaker after weight loss (r = -0.47, p=0.07 for both). IL-6 correlated with abdominal subcutaneous adipose tissue masses before and after weight loss (r = 0.52 and 0.57, respectively; p<0.04 for both). There was also an inverse correlation between IL-6 and abdominal subcutaneous insulin-stimulated glucose uptake before and after weight loss (r = -0.75 and -0.68, respectively; p<0.004 for both).

6.3. Effects of weight loss on liver metabolism

Serum lipids, gamma-GT and high sensitivity c-reactive protein decreased (**Table 2, Table 4.**). There was no significant change in OGTT 2-hour values. Effects of weight loss on liver metabolism are presented in **Table 5**.

Table 5. Effects of weight loss on glucose uptake and insulin clearance rates, n=16 (study II). Data are mean ± SE. P-values compare pre/post treatment values using non-parametric Wilcoxon signed rank test.

Value	Baseline	After weight loss	P-value
Liver insulin-mediated glucose uptake, µmol/min/100ml	2.3 ± 0.1	2.2 ± 0.1	0.86
P-glucose during clamp, mmol/L	5.1 ± 0.1	5.2 ± 0.1	0.14
S-insulin during clamp, pmol/l	458 ± 16.1	441 ± 12.1	0.25
Endogenous glucose production (mmol/min)	0.65 ± 0.06	0.43 ± 0.08	<0.04
Insulin clearance rate in OGTT, L/min/m^2	1.27 ± 0.09	1.49 ± 0.09	<0.006
Insulin clearance rate in clamp, L/min/m^2	0.55 ± 0.03	0.63 ± 0.07	0.45

6.3.1. Liver glucose and FFA uptake

Liver volume decreased by 11 % (from 1600 ± 81 ml to 1420 ± 73 ml, p<0.002). This was partly explained by liver triglyceride content, which decreased by 60 % (p<0.0001). Although serum FFA concentrations did not differ, liver FFA uptake was 26 % lower after the dieting period (p<0.003) **(Figure 5)**, whereas insulin-mediated hepatic glucose uptake did not change. The endogenous glucose production, as determined from the FDG plasma radioactivity curves of 15 subjects, was decreased by 40 % (p<0.04) (**Table 5**). Consequently, the net balance of glucose across the liver (i.e. difference between uptake and release) was changed in favour of glucose retention as the organ demonstrated an improvement in hepatic insulin resistance (p<0.05). In addition, hepatic insulin fractional extraction (n=16) increased by 9 % (p<0.05).

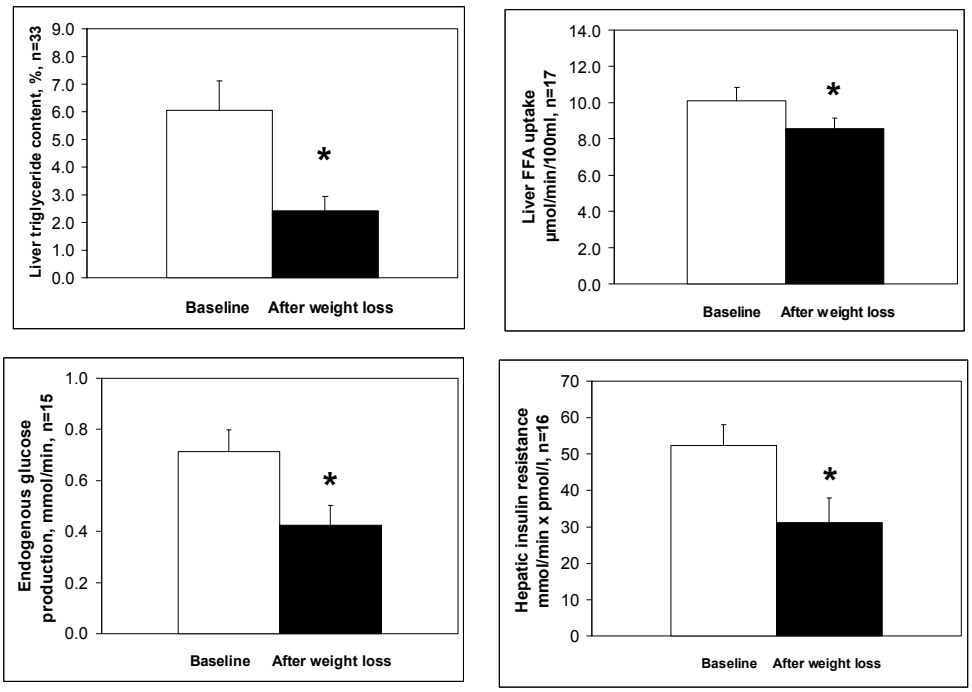

Figure 5. Effects of weight loss on liver metabolism (study II). White bar is before and black bar is after weight loss. Data are mean ± SE. * $p < 0.05$ compared to baseline. FFA = free fatty acid.

6.3.2. Associations of liver steatosis

In univariate analysis, liver fat content was positively associated with whole-liver FFA uptake, visceral fat mass, fasting serum FFA, HbA_{1C} and fasting plasma triglyceride concentrations (**Table 6**).

Liver fat content was negatively associated with skeletal muscle insulin-stimulated glucose uptake both before and after the intervention ($r=-0.60$, $p<0.02$ and $r=-0.68$, $p<0.01$) and with the OGIS index ($r=-0.43$, $p<0.04$ and $r=-0.57$, $p<0.003$, respectively). Baseline values and changes in hepatic insulin fractional extraction were associated with those in skeletal muscle glucose uptake ($r=0.72$, $p<0.002$ and $r=0.75$, $p<0.002$ respectively) and the OGIS index ($r=0.60$, $p<0.02$ and $r=0.56$, $p<0.03$ respectively).

Table 6. Correlations of liver triglyceride content (study II).

	Liver triglyceride content	
	Baseline	Changes after weight loss
Whole liver FFA uptake, n=17	r = 0.50, p<0.05	r = 0.54, p<0.03
Visceral fat mass, n=33	r = 0.53, p<0.002	r = 0.39, p<0.04
Serum FFA levels, n=33	r = 0.36, p<0.04	r = 0.41, p<0.02
HbA_{1C}, n=33	r = 0.50, p<0.003	r = 0.37, p<0.04
Serum triglycerides, n=33	r = 0.40, p<0.02	r = 0.36, p<0.05

6.4. Effects of weight loss on heart metabolism

Effects of weight loss on hemodynamics, myocardial structure and function are presented in **Table 7**. Standard electrocardiographs were normal before and after weight loss in all subjects. There was significant reduction in blood pressure and heart rate. Left ventricle mass decreased by 7 % ($p<0.005$). Cardiac output decreased by 18 % ($p<0.0005$) while stroke volume did not change ($p=0.15$) Cardiac work decreased by 26 % ($p<0.0001$).

Table 7. Hemodynamic parameters, cardiac structure and function in 17 subjects (study III). Data are mean ± SE. P-values compare pre/post treatment values using non-parametric Wilcoxon signed rank test.

Value	Baseline	After weight loss	P value
Hemodynamics			
Heart rate, bpm	70 ± 2	60 ± 2	0.0003
Systolic blood pressure, mmHg	145 ± 4	132 ± 4	0.0007
Diastolic blood pressure, mmHg	95 ± 2	85 ± 2	0.0002
Mean arterial pressure, mmHg	112 ± 2	100 ± 3	0.0001
Rate pressure product, mmHg · bpm	10100 ± 403	7800 ± 455	<0.0001
Structure			
Left ventricle mass, g	109 ± 7	101 ± 6	0.0043
Function			
Cardiac output, L/min	8.3 ± 0.4	6.8 ± 0.3	0.0004
Cardiac index, $L \cdot min^{-1} \cdot m^{-2}$	3.8 ± 0.1	3.3 ± 0.2	0.0067
Stroke volume, L/beat	0.12 ± 0.01	0.12 ± 0.01	0.15
Cardiac work, $mmHg \cdot L \cdot min^{-1}$	1248 ± 56	921 ± 49	<0.0001

6.4.1. Heart glucose and FFA uptake

Myocardial fatty acid uptake decreased by 24 % ($p<0.0001$) and myocardial triglyceride content decreased by 31 % ($p=0.076$) (**Figure 6).** Insulin-stimulated myocardial glucose uptake remained unchanged when whole-body insulin sensitivity increased by 33 % (from 24.2 ± 2.5 to 31.9 ± 3.9 μmol/kg/min, $p<0.01$). Basal myocardial blood flow decreased by 8 % ($p<0.04$), when adenosine stimulated blood flow remained unchanged (from 5.1 ± 0.3 to 5.1 ± 0.3 ml/g/min, $p=0.82$). Coronary flow reserve (from 4.3 ± 0.4 to 4.2 ± 0.4 dimensionless, $p=0.98$) and myocardial glucose extraction rate (from 0.32 ± 0.04 to 0.37 ± 0.06 μmol/ml, $p=0.18$) remained unchanged.

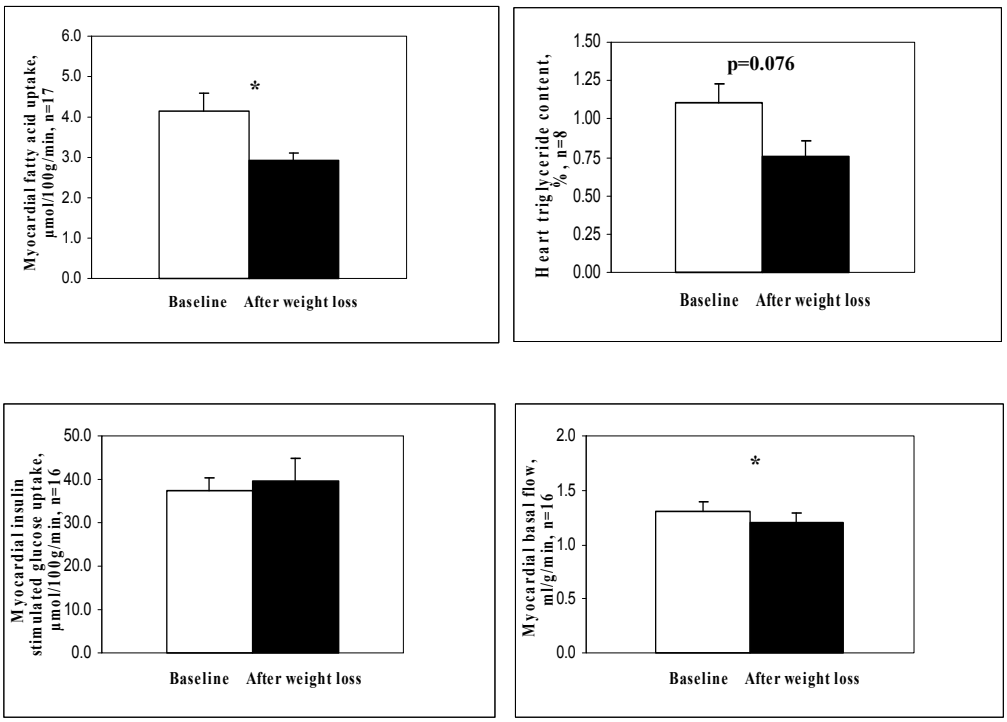

Figure 6. Effects of weight loss on myocardial substrate metabolism, triglyceride content and blood flow. Data are mean ± SE. * $p<0.05$ for Wilcoxon signed rank test.

7 DISCUSSION

7.1. Synopsis

The rapid loss of body weight during a six weeks of a very-low-energy diet leads to the elevation of whole-body insulin sensitivity and skeletal muscle glucose uptake in humans. The present study demonstrates the different regulation between perfusion and metabolism in visceral and abdominal subcutaneous adipose tissue in. Marked weight reduction after very-low-energy diet decreased adipose tissue perfusion per depot in both visceral and abdominal subcutaneous adipose tissue compartments, whereas insulin-stimulated glucose uptakes remained unchanged.

To our knowledge, this is the first study where liver glucose and fatty acid metabolism, together with liver triglyceride content, were evaluated simultaneously before and after weight loss induced by a very-low-energy diet. As could be expected, liver triglyceride content per volume decreased and liver volume decreased with dieting. When the metabolic rates before and after the six-week energy restriction were compared, liver insulin resistance and especially hepatic fatty acid uptake were significantly decreased. These findings are accompanied by improved skeletal muscle insulin sensitivity and amelioration of all measured metabolic and hemodynamic variables. We could show that myocardial uptake of FFA was reduced after a short-term very-low-energy diet. Furthermore, these changes were paralleled be a reduction in left ventricle mass, cardiac work and resting perfusion and borderline reduction in myocardial triglyceride content. Short-term weight loss did not change myocardial insulin-stimulated glucose uptake or maximal vasodilatatory capacity.

To minimize the radiation burden of the healthy volunteers, we did not perform all PET studies in all subjects. Methods that were used were previously validated and feasible, but time-consuming and expensive to use in clinical work. Furthermore, these studies did not have a control group and the number of study subject studied was relatively small with mixed age and sex. Technical issues limited the number of heart MRS examinations. Use of very-low-energy diet for 6 weeks was effective, but continuing of the diet could have changed some borderline results.

7.2. Weight loss and adipose tissue metabolism

Adipose tissue perfusion and insulin-stimulated glucose uptake per gram of tissue were found to be higher in the visceral area as compared to abdominal subcutaneous fat. This observation is in line with previous flow results in animals (Enevoldsen *et al.*, 2000) and in humans (Virtanen *et al.*, 2002), and glucose uptake results in humans (Virtanen *et al.*, 2002). Adipose tissue masses and blood flow values in adipose tissue were of the same order of magnitude as previously found in obese subjects using this or other techniques (Frayn *et al.*, 2003; Virtanen *et al.*, 2002). Higher metabolic activity in visceral adipose tissue is thought to reflect the dense

innervation and circulation in visceral depots (West *et al.*, 1989) since adipose tissue blood flow has a regulatory role in adipose metabolism (Frayn *et al.*, 1997). Visceral adipose tissue has also been found to be particularly active in lipid metabolism (Mårin *et al.*, 1992). Moreover, adipose tissue delivers vasoactive substances, adipokines and cytokines, their secretion being depot-dependent (Montague *et al.*, 1997; Orel *et al.*, 2004; Lefebvre *et al.*, 1998). Furthermore, visceral obesity is the main indicator of dysfunctional adipose tissue as part of a cluster of metabolic abnormalities defined as a metabolic syndrome (Despres & Lemieux, 2006). Although our data show visceral fat to be more metabolically active, the abdominal subcutaneous fat mass was four times larger than the visceral fat mass. Because the total subcutaneous adipose tissue mass is much larger than the intra-abdominal one, subcutaneous adipose depot is expected to have a stronger impact on the metabolism of the whole body (Jensen, 2006). The inverse relationship between abdominal subcutaneous and whole-body insulin sensitivity observed in this study provides support to this concept.

We have previously reported in non-obese and obese non-diabetic and diabetic subjects, an inverse relationship between adipose tissue mass and adipose tissue glucose uptake in the intra-abdominal and abdominal subcutaneous depot, indicating that down-regulation of insulin sensitivity in expanding fat depots may be a general phenomenon (Virtanen *et al.*, 2002; Virtanen *et al.*, 2005). The changes in cellular phenotype that occur as adipocytes hypertrophy include a strictly mechanical effect, whereby an enlarged lipid droplet pushes cell organelles, such as mitochondria, against the cell surface. However, when calculated per tissue depot, glucose uptake in each scanned fat depot, intra-abdominal, subcutaneous, and total, was similar (Virtanen *et al.*, 2005). In concert with this, total abdominal subcutaneous or intra-abdominal adipose tissue glucose uptake during insulin stimulation was identical before and after dieting in the present study.

Our data document how the total perfusion through both abdominal subcutaneous and visceral depots during euglycemic hyperinsulinemic conditions accounts for a large proportion of the estimated cardiac output. If we assume that the perfusion is similar throughout the subcutaneous adipose tissue in the body, and the average flow rates measured here are used, in a 100 kg subject with 27 % body fat (Virtanen *et al.*, 2002), the whole-body adipose tissue flow would be approximately 900 ml/min, corresponding to ~18 % of average cardiac output (5 L/min). Furthermore, a 10 kg weight reduction connected with a moderate -10 % fat mass decrement would then result in a whole-body adipose tissue flow of 620 ml/min, representing 12 % of average cardiac output. These estimations suggest that the adipose tissue has an important impact on cardiac workload, and that the weight reduction can also reduce the adipose tissue-dependent part of it. In accordance with this, both blood pressure values and the rate pressure product, which are indirect indicators of cardiac work and known correlates of myocardial oxygen consumption (Gobel *et al.*, 1978), decreased in this study.

7.2.1. Adipokines and cytokines

In this study, serum leptin levels decreased after weight loss as has been previously found (Considine *et al.*, 1996) and leptin correlated with abdominal subcutaneous adipose tissue mass before and after weight loss. Leptin acts as a peripheral signal to the central nervous system in the hypothalamic regulation of eating behaviour and metabolic homeostasis (Havel, 2001; Porte, Jr. *et al.*, 2002; Niswender & Schwartz, 2003). In addition, direct autocrine/paracrine functions of leptin have been described (Frühbeck *et al.*, 1997). In dieting, hunger is related to magnitude of leptin decrement (Keim *et al.*, 1998), and in one study, leptin treatment reduced appetite (Westerterp-Plantenga *et al.*, 2001). Leptin gene expression is elevated in obesity (Frühbeck *et al.*, 1997), and leptin receptor defects result in extreme hyperphagia and obesity in humans (Clement *et al.*, 1998). Interestingly, leptin mRNA levels (Lefebvre *et al.*, 1998; Montague *et al.*, 1997) and secretion of leptin are higher in subcutaneous than in visceral adipocytes (Orel *et al.*, 2004), which may account for our finding that circulating leptin levels were more strongly associated with abdominal subcutaneous than with visceral fat metabolism and perfusion.

Circulating leptin correlated inversely with abdominal subcutaneous adipose tissue blood flow before weight loss in this study. Leptin receptors are found in the vasculature, and long-term infusion of leptin is found to increase arterial blood pressure (Shek *et al.*, 1998) and lead to endothelial dysfunction (Knudson *et al.*, 2005), as well as play a role in angiogenesis (Sierra-Honigmann *et al.*, 1998). Leptin is also proposed to be involved in control of vascular tone, and blood pressure, by simultaneously producing a neurogenic pressor effect and an opposing nitric oxide-mediated depressor effect (Fruhbeck, 1999). Thus, leptin might be one obesity-associated factor contributing to reduced blood flow, and as a consequence of increased blood pressure, to increased cardiovascular morbidity and mortality in obesity. In this sense, leptin is also a potential link between weight loss and beneficial vascular effects.

In our dataset, circulating IL-6 levels decreased reflecting improvement of inflammation, possibly involving adipose tissue. IL-6 levels correlated with abdominal subcutaneous adipose tissue masses throughout this study, and they also correlated inversely with insulin-stimulated abdominal subcutaneous adipose tissue glucose uptake. About 30 % of all IL-6 is secreted by adipose tissue (Mohamed-Ali *et al.*, 1997), and 10 % is produced by adipocytes (Fried *et al.*, 1998). IL-6 is reduced after weight loss (Esposito *et al.*, 2003), and is associated with insulin resistance and obesity (Vozarova *et al.*, 2001). IL-6 gene expression is also elevated in obesity (Vgontzas *et al.*, 1997) when it is expressed in adipose tissue, and is thought to be related to macrophage infiltration of adipose tissue (Fantuzzi, 2005). In turn, the infiltration of inflammatory cells into adipose tissue depots further stimulates adipocytes to secrete inflammatory mediators and adipokines, thus promoting a vicious circle (Di Gregorio *et al.*, 2005).

Circulating total adiponectin level was not altered by dieting in this study, as has previously been reported in very-low-energy diet interventions (Garaulet *et al.*, 2004;

Anderlova *et al.*, 2006; Kim *et al.*, 2006). In a one-year-long weight loss intervention, elevation of adiponectin levels was observed (Coppola *et al.*, 2008). Low adiponectin levels and gene expression are found in obesity (Arita *et al.*, 1999;Weyer *et al.*, 2001), and it has been found that adiponectin gene expression is higher in visceral adipose tissue than in subcutaneous adipose tissue (Motoshima *et al.*, 2002). Adiponectin administration lowers glucose levels in healthy mice and in mouse models of diabetes (Berg *et al.*, 2001), and it lowers hepatic glucose production and liver fat in mice (Combs *et al.*, 2001; Xu *et al.*, 2003). Adiponectin also enhances insulin action to decrease glucose production in isolated hepatocytes (Yamauchi *et al.*, 2001). These findings and our results indicate that adipose tissue glucose metabolism could be partly mediated by adiponectin in an autocrine manner. Other measured cytokines; tumor necrosis factor-α, IL-4, IL-10 and interferon-γ, did not change after weight loss, and there were no associations between these cytokines and adipose tissue masses, glucose uptake values or blood flow values in line with the notion that they are derived mainly from tissues other than fat.

7.3. Weight loss and liver metabolism

Liver triglyceride content was, on average, 6.1 % at baseline with a mean BMI of 33.7 kg/m^2, and almost half of the subjects had values in excess of 5 %, i.e. the diagnostic cut-off of steatosis (Hoyumpa, Jr. *et al.*, 1975; Thomsen *et al.*, 1994). After a very-low-energy diet of six weeks, mean triglyceride content was 2.4 % with a BMI of 29.9 kg/m^2, and only one-eighth of subjects had levels within steatotic range. Triglyceride content decreased in parallel with a decrease in visceral fat mass. This was consistent with the observation that 80 % of the total loss in hepatic triglycerides induced by a very-low-energy regimen occurs within two weeks from the start of energy restriction, as compared with a progressive, and slower decline in the visceral adipose tissue mass (Colles *et al.*, 2006). The decrease in liver volume with dieting has previously been reported in the severely obese (Lewis *et al.*, 2006), but was also demonstrated here. Interestingly, it could only partly be explained by reduced triglyceride content. This might suggest that the volume is also decreased due to reduced water content or inflammation of the liver. In agreement with previous studies, we found lower circulating concentrations of high sensitivity C-reactive protein, as well as gamma-GT levels (Raitakari *et al.*, 2004).

Liver FFA uptake was decreased in parallel with a decrease in liver fat when measured after a week´s normal energy diet following the very-low-energy diet period. The circulating triglyceride levels correlated significantly with liver fat content, indicating excess dietary fat and increased substrate delivery in the development of hepatic steatosis (Utzschneider & Kahn, 2006), thus supporting this mechanistic link in the association of insulin resistance with liver fat content. Because fasting serum fatty acid concentrations were similar after the dieting period, the change in the uptake was due to direct effects on the intrinsic regulation of fatty acid uptake by the liver. The findings of a reduced fatty acid uptake, together with a lower lipid content in the liver, resemble those recently ascribed to increased physical activity by Hannukainen et al

(Hannukainen *et al.*, 2007). In our current study, participants were instructed not to change their physical activity to avoid the overlapping effects of exercise, thus energy restriction or increased physical activity alone may share similar outcomes in terms of hepatic lipid storage.

7.3.1. Hepatic glucose and insulin metabolism

Mean fasting C-peptide levels decreased and fasting insulin levels tended to decrease with the diet intervention in parallel with increased hepatic insulin fractional extraction. Importantly, the endogenous glucose production and liver insulin sensitivity were improved by >20 % after the intervention, consistent with the described relationship linking liver steatosis and hepatic glucose production (Seppälä-Lindroos *et al.*, 2002), and with the decline in endogenous glucose production induced by a two-day very-low-energy regimen in patients with diabetes (Jazet *et al.*, 2005). Insulin-mediated hepatic glucose uptake was not changed. This evidence is compatible with our previous findings (Iozzo *et al.*, 2003a) showing that insulin-mediated glucose uptake in the liver is much less dependent on insulin resistance than it is in the whole body, and does not differ between non-diabetic insulin-resistant and insulin-sensitive subjects. Liver glucose uptake is modulated by plasma glucose and fatty acid concentrations (Iozzo *et al.*, 2003b; Iozzo *et al.*, 2004a), neither of which changed in our study. Altogether, the above observations can be summarized as follows: the insulin-mediated retention of glucose by the liver - accounting for the balance between output and uptake of glucose - is enhanced due to energy restriction, and this effect is probably mediated, at least in part, through the lowering of fatty acid uptake and accumulation in the organ. This may be the mechanism explaining the association between changes in glycosylated hemoglobin and in liver fat content observed in this study.

Hepatic insulin fractional extraction was associated with insulin sensitivity. The liver removes approximately 50 % of secreted insulin during the first portal passage (Sato *et al.*, 1991; Duckworth *et al.*, 1988) and reduced hepatic insulin clearance is a typical finding in insulin resistance (Letiexhe *et al.*, 1993). Although a decrease in insulin clearance through saturation of the insulin-removal processes may be a consequence of the increased insulin levels following insulin resistance, a primary role of the liver in controlling insulin action by modulating the supply of insulin to the systemic circulation has been also proposed (Duckworth *et al.*, 1988). In support of this hypothesis - implicating visceral fat as the regulatory element – either a selective elevation of fatty acid levels in the portal vein or the accumulation of triglycerides in the liver has been shown to negatively affect hepatic insulin clearance, causing hyperinsulinemia (Björntorp, 1995; Yoshii *et al.*, 2006), while chronic hyperinsulinemia is a known down-regulator of whole-body insulin sensitivity (Iozzo *et al.*, 2001). Our data may support either of these alternative hypotheses, suggesting that they may be simultaneously operative.

7.4. Weight loss and heart metabolism

Earlier studies have demonstrated changes in cardiac metabolism in obesity. Peterson et al. (Peterson *et al.*, 2004) compared healthy obese and lean subjects, and found that myocardial fatty acid uptake was increased and myocardial efficiency decreased in obese subjects compared to lean ones, but there were no differences in glucose uptake, blood flow or serum FFA concentrations. They concluded that fatty acid supply could not explain differences in myocardial fatty acid uptake. Similarly in the present study, serum FFA concentration did not change after weight loss. This suggests that other mechanisms are involved in the reduction of myocardial FFA uptake than a mass effect. We measured myocardial fatty acid uptake using [^{18}F]FTHA and positron emission tomography, which are suitable and validated methods for measurement of fatty acid uptake in the heart (Takala *et al.*, 2002). In the heart, approximally 80% of [^{18}F]FTHA is accumulates in mitochondria after the initial step of beta-oxidation (Takala *et al.*, 2002). Thus, although the uptake of [^{18}F]FTHA can be considered to reflect mainly FFA beta-oxidation, the fate of FFA (i.e. triglyceride pool vs. oxidation) after entering the cell cannot be determined with this tracer.

Previously, a relation has been found between FFA levels and diastolic dysfunction in obese humans, suggesting a lipotoxic effect of circulating FFA (Leichman *et al.*, 2006; Borradaile & Schaffer, 2005). In the present study, weight loss was associated with a reduction in myocardial FFA uptake and parallel changes in cardiac work and myocardial triglycerides content. Accumulation of triglycerides in cardiomyocytes impairs left ventricular function and promotes fibrosis and apoptosis in animal studies (Zhou *et al.*, 2000; Carroll & Tyagi, 2005; Christoffersen *et al.*, 2003; Ouwens *et al.*, 2005). In obese rats, cardiomyocyte apoptosis leads to decrease in myocardial contractility (Zhou *et al.*, 2000). In the same study, an increase in the myocardial ceramide content suggested that non-oxidative fatty acid metabolites could lead to cell apoptosis. In a previous human study by Hammer et al.(Hammer *et al.*, 2008), a 16-week very-low-energy diet decreased myocardial triglyceride content significantly and improved diastolic function in patients with type 2 diabetes. In the present study, myocardial triglyceride content decreased after weight loss in parallel with changes in myocardial FFA uptake and cardiac work. However, we were unable to demonstrate significant associations between cardiac metabolism, triglyceride content and cardiac function within the study sample possible due to the low number of subjects studied. We have previously shown that myocardial triglyceride content measured with magnetic resonance spectroscopy is increased in obesity and correlates with circulating FFA levels (Kankaanpää *et al.*, 2006). In the same study, FFA levels also correlated with left ventricle mass, and it was concluded that increased fat content in the myocardium could lead to left ventricle overload and hypertrophy.

7.4.1. Heart glucose metabolism

We found that insulin-stimulated glucose uptake in the myocardium was not changed after weight loss. Similarly, in the study by Peterson et al., there was no difference in glucose uptake between obese and lean subjects (Peterson *et al.*, 2004). Myocardial

glucose uptake is regulated by several factors, such as circulating concentrations of alternative substrates including FFA and lactate, hormonal status (insulin, glucagon) and cardiac work (Opie, 1998). Circulating FFA levels and cardiac work are the major regulators of myocardial glucose uptake (Nuutila *et al.*, 1992; Knuuti *et al.*, 1995). In the present study, there was a decrease in cardiac work after weight loss, but this was not reflected in myocardial glucose uptake. Taken together, the available data suggest that obesity alone is not associated with myocardial insulin resistance and in line with this, rapid weight loss has no effect on myocardial glucose metabolism.

8 SUMMARY AND CONCLUSIONS

I

Weight loss with a 6-week very-low-energy diet enhances skeletal muscle glucose uptake and this leads to an increase in whole-body insulin sensitivity in obese humans. Perfusion and glucose uptake are higher in visceral adipose tissue compared to subcutaneous adipose tissue, and perfusion decreases in both compartments when glucose uptake is preserved after weight loss. Leptin and IL-6 levels are associated with abdominal subcutaneous and intra-abdominal adipose tissue insulin resistance.

II

The liver responds to a six-week period of energy restriction with a parallel reduction in lipid uptake and storage. Liver fat content decreases. This is accompanied by enhancement of hepatic insulin sensitivity and clearance and a decrease in circulating inflammatory markers, glycosylated haemoglobin, and liver enzymes. Also liver volume decreases.

III

Weight reduction decreases myocardial fatty acid uptake in parallel with myocardial mass and cardiac work. These results show that the increased fatty acid uptake found in the obese heart can be reversed by weight loss in humans. Also myocardial fat content decreased. There was a decrease in cardiac work after weight loss, but this was not reflected in myocardial glucose uptake. Taken together, the available data suggest that obesity alone is not associated with myocardial insulin resistance.

In conclusion, weight loss in obese subjects without co-morbidies leads to change in fatty acid metabolism and decrease in fat stores and ectopic fat. More detailed studies are needed to understand long term effects and cellular mechanisms of these changes. Doctors in clinical work should encourage their obese patients to lose weight, for example with very-low-energy diet, as it seems to be effective and beneficial for health as the lipid profile improves and HbA_{1c} and blood pressure decreases. These positive changes might delay or prevent possible onset of chronic diseases, such as type 2 diabetes.

9 ACKNOWLEDGEMENTS

This study was carried out at the Department of Medicine, and the Department of Clinical Physiology and Nuclear Medicine, University of Turku, Finland, during the years 2003-2009. I wish to express my sincerest gratitude to heads of the departments, professor Jorma Viikari and docent Jaakko Hartiala, director of Turku PET Centre, professor Juhani Knuuti, for the use of their departments´ facilities.

I am deeply indebted to my supervisors professor Pirjo Nuutila, docent Patricia Iozzo and professor Olli Raitakari, for introducing me to the world of PET, metabolic research and scientific writing. Thank you all for ever so rapid responses and answers for all the problems and questions that I have faced during the years.

I warmly thank the reviewers docent Katriina Kukkonen-Harjula and docent Jukka Westerbacka for encouragement and constructive criticism during the final preparation of this thesis.

I wish to thank Riikka Lautamäki, MD, PhD and Mikko Järvisalo, MD, PhD, for teaching me PET scanning basics and Mikko Kankaanpää, MSc, for teaching me MRI and MRS basics, I wish you Mikko strenght for rehabilitation. I wish to thank all the co-workers in the cardiometabolic study group, especially Kirsi Virtanen, MD, PhD, Ronald Borra, MD, and Anna Karmi, BM, for practical help in many time consuming analyzes.

I also wish to thank all my co-authors docent Riitta Parkkola, docent Risto Huupponen, docent Terho Lehtimäki, professor Tapani Rönnemaa, Letizia Guiducci, MD, PhD, Andrea Mari, MD, PhD, Virva Lepomäki, MSc, Jussi Pärkkä, MD, and Markku Taittonen, MD, PhD, for fruitfull collaboration.

I have been preveledged to work with a great number of talented individuals in the Turku PET centre. You are too many to be mentioned by name. Thank you all.

I express my greatest gratitude to my parents Anna and Pertti for their love, support and guidance throughout my life. I thank my brother Jussi for being my oldest and dearest friend and always being there for me.

From the bottom of my heart I thank my beloved wife Susanna for understanding and support during these years. This work would have never been finished without your love and dedication. I am grateful for each moment we share. Our lovely daughter Helmi has brought overwhelming joy and happiness to my life.

This study was financially supported by the HEPADIP EU FP6 program, the Novo Nordisk foundation, the Yrjö Jahnsson Foundation, the Juho Vainio Foundation, the Finnish Diabetes Foundation, the Orion Group Research Foundation, the Aarne Koskelo Foundation, the Finnish Medical Society Duodecim, the EFSD/Eli-Lilly Fellowship in diabetes and metabolism, and the Hospital District of Southwest Finland. These studies were conducted within the Centre of Excellence in Molecular Imaging in Cardiovascular and Metabolic Research supported by the Academy of Finland, University of Turku, Turku University Hospital and Åbo Academy.

Antti Vilsäinen

Turku, August 2009

10 REFERENCES

Aasum, E., Hafstad, A. D., Severson, D. L., & Larsen, T. S. (2003). Age-dependent changes in metabolism, contractile function, and ischemic sensitivity in hearts from db/db mice. *Diabetes* **52**, 434-441.

Abate, N., Garg, A., Coleman, R., Grundy, S. M., & Peshock, R. M. (1997). Prediction of total subcutaneous abdominal, intraperitoneal, and retroperitoneal adipose tissue masses in men by a single axial magnetic resonance imaging slice. *Am.J.Clin.Nutr.* **65**, 403-408.

Abate, N., Garg, A., Peshock, R. M., Stray-Gundersen, J., & Grundy, S. M. (1995). Relationships of generalized and regional adiposity to insulin sensitivity in men. *J.Clin.Invest* **96**, 88-98.

Abu, K. M., McCutcheon, M. J., Reddy, S., Pearman, P. L., Hunter, G. R., & Weinsier, R. L. (1988). Electrical impedance in assessing human body composition: the BIA method. *Am.J.Clin.Nutr.* **47**, 789-792.

Alenius, S. & Ruotsalainen, U. (1997). Bayesian image reconstruction for emission tomography based on median root prior. *European Journal of Nuclear Medicine* **24**, 258-265.

Alexander, J. K. (2001). Obesity and coronary heart disease. *Am.J Med.Sci.* **321**, 215-224.

Alfakih, K., Reid, S., Jones, T., & Sivananthan, M. (2004). Assessment of ventricular function and mass by cardiac magnetic resonance imaging. *Eur.Radiol.* **14**, 1813-1822.

Amatruda, J. M., Richeson, J. F., Welle, S. L., Brodows, R. G., & Lockwood, D. H. (1988). The safety and efficacy of a controlled low-energy ('very-low-calorie') diet in the treatment of non-insulin-dependent diabetes and obesity. *Arch.Intern.Med.* **148**, 873-877.

Anderlova, K., Kremen, J., Dolezalova, R., Housova, J., Haluzikova, D., Kunesova, M., & Haluzik, M. (2006). The influence of very-low-calorie-diet on serum leptin, soluble leptin receptor, adiponectin and resistin levels in obese women. *Physiol Res.* **55**, 277-283.

Andersen, T., Gluud, C., Franzmann, M. B., & Christoffersen, P. (1991). Hepatic effects of dietary weight loss in morbidly obese subjects. *J.Hepatol.* **12**, 224-229.

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoka, K., Kuriyama, H., Nishida, M., Yamashita, S., Okubo, K., Matsubara, K., Muraguchi, M., Ohmoto, Y., Funahashi, T., & Matsuzawa, Y. (1999). Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem.Biophys.Res.Commun.* **257**, 79-83.

Arner, P. (1997). Regional adipocity in man. *J Endocrinol.* **155**, 191-192.

Avenell, A., Brown, T. J., McGee, M. A., Campbell, M. K., Grant, A. M., Broom, J., Jung, R. T., & Smith, W. C. (2004). What are the long-term benefits of weight reducing diets in adults? A systematic review of randomized controlled trials. *J Hum.Nutr.Diet.* **17**, 317-335.

Avenell, A., Sattar, N., & Lean, M. (2006). ABC of obesity. Management: Part I--behaviour change, diet, and activity. *BMJ* **333**, 740-743.

Ayyad, C. & Andersen, T. (2000). Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1931 and 1999. *Obes.Rev.* **1**, 113-119.

Baron, A. D., Laakso, M., Brechtel, G., & Edelman, S. V. (1991). Mechanism of insulin resistance in insulin-dependent diabetes mellitus: a major role for reduced skeletal muscle blood flow. *J Clin.Endocrinol.Metab* **73**, 637-643.

Bellentani, S., Saccoccio, G., Masutti, F., Croce, L. S., Brandi, G., Sasso, F., Cristanini, G., & Tiribelli, C. (2000). Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann.Intern.Med.* **132**, 112-117.

Bender, R., Jockel, K. H., Trautner, C., Spraul, M., & Berger, M. (1999). Effect of age on excess mortality in obesity. *JAMA* **281**, 1498-1504.

Berg, A. H., Combs, T. P., Du, X., Brownlee, M., & Scherer, P. E. (2001). The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat.Med.* **7**, 947-953.

Bergmann, S. R., Weinheimer, C. J., Markham, J., & Herrero, P. (1996). Quantitation of myocardial fatty acid metabolism using PET. *J Nucl Med* **37**, 1723-1730.

Bergström, A., Pisani, P., Tenet, V., Wolk, A., & Adami, H. O. (2001). Overweight as an avoidable cause of cancer in Europe. *Int.J Cancer* **91**, 421-430.

Berk, P. D. (2008). Regulatable fatty acid transport mechanisms are central to the pathophysiology of obesity, fatty liver, and metabolic syndrome. *Hepatology* **48**, 1362-1376.

Berk, P. D. & Stump, D. D. (1999). Mechanisms of cellular uptake of long chain free fatty acids. *Mol.Cell Biochem.* **192**, 17-31.

Berne, R. Regulation of coronary blood flow. Physiol Rev 44, 1-29. 1964.

Ref Type: Generic

Björntorp, P. (1990). "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis* **10**, 493-496.

Björntorp, P. (1992). Abdominal obesity and the metabolic syndrome. *Ann.Med.* **24**, 465-468.

Björntorp, P. (1995). Liver triglycerides and metabolism. *Int.J Obes.Relat Metab Disord.* **19**, 839-840.

Björntorp, P. (1996). The regulation of adipose tissue distribution in humans. *Int.J.Obes.Relat Metab Disord.* **20**, 291-302.

Björntorp, P. (1997). Obesity and Diabetes Mellitus. In *Ellenberg & Rifkin's Diabetes Mellitus*, eds. Porte, D. J. & Sherwin, R. S., pp. 553-564. Appleton & Lange, Stamford.

Blair, A., Shaul, P. W., Yuhanna, I. S., Conrad, P. A., & Smart, E. J. (1999). Oxidized low density lipoprotein displaces endothelial nitric-oxide synthase (eNOS) from plasmalemmal caveolae and impairs eNOS activation. *J Biol Chem* **274**, 32512-32519.

Bluher, M., Michael, M. D., Peroni, O. D., Ueki, K., Carter, N., Kahn, B. B., & Kahn, C. R. (2002). Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev.Cell* **3**, 25-38.

Boden, G., Cheung, P., Stein, T. P., Kresge, K., & Mozzoli, M. (2002). FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. *Am J Physiol Endocrinol Metab* **283**, E12-E19.

Boden, G., Lebed, B., Schatz, M., Homko, C., & Lemieux, S. (2001). Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes* **50**, 1612-1617.

Bonadonna, R. C., Groop, L., Kraemer, N., Ferrannini, E., Del Prato, S., & DeFronzo, R. A. (1990). Obesity and insulin resistance in humans: a dose-response study. *Metabolism* **39**, 452-459.

Bondini, S., Kleiner, D. E., Goodman, Z. D., Gramlich, T., & Younossi, Z. M. (2007). Pathologic assessment of non-alcoholic fatty liver disease. *Clin.Liver Dis.* **11**, 17-23, vii.

Borradaile, N. M. & Schaffer, J. E. (2005). Lipotoxicity in the heart. *Curr.Hypertens.Rep.* **7**, 412-417.

Bradbury, M. W. & Berk, P. D. (2004). Lipid metabolism in hepatic steatosis. *Clin.Liver Dis.* **8**, 639-71, xi.

Bruning, J. C., Michael, M. D., Winnay, J. N., Hayashi, T., Horsch, D., Accili, D., Goodyear, L. J., & Kahn, C. R. (1998). A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol.Cell* **2**, 559-569.

Brunt, E. M. (2001). Nonalcoholic steatohepatitis: definition and pathology. *Semin.Liver Dis.* **21**, 3-16.

Bugianesi, E., Gastaldelli, A., Vanni, E., Gambino, R., Cassader, M., Baldi, S., Ponti, V., Pagano, G., Ferrannini, E., & Rizzetto, M. (2005). Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia.* **48**, 634-642.

Burke, A. & Lucey, M. R. (2004). Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis and orthotopic liver transplantation. *Am.J Transplant.* **4**, 686-693.

Capaldo, B., Lembo, G., Napoli, R., Rendina, V., Albano, G., Sacca, L., & Trimarco, B. (1991). Skeletal muscle is a primary site of insulin resistance in essential hypertension. *Metabolism* **40**, 1320-1322.

Carroll, J. F. & Tyagi, S. C. (2005). Extracellular matrix remodeling in the heart of the homocysteinemic obese rabbit. *Am.J Hypertens.* **18**, 692-698.

Chan, J. M., Rimm, E. B., Colditz, G. A., Stampfer, M. J., & Willett, W. C. (1994). Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* **17**, 961-969.

Charlton, M., Kasparova, P., Weston, S., Lindor, K., Maor-Kendler, Y., Wiesner, R. H., Rosen, C. B., & Batts, K. P. (2001). Frequency of nonalcoholic steatohepatitis as a cause of advanced liver disease. *Liver Transpl.* **7**, 608-614.

Chen, X., Iqbal, N., & Boden, G. (1999). The effects of free fatty acids on gluconeogenesis and glycogenolysis in normal subjects. *J Clin.Invest* **103**, 365-372.

Chilian, W. M. (1991). Functional distribution of alpha 1- and alpha 2-adrenergic receptors in the coronary microcirculation. *Circulation* **84**, 2108-2122.

Chilian, W. M. & Layne, S. M. (1990). Coronary microvascular responses to reductions in perfusion pressure. Evidence for persistent arteriolar vasomotor tone during coronary hypoperfusion. *Circ.Res.* **66**, 1227-1238.

Chirieac, D. V., Chirieac, L. R., Corsetti, J. P., Cianci, J., Sparks, C. E., & Sparks, J. D. (2000). Glucose-stimulated insulin secretion suppresses hepatic triglyceride-rich lipoprotein and apoB production. *Am.J.Physiol Endocrinol.Metab* **279**, E1003-E1011.

Christoffersen, C., Bollano, E., Lindegaard, M. L., Bartels, E. D., Goetze, J. P., Andersen, C. B., & Nielsen, L. B. (2003). Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* **144**, 3483-3490.

Chu, C. A., Sherck, S. M., Igawa, K., Sindelar, D. K., Neal, D. W., Emshwiller, M., & Cherrington, A. D. (2002). Effects of free fatty acids on hepatic glycogenolysis and gluconeogenesis in conscious dogs. *Am J Physiol Endocrinol Metab* **282**, E402-E411.

Clarke, N. E. & Mosher, R. E. (1952). The water and electrolyte content of the human heart in congestive heart failure with and without digitalization. *Circulation* **5**, 907-914.

Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., Gourmelen, M., Dina, C., Chambaz, J., Lacorte, J. M., Basdevant, A., Bougneres, P., Lebouc, Y., Froguel, P., & Guy-Grand, B. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* **392**, 398-401.

Clore, J. N., Glickman, P. S., Nestler, J. E., & Blackard, W. G. (1991). In vivo evidence for hepatic autoregulation during FFA-stimulated gluconeogenesis in normal humans. *Am.J Physiol* **261**, E425-E429.

Colditz, G. A. & Coakley, E. (1997). Weight, weight gain, activity, and major illnesses: the Nurses' Health Study. *Int.J Sports Med.* **18 Suppl 3**, S162-S170.

Colditz, G. A., Willett, W. C., Stampfer, M. J., Manson, J. E., Hennekens, C. H., Arky, R. A., & Speizer, F. E. (1990). Weight as a risk factor for clinical diabetes in women. *Am J Epidemiol.* **132**, 501-513.

Colles, S. L., Dixon, J. B., Marks, P., Strauss, B. J., & O'Brien, P. E. (2006). Preoperative weight loss with a very-low-energy diet: quantitation of changes in liver and abdominal fat by serial imaging. *Am.J.Clin.Nutr.* **84**, 304-311.

Colquitt, J. L., Picot, J., Loveman, E., & Clegg, A. J. (2009). Surgery for obesity. *Cochrane.Database.Syst.Rev.* CD003641.

Combs, T. P., Berg, A. H., Obici, S., Scherer, P. E., & Rossetti, L. (2001). Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* **108**, 1875-1881.

Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J., Bauer, T. L., & . (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N.Engl.J Med.* **334**, 292-295.

Coppola, A., Marfella, R., Coppola, L., Tagliamonte, E., Fontana, D., Liguori, E., Cirillo, T., Cafiero, M., Natale, S., & Astarita, C. (2008). Effect of weight loss on coronary circulation and adiponectin levels in obese women. *Int.J Cardiol.*

Dahlman, I. & Arner, P. (2007). Obesity and polymorphisms in genes regulating human adipose tissue. *Int.J Obes.(Lond)* **31**, 1629-1641.

Das, U. N. (2001). Is obesity an inflammatory condition? *Nutrition* **17**, 953-966.

Dattilo, A. M. & Kris-Etherton, P. M. (1992). Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *Am.J Clin.Nutr.* **56**, 320-328.

Davey-Smith G., Shipley, M. J., Batty, G. D., Morris, J. N., & Marmot, M. (2000). Physical activity and cause-specific mortality in the Whitehall study. *Public Health* **114**, 308-315.

Dayanikli, F., Grambow, D., Muzik, O., Mosca, L., Rubenfire, M., & Schwaiger, M. (1994). Early detection of abnormal coronary flow reserve in asymptomatic men at high risk for coronary artery disease using positron emission tomography. *Circulation* **90**, 808-817.

DeFronzo, R. A. & Ferrannini, E. (1991). Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* **14**, 173-194.

DeFronzo, R. A., Tobin, J. D., & Andres, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am.J.Physiol* **237**, E214-E223.

DeGrado, T. R., Coenen, H. H., & Stocklin, G. (1991). 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (FTHA): evaluation in mouse of a new probe of myocardial utilization of long chain fatty acids. *J Nucl Med* **32**, 1888-1896.

Depre, C., Vanoverschelde, J. L., & Taegtmeyer, H. (1999). Glucose for the heart. *Circulation* **99**, 578-588.

Despres, J. P., Lamarche, B., Mauriege, P., Cantin, B., Dagenais, G. R., Moorjani, S., & Lupien, P. J. (1996). Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* **334**, 952-957.

Despres, J. P. & Lemieux, I. (2006). Abdominal obesity and metabolic syndrome. *Nature* **444**, 881-887.

Despres, J. P., Nadeau, A., Tremblay, A., Ferland, M., Moorjani, S., Lupien, P. J., Theriault, G., Pinault, S., & Bouchard, C. (1989). Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. *Diabetes* **38**, 304-309.

Di Gregorio, G. B., Yao-Borengasser, A., Rasouli, N., Varma, V., Lu, T., Miles, L. M., Ranganathan, G., Peterson, C. A., McGehee, R. E., & Kern, P. A. (2005). Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* **54**, 2305-2313.

Diehl, A. M. (2005). Lessons from animal models of NASH. *Hepatol.Res.* **33**, 138-144.

Dohm, G. L., Tapscott, E. B., Pories, W. J., Dabbs, D. J., Flickinger, E. G., Meelheim, D., Fushiki, T., Atkinson, S. M., Elton, C. W., & Caro, J. F. (1988). An in vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J Clin.Invest* **82**, 486-494.

Donnelly, K. L., Smith, C. I., Schwarzenberg, S. J., Jessurun, J., Boldt, M. D., & Parks, E. J. (2005). Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J.Clin.Invest* **115**, 1343-1351.

Drenick, E. J., Simmons, F., & Murphy, J. F. (1970). Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. *N.Engl.J.Med.* **282**, 829-834.

Duckworth, W. C., Hamel, F. G., & Peavy, D. E. (1988). Hepatic metabolism of insulin. *Am.J Med.* **85**, 71-76.

Durnin, J. V. & Rahaman, M. M. (1967). The assessment of the amount of fat in the human body from measurements of skinfold thickness. *Br.J Nutr.* **21**, 681-689.

Enevoldsen, L. H., Stallknecht, B., Fluckey, J. D., & Galbo, H. (2000). Effect of exercise training on in vivo insulin-stimulated glucose uptake in intra-abdominal adipose tissue in rats. *Am.J.Physiol Endocrinol.Metab* **278**, E25-E34.

Esposito, K., Pontillo, A., Di Palo, C., Giugliano, G., Masella, M., Marfella, R., & Giugliano, D. (2003). Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* **289**, 1799-1804.

Everhart, J. E., Pettitt, D. J., Bennett, P. H., & Knowler, W. C. (1992). Duration of obesity increases the incidence of NIDDM. *Diabetes* **41**, 235-240.

Fantuzzi, G. (2005). Adipose tissue, adipokines, and inflammation. *J Allergy Clin.Immunol.* **115**, 911-919.

Farese, R. V., Jr., Yost, T. J., & Eckel, R. H. (1991). Tissue-specific regulation of lipoprotein lipase activity by insulin/glucose in normal-weight humans. *Metabolism* **40**, 214-216.

Farooqi, I. S., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., Sanna, V., Jebb, S. A., Perna, F., Fontana, S., Lechler, R. I., DePaoli, A. M., & O'Rahilly, S. (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin.Invest* **110**, 1093-1103.

Farrell, G. C. & Larter, C. Z. (2006). Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* **43**, S99-S112.

Ferrannini, E., Buzzigoli, G., Bonadonna, R., Giorico, M. A., Oleggini, M., Graziadei, L., Pedrinelli, R., Brandi, L., & Bevilacqua, S. (1987). Insulin resistance in essential hypertension. *N Engl J Med* **317**, 350-357.

Ferrannini, E. & Camastra, S. (1998). Relationship between impaired glucose

tolerance, non-insulin-dependent diabetes mellitus and obesity. *Eur.J.Clin.Invest* **28** Suppl 2:3-6; discussion 6-7., 3-6.

Ferrannini, E., Natali, A., Bell, P., Cavallo-Perin, P., Lalic, N., & Mingrone, G. (1997). Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *J Clin.Invest* **100**, 1166-1173.

Ferrannini, E., Santoro, D., Bonadonna, R., Natali, A., Parodi, O., & Camici, P. G. (1993). Metabolic and hemodynamic effects of insulin on human hearts. *Am J Physiol* **264**, E308-E315.

Finck, B. N., Han, X., Courtois, M., Aimond, F., Nerbonne, J. M., Kovacs, A., Gross, R. W., & Kelly, D. P. (2003). A critical role for PPARalpha-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. *Proc.Natl.Acad.Sci.U.S.A* **100**, 1226-1231.

Frayn, K. N., Coppack, S. W., Humphreys, S. M., & Whyte, P. L. (1989). Metabolic characteristics of human adipose tissue in vivo. *Clin.Sci.* **76**, 509-516.

Frayn, K. N., Fielding, B. A., & Summers, L. K. (1997). Investigation of human adipose tissue metabolism in vivo. *J Endocrinol.* **155**, 187-189.

Frayn, K. N., Karpe, F., Fielding, B. A., Macdonald, I. A., & Coppack, S. W. (2003). Integrative physiology of human adipose tissue. *Int.J.Obes.Relat Metab Disord.* **27**, 875-888.

French, S. A., Folsom, A. R., Jeffery, R. W., Zheng, W., Mink, P. J., & Baxter, J. E. (1997). Weight variability and incident disease in older women: the Iowa Women's Health Study. *Int.J Obes.Relat Metab Disord.* **21**, 217-223.

Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab* **83**, 847-850.

Fruhbeck, G. (1999). Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes* **48**, 903-908.

Frühbeck, G., Aguado, M., & Martinez, J. A. (1997). In vitro lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. *Biochem.Biophys.Res.Commun.* **240**, 590-594.

Garaulet, M., Viguerie, N., Porubsky, S., Klimcakova, E., Clement, K., Langin, D., & Stich, V. (2004). Adiponectin gene expression and plasma values in obese women during very-low-calorie diet. Relationship with cardiovascular risk factors and insulin resistance. *J Clin.Endocrinol.Metab* **89**, 756-760.

Garbow, J. R., Lin, X., Sakata, N., Chen, Z., Koh, D., & Schonfeld, G. (2004). In vivo MRS measurement of liver lipid levels in mice. *J.Lipid Res.* **45**, 1364-1371.

Garrow, J. S. & Webster, J. (1985). Quetelet's index (W/H2) as a measure of fatness. *Int.J Obes.* **9**, 147-153.

Gilden, T. A. & Wadden, T. A. (2006). The evolution of very-low-calorie diets: an update and meta-analysis. *Obesity.(Silver.Spring)* **14**, 1283-1293.

Gobel, F. L., Norstrom, L. A., Nelson, R. R., Jorgensen, C. R., & Wang, Y. (1978). The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. *Circulation* **57**, 549-556.

Goodman-Gruen, D. & Barrett-Connor, E. (1996). Sex differences in measures of body fat and body distribution in the elderly. *Am J Epidemiol.* **143**, 898-906.

Goodpaster, B. H., Kelley, D. E., Wing, R. R., Meier, A., & Thaete, F. L. (1999). Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* **48**, 839-847.

Goodpaster, B. H., Thaete, F. L., Simoneau, J. A., & Kelley, D. E. (1997). Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. *Diabetes* **46**, 1579-1585.

Goodyear, L. J., Giorgino, F., Sherman, L. A., Carey, J., Smith, R. J., & Dohm, G. L. (1995). Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *J Clin.Invest* **95**, 2195-2204.

Greco, A. V., Mingrone, G., Giancaterini, A., Manco, M., Morroni, M., Cinti, S., Granzotto, M., Vettor, R., Camastra, S., & Ferrannini, E. (2002). Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes* **51**, 144-151.

Greco, D., Kotronen, A., Westerbacka, J., Puig, O., Arkkila, P., Kiviluoto, T., Laitinen, S.,

Kolak, M., Fisher, R. M., Hamsten, A., Auvinen, P., & Yki-Järvinen, H. (2008). Gene expression in human NAFLD. *Am.J Physiol Gastrointest.Liver Physiol* **294**, G1281-G1287.

Green, A. & Newsholme, E. A. (1979). Sensitivity of glucose uptake and lipolysis of white adipocytes of the rat to insulin and effects of some metabolites. *Biochem.J* **180**, 365-370.

Hagström-Toft, E., Arner, P., Johansson, U., Eriksson, L. S., Ungerstedt, U., & Bolinder, J. (1992). Effect of insulin on human adipose tissue metabolism in situ. Interactions with beta-adrenoceptors. *Diabetologia* **35**, 664-670.

Hall, J. E., Guyton, A. C., & Brands, M. W. (1996). Pressure-volume regulation in hypertension. *Kidney Int.Suppl* **55**, S35-S41.

Hällsten, K., Yki-Järvinen, H., Peltoniemi, P., Oikonen, V., Takala, T., Kemppainen, J., Laine, H., Bergman, J., Bolli, G. B., Knuuti, J., & Nuutila, P. (2003). Insulin- and exercise-stimulated skeletal muscle blood flow and glucose uptake in obese men. *Obes.Res* **11**, 257-265.

Hamacher, K., Coenen, H. H., & Stocklin, G. (1986). Efficient stereospecific synthesis of no-carrier-added 2-[18F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. *J.Nucl.Med.* **27**, 235-238.

Hammer, S., Snel, M., Lamb, H. J., Jazet, I. M., van der Meer, R. W., Pijl, H., Meinders, E. A., Romijn, J. A., de Roos, A., & Smit, J. W. (2008). Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am.Coll.Cardiol.* **52**, 1006-1012.

Han, T. S., van Leer, E. M., Seidell, J. C., & Lean, M. E. (1995). Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. *BMJ* **311**, 1401-1405.

Hannukainen, J. C., Nuutila, P., Ronald, B., Kaprio, J., Kujala, U. M., Janatuinen, T., Heinonen, O. J., Kapanen, J., Viljanen, T., Haaparanta, M., Ronnemaa, T., Parkkola, R., Knuuti, J., & Kalliokoski, K. K. (2007). Increased physical activity decreases hepatic free fatty acid uptake: a study in human monozygotic twins. *J Physiol* **578**, 347-358.

Hattori, K., Numata, N., Ikoma, M., Matsuzaka, A., & Danielson, R. R. (1991). Sex differences in the distribution of subcutaneous and internal fat. *Hum.Biol.* **63**, 53-63.

Havel, P. J. (2001). Peripheral signals conveying metabolic information to the brain: short-term and long-term regulation of food intake and energy homeostasis. *Exp.Biol.Med.(Maywood.)* **226**, 963-977.

Himms-Hagen, J. (1999). Physiological roles of the leptin endocrine system: differences between mice and humans. *Crit Rev.Clin.Lab Sci* **36**, 575-655.

Horvath, K., Jeitler, K., Siering, U., Stich, A. K., Skipka, G., Gratzer, T. W., & Siebenhofer, A. (2008). Long-term effects of weight-reducing interventions in hypertensive patients: systematic review and meta-analysis. *Arch.Intern.Med.* **168**, 571-580.

Hoyumpa, A. M., Jr., Greene, H. L., Dunn, G. D., & Schenker, S. (1975). Fatty liver: biochemical and clinical considerations. *Am.J.Dig.Dis.* **20**, 1142-1170.

Hu, F. B., Rimm, E. B., Stampfer, M. J., Ascherio, A., Spiegelman, D., & Willett, W. C. (2000). Prospective study of major dietary patterns and risk of coronary heart disease in men. *Am.J Clin.Nutr.* **72**, 912-921.

Iida, H., Rhodes, C. G., de Silva, R., Araujo, L. I., Bloomfield, P. M., Lammertsma, A. A., & Jones, T. (1992). Use of the left ventricular time-activity curve as a noninvasive input function in dynamic oxygen-15-water positron emission tomography. *J.Nucl Med.* **33**, 1669-1677.

Iida, H., Takahashi, A., Tamura, Y., Ono, Y., & Lammertsma, A. A. (1995). Myocardial blood flow: comparison of oxygen-15-water bolus injection, slow infusion and oxygen-15-carbon dioxide slow inhalation. *J.Nucl Med.* **36**, 78-85.

Iozzo, P., Gastaldelli, A., Jarvisalo, M. J., Kiss, J., Borra, R., Buzzigoli, E., Viljanen, A., Naum, G., Viljanen, T., Oikonen, V., Knuuti, J., Savunen, T., Salvadori, P. A., Ferrannini, E., & Nuutila, P. (2006). 18F-FDG assessment of glucose disposal and production rates during fasting and insulin stimulation: a validation study. *J Nucl.Med.* **47**, 1016-1022.

Iozzo, P., Geisler, F., Oikonen, V., Mäki, M., Takala, T., Solin, O., Ferrannini, E., Knuuti, J., & Nuutila, P. (2003a). Insulin stimulates liver glucose uptake in humans: an 18F-FDG PET Study. *J.Nucl.Med.* **44**, 682-689.

Iozzo, P., Hällsten, K., Oikonen, V., Virtanen, K. A., Kemppainen, J., Solin, O., Ferrannini, E.,

Knuuti, J., & Nuutila, P. (2003b). Insulin-mediated hepatic glucose uptake is impaired in type 2 diabetes: evidence for a relationship with glycemic control. *J.Clin.Endocrinol.Metab* **88**, 2055-2060.

Iozzo, P., Järvisalo, M. J., Kiss, J., Borra, R., Naum, G. A., Viljanen, A., Viljanen, T., Gastaldelli, A., Buzzigoli, E., Guiducci, L., Barsotti, E., Savunen, T., Knuuti, J., Haaparanta-Solin, M., Ferrannini, E., & Nuutila, P. (2007). Quantification of liver glucose metabolism by positron emission tomography: validation study in pigs. *Gastroenterology* **132**, 531-542.

Iozzo, P., Lautamaki, R., Geisler, F., Virtanen, K. A., Oikonen, V., Haaparanta, M., Yki-Jarvinen, H., Ferrannini, E., Knuuti, J., & Nuutila, P. (2004a). Non-esterified fatty acids impair insulin-mediated glucose uptake and disposition in the liver. *Diabetologia* **47**, 1149-1156.

Iozzo, P., Pratipanawatr, T., Pijl, H., Vogt, C., Kumar, V., Pipek, R., Matsuda, M., Mandarino, L. J., Cusi, K. J., & DeFronzo, R. A. (2001). Physiological hyperinsulinemia impairs insulin-stimulated glycogen synthase activity and glycogen synthesis. *Am J Physiol Endocrinol Metab* **280**, E712-E719.

Iozzo, P., Turpeinen, A. K., Takala, T., Oikonen, V., Bergman, J., Gronroos, T., Ferrannini, E., Nuutila, P., & Knuuti, J. (2004b). Defective liver disposal of free fatty acids in patients with impaired glucose tolerance. *J Clin.Endocrinol.Metab* **89**, 3496-3502.

Iozzo, P., Turpeinen, A. K., Takala, T., Oikonen, V., Solin, O., Ferrannini, E., Nuutila, P., & Knuuti, J. (2003c). Liver uptake of free fatty acids in vivo in humans as determined with 14(R, S)-[18F]fluoro-6-thia-heptadecanoic acid and PET. *Eur J Nucl Med Mol Imaging* **30**, 1160-1164.

Jackson, A. S., Stanforth, P. R., Gagnon, J., Rankinen, T., Leon, A. S., Rao, D. C., Skinner, J. S., Bouchard, C., & Wilmore, J. H. (2002). The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study. *Int.J Obes.Relat Metab Disord.* **26**, 789-796.

Jackson-Leach, R. & Lobstein, T. (2006). Estimated burden of paediatric obesity and co-morbidities in Europe. Part 1. The increase in the prevalence of child obesity in Europe is itself increasing. *Int.J Pediatr.Obes.* **1**, 26-32.

Jacob, S., Machann, J., Rett, K., Brechtel, K., Volk, A., Renn, W., Maerker, E., Matthaei, S., Schick, F., Claussen, C. D., & Haring, H. U. (1999). Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* **48**, 1113-1119.

Jazet, I. M., Pijl, H., Frolich, M., Romijn, J. A., & Meinders, A. E. (2005). Two days of a very low calorie diet reduces endogenous glucose production in obese type 2 diabetic patients despite the withdrawal of blood glucose-lowering therapies including insulin. *Metabolism* **54**, 705-712.

Jensen, M. D. (2006). Is visceral fat involved in the pathogenesis of the metabolic syndrome? Human model. *Obesity.(Silver.Spring)* **14 Suppl 1**, 20S-24S.

Jones, P. R. & Edwards, D. A. (1999). Areas of fat loss in overweight young females following an 8-week period of energy intake reduction. *Ann.Hum.Biol.* **26**, 151-162.

Jou, J., Choi, S. S., & Diehl, A. M. (2008). Mechanisms of disease progression in nonalcoholic fatty liver disease. *Semin.Liver Dis.* **28**, 370-379.

Jousilahti, P., Tuomilehto, J., Vartiainen, E., Pekkanen, J., & Puska, P. (1996). Body weight, cardiovascular risk factors, and coronary mortality. 15-year follow-up of middle-aged men and women in eastern Finland. *Circulation* **93**, 1372-1379.

Jousilahti, P., Tuomilehto, J., Vartiainen, E., Valle, T., & Nissinen, A. (1995). Body mass index, blood pressure, diabetes and the risk of anti-hypertensive drug treatment: 12-year follow-up of middle-aged people in eastern Finland. *J Hum.Hypertens.* **9**, 847-854.

Kahn, B. B. (1992). Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. *J Clin Invest* **89**, 1367-1374.

Kankaanpää, M., Lehto, H. R., Pärkkä, J. P., Komu, M., Viljanen, A., Ferrannini, E., Knuuti, J., Nuutila, P., Parkkola, R., & Iozzo, P. (2006). Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin.Endocrinol.Metab* **91**, 4689-4695.

Kannel, W. B. (1996). Blood pressure as a cardiovascular risk factor: prevention and treatment. *JAMA* **275**, 1571-1576.

Karpe, F., Fielding, B. A., Ilic, V., Macdonald, I. A., Summers, L. K., & Frayn, K. N. (2002). Impaired postprandial adipose tissue blood flow

response is related to aspects of insulin sensitivity. *Diabetes* **51**, 2467-2473.

Karvetti, R. L. & Hakala, P. (1992). A seven-year follow-up of a weight reduction programme in Finnish primary health care. *Eur.J Clin.Nutr.* **46**, 743-752.

Katzel, L. I., Bleecker, E. R., Colman, E. G., Rogus, E. M., Sorkin, J. D., & Goldberg, A. P. (1995). Effects of weight loss vs aerobic exercise training on risk factors for coronary disease in healthy, obese, middle-aged and older men. A randomized controlled trial. *JAMA* **274**, 1915-1921.

Katzen, H. M., Soderman, D. D., & Wiley, C. E. (1970). Multiple forms of hexokinase. Activities associated with subcellular particulate and soluble fractions of normal and streptozotocin diabetic rat tissues. *J Biol Chem* **245**, 4081-4096.

Keim, N. L., Stern, J. S., & Havel, P. J. (1998). Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *Am.J Clin.Nutr.* **68**, 794-801.

Kim, J. K., Fillmore, J. J., Chen, Y., Yu, C., Moore, I. K., Pypaert, M., Lutz, E. P., Kako, Y., Velez-Carrasco, W., Goldberg, I. J., Breslow, J. L., & Shulman, G. I. (2001). Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc.Natl.Acad.Sci U.S.A* **98**, 7522-7527.

Kim, M. J., Maachi, M., Debard, C., Loizon, E., Clement, K., Bruckert, E., Hainque, B., Capeau, J., Vidal, H., & Bastard, J. P. (2006). Increased adiponectin receptor-1 expression in adipose tissue of impaired glucose-tolerant obese subjects during weight loss. *Eur.J Endocrinol.* **155**, 161-165.

Kip, K. E., Marroquin, O. C., Kelley, D. E., Johnson, B. D., Kelsey, S. F., Shaw, L. J., Rogers, W. J., & Reis, S. E. (2004). Clinical importance of obesity versus the metabolic syndrome in cardiovascular risk in women: a report from the Women's Ischemia Syndrome Evaluation (WISE) study. *Circulation* **109**, 706-713.

Kleiner, D. E., Brunt, E. M., Van Natta, M., Behling, C., Contos, M. J., Cummings, O. W., Ferrell, L. D., Liu, Y. C., Torbenson, M. S., Unalp-Arida, A., Yeh, M., McCullough, A. J., & Sanyal, A. J. (2005). Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* **41**, 1313-1321.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., & Nathan, D. M. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N.Engl.J Med* **346**, 393-403.

Knudson, J. D., Dincer, U. D., Zhang, C., Swafford, A. N., Jr., Koshida, R., Picchi, A., Focardi, M., Dick, G. M., & Tune, J. D. (2005). Leptin receptors are expressed in coronary arteries, and hyperleptinemia causes significant coronary endothelial dysfunction. *Am.J Physiol Heart Circ.Physiol* **289**, H48-H56.

Knuuti, M. J., Maki, M., Yki-Jarvinen, H., Voipio-Pulkki, L. M., Harkonen, R., Haaparanta, M., & Nuutila, P. (1995). The effect of insulin and FFA on myocardial glucose uptake. *J Mol Cell Cardiol* **27**, 1359-1367.

Kolterman, O. G., Insel, J., Saekow, M., & Olefsky, J. M. (1980). Mechanisms of insulin resistance in human obesity: evidence for receptor and postreceptor defects. *J Clin Invest* **65**, 1272-1284.

Koskenvuo, J. W., Karra, H., Lehtinen, J., Niemi, P., Parkka, J., Knuuti, J., & Hartiala, J. J. (2007). Cardiac MRI: accuracy of simultaneous measurement of left and right ventricular parameters using three different sequences. *Clin.Physiol Funct.Imaging* **27**, 385-393.

Kraegen, E. W., Cooney, G. J., Ye, J. M., Thompson, A. L., & Furler, S. M. (2001). The role of lipids in the pathogenesis of muscle insulin resistance and beta cell failure in type II diabetes and obesity. *Exp.Clin.Endocrinol.Diabetes* **109 Suppl 2**, S189-S201.

Laakso, M., Edelman, S. V., Brechtel, G., & Baron, A. D. (1992). Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* **41**, 1076-1083.

Lahti-Koski, M., Pietinen, P., Heliövaara, M., & Vartiainen, E. (2002). Associations of body mass index and obesity with physical activity, food choices, alcohol intake, and smoking in the 1982-1997 FINRISK Studies. *Am J Clin Nutr.* **75**, 809-817.

Laine, H., Raitakari, O. T., Niinikoski, H., Pitkänen, O. P., Iida, H., Viikari, J., Nuutila, P., & Knuuti, J. (1998a). Early impairment of coronary flow reserve in young men with borderline hypertension. *J.Am.Coll.Cardiol.* **32**, 147-153.

Laine, H., Yki-Järvinen, H., Kirvelä, O., Tolvanen, T., Raitakari, M., Solin, O., Haaparanta, M., Knuuti, J., & Nuutila, P. (1998b). Insulin resistance of glucose uptake in skeletal muscle cannot be ameliorated by enhancing endothelium-dependent blood flow in obesity. *J.Clin.Invest* **101**, 1156-1162.

Leeson, C. R. & Leeson, T. S. (1976). *Histology*, 3rd ed., pp. 113-131. W.B. Saunders Company, Philadelphia, London, Toronto.

Lefebvre, A. M., Laville, M., Vega, N., Riou, J. P., van Gaal, L., Auwerx, J., & Vidal, H. (1998). Depot-specific differences in adipose tissue gene expression in lean and obese subjects. *Diabetes* **47**, 98-103.

Lehtimäki, T., Ojala, P., Rontu, R., Goebeler, S., Karhunen, P. J., Jylhä, M., Mattila, K., Metso, S., Jokela, H., Nikkilä, M., Wuolijoki, E., Hervonen, A., & Hurme, M. (2005). Interleukin-6 modulates plasma cholesterol and C-reactive protein concentrations in nonagenarians. *J Am.Geriatr.Soc.* **53**, 1552-1558.

Leichman, J. G., Aguilar, D., King, T. M., Vlada, A., Reyes, M., & Taegtmeyer, H. (2006). Association of plasma free fatty acids and left ventricular diastolic function in patients with clinically severe obesity. *Am.J Clin.Nutr.* **84**, 336-341.

Lelliott, C. & Vidal-Puig, A. J. (2004). Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation. *Int.J Obes.Relat Metab Disord.* **28 Suppl 4**, S22-S28.

Lemieux, S., Prud'homme, D., Bouchard, C., Tremblay, A., & Despres, J. P. (1996). A single threshold value of waist girth identifies normal-weight and overweight subjects with excess visceral adipose tissue. *Am.J Clin.Nutr.* **64**, 685-693.

Letiexhe, M. R., Scheen, A. J., Gerard, P. L., Bastens, B. H., Pirotte, J., Belaiche, J., & Lefebvre, P. J. (1993). Insulin secretion, clearance, and action on glucose metabolism in cirrhotic patients. *J.Clin.Endocrinol.Metab* **77**, 1263-1268.

Lewis, M. C., Phillips, M. L., Slavotinek, J. P., Kow, L., Thompson, C. H., & Toouli, J. (2006). Change in liver size and fat content after treatment with Optifast very low calorie diet. *Obes.Surg.* **16**, 697-701.

Liedtke, A. J. (1981). Alterations of carbohydrate and lipid metabolism in the acutely ischemic heart. *Prog.Cardiovasc.Dis.* **23**, 321-336.

Lönnroth, P. & Smith, U. (1992). Intermediary Metabolism with an Emphasis on Lipid Metabolism, Adipose Tissue, and Fat Cell Metabolism: A Review. In *Obesity*, eds. Björntorp, P. & Brodoff, B. N., pp. 3-14. J. B. Lippincott Company, Philadelphia.

Lopaschuk, G. D., Belke, D. D., Gamble, J., Itoi, T., & Schonekess, B. O. (1994). Regulation of fatty acid oxidation in the mammalian heart in health and disease. *Biochim.Biophys Acta* **1213**, 263-276.

Lord, G. M., Matarese, G., Howard, J. K., Baker, R. J., Bloom, S. R., & Lechler, R. I. (1998). Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* **394**, 897-901.

Ludwig, J., Viggiano, T. R., McGill, D. B., & Oh, B. J. (1980). Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin.Proc.* **55**, 434-438.

Lukaski, H. C. (1993). Soft tissue composition and bone mineral status: evaluation by dual-energy X-ray absorptiometry. *J Nutr.* **123**, 438-443.

MacMahon, S., Peto, R., Cutler, J., Collins, R., Sorlie, P., Neaton, J., Abbott, R., Godwin, J., Dyer, A., & Stamler, J. (1990). Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* **335**, 765-774.

Mäki, M. T., Haaparanta, M., Nuutila, P., Oikonen, V., Luotolahti, M., Eskola, O., & Knuuti, J. M. (1998). Free fatty acid uptake in the myocardium and skeletal muscle using fluorine-18-fluoro-6-thia-heptadecanoic acid. *J Nucl Med* **39**, 1320-1327.

Malhi, H. & Gores, G. J. (2008). Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. *Semin.Liver Dis.* **28**, 360-369.

Mantena, S. K., King, A. L., Andringa, K. K., Eccleston, H. B., & Bailey, S. M. (2008). Mitochondrial dysfunction and oxidative stress in the pathogenesis of alcohol- and obesity-induced fatty liver diseases. *Free Radic.Biol.Med.* **44**, 1259-1272.

Marceau, P., Hould, F. S., Simard, S., Lebel, S., Bourque, R. A., Potvin, M., & Biron, S. (1998).

Biliopancreatic diversion with duodenal switch. *World J Surg.* **22**, 947-954.

Marchesini, G., Brizi, M., Morselli-Labate, A. M., Bianchi, G., Bugianesi, E., McCullough, A. J., Forlani, G., & Melchionda, N. (1999). Association of nonalcoholic fatty liver disease with insulin resistance. *Am.J.Med.* **107**, 450-455.

Mari, A., Pacini, G., Murphy, E., Ludvik, B., & Nolan, J. J. (2001). A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care.* **24**, 539-548.

Mårin, P., Andersson, B., Ottosson, M., Olbe, L., Chowdhury, B., Kvist, H., Holm, G., Sjöstrom, L., & Björntorp, P. (1992). The morphology and metabolism of intraabdominal adipose tissue in men. *Metabolism* **41**, 1242-1248.

Matsuda, M. & DeFronzo, R. A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* **22**, 1462-1470.

Mazumder, P. K., O'Neill, B. T., Roberts, M. W., Buchanan, J., Yun, U. J., Cooksey, R. C., Boudina, S., & Abel, E. D. (2004). Impaired cardiac efficiency and increased fatty acid oxidation in insulin-resistant ob/ob mouse hearts. *Diabetes* **53**, 2366-2374.

McCullough, A. J. (2006). Pathophysiology of nonalcoholic steatohepatitis. *J Clin.Gastroenterol.* **40 Suppl 1**, S17-S29.

McGarry, J. D. & Dobbins, R. L. (1999). Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* **42**, 128-138.

McGee, D. L. (2005). Body mass index and mortality: a meta-analysis based on person-level data from twenty-six observational studies. *Ann.Epidemiol.* **15**, 87-97.

Merriman, R. B., Aouizerat, B. E., & Bass, N. M. (2006). Genetic influences in nonalcoholic fatty liver disease. *J Clin.Gastroenterol.* **40 Suppl 1**, S30-S33.

Metz, J. A., Stern, J. S., Kris-Etherton, P., Reusser, M. E., Morris, C. D., Hatton, D. C., Oparil, S., Haynes, R. B., Resnick, L. M., Pi-Sunyer, F. X., Clark, S., Chester, L., McMahon, M., Snyder, G. W., & McCarron, D. A. (2000). A randomized trial of improved weight loss with a prepared meal plan in overweight and obese patients: impact on cardiovascular risk reduction. *Arch.Intern.Med.* **160**, 2150-2158.

Michael, M. D., Kulkarni, R. N., Postic, C., Previs, S. F., Shulman, G. I., Magnuson, M. A., & Kahn, C. R. (2000). Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol.Cell* **6**, 87-97.

Mikkelsen, K. L., Heitmann, B. L., Keiding, N., & Sorensen, T. I. (1999). Independent effects of stable and changing body weight on total mortality. *Epidemiology* **10**, 671-678.

Minehira, K., Young, S. G., Villanueva, C. J., Yetukuri, L., Oresic, M., Hellerstein, M. K., Farese, R. V., Jr., Horton, J. D., Preitner, F., Thorens, B., & Tappy, L. (2008). Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. *J Lipid Res.* **49**, 2038-2044.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., Klein, S., & Coppack, S. W. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin.Endocrinol.Metab* **82**, 4196-4200.

Moloney, M. (2000). Dietary treatments of obesity. *Proc.Nutr.Soc.* **59**, 601-608.

Montague, C. T., Prins, J. B., Sanders, L., Digby, J. E., & O'Rahilly, S. (1997). Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes* **46**, 342-347.

Moore, L. L., Visioni, A. J., Wilson, P. W., D'Agostino, R. B., Finkle, W. D., & Ellison, R. C. (2000). Can sustained weight loss in overweight individuals reduce the risk of diabetes mellitus? *Epidemiology* **11**, 269-273.

Motoshima, H., Wu, X., Sinha, M. K., Hardy, V. E., Rosato, E. L., Barbot, D. J., Rosato, F. E., & Goldstein, B. J. (2002). Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. *J Clin.Endocrinol.Metab* **87**, 5662-5667.

Muller, J. M., Davis, M. J., & Chilian, W. M. (1996). Integrated regulation of pressure and flow in the coronary microcirculation. *Cardiovascular Research* **32**, 668-678.

Narishige, T., Egashira, K., Akatsuka, Y., Katsuda, Y., Numaguchi, K., Sakata, M., & Takeshita, A. (1993). Glibenclamide, a putative ATP-sensitive K+ channel blocker, inhibits coronary autoregulation in anesthetized dogs. *Circ.Res.* **73**, 771-776.

Natali, A., Santoro, D., Palombo, C., Cerri, M., Ghione, S., & Ferrannini, E. (1991). Impaired insulin action on skeletal muscle metabolism in essential hypertension. *Hypertension* **17**, 170-178.

Neely, J. R. & Morgan, H. E. (1974). Relationship Between Carbohydrate and Lipid-Metabolism and Energy-Balance of Heart-Muscle. *Annual Review of Physiology* **36**, 413-459.

Nellis, S. H., Liedtke, A. J., & Renstrom, B. (1992). Fatty acid kinetics in aerobic myocardium: characteristics of tracer carbon entry and washout and influence of metabolic demand. *J Nucl Med* **33**, 1864-1874.

Neuschwander-Tetri, B. A. & Caldwell, S. H. (2003). Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* **37**, 1202-1219.

Ng, C. K., Soufer, R., & McNulty, P. H. (1998). Effect of hyperinsulinemia on myocardial fluorine-18-FDG uptake. *J.Nucl.Med.* **39**, 379-383.

Niesler, C. U., Siddle, K., & Prins, J. B. (1998). Human preadipocytes display a depot-specific susceptibility to apoptosis. *Diabetes* **47**, 1365-1368.

Nikolaidis, M. G., Petridou, A., & Mougios, V. (2006). Comparison of the phospholipid and triacylglycerol fatty acid profile of rat serum, skeletal muscle and heart. *Physiol Res.* **55**, 259-265.

Niswender, K. D. & Schwartz, M. W. (2003). Insulin and leptin revisited: adiposity signals with overlapping physiological and intracellular signaling capabilities. *Front Neuroendocrinol.* **24**, 1-10.

Noakes, M. & Clifton, P. M. (2000). Weight loss and plasma lipids. *Curr.Opin.Lipidol.* **11**, 65-70.

Norris, S. L., Zhang, X., Avenell, A., Gregg, E., Bowman, B., Schmid, C. H., & Lau, J. (2005). Long-term effectiveness of weight-loss interventions in adults with pre-diabetes: a review. *Am.J Prev.Med.* **28**, 126-139.

Norris, S. L., Zhang, X., Avenell, A., Gregg, E., Bowman, B., Serdula, M., Brown, T. J., Schmid, C. H., & Lau, J. (2004). Long-term effectiveness of lifestyle and behavioral weight loss interventions in adults with type 2 diabetes: a meta-analysis. *Am.J Med.* **117**, 762-774.

Nuutila, P., Koivisto, V. A., Knuuti, J., Ruotsalainen, U., Teräs, M., Haaparanta, M., Bergman, J., Solin, O., Voipio-Pulkki, L. M., Wegelius, U., & . (1992). Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. *J Clin Invest* **89**, 1767-1774.

Ogawa, A., Kurita, K., Ikezawa, Y., Igarashi, M., Kuzumaki, T., Daimon, M., Kato, T., Yamatani, K., & Sasaki, H. (1996). Functional localization of glucose transporter 2 in rat liver. *J Histochem.Cytochem.* **44**, 1231-1236.

Ohlson, L. O., Larsson, B., Svardsudd, K., Welin, L., Eriksson, H., Wilhelmsen, L., Bjorntorp, P., & Tibblin, G. (1985). The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up of the participants in the study of men born in 1913. *Diabetes* **34**, 1055-1058.

Opie LH. Oxygen supply, coronary flow. In: Opie LH, ed. The Heart.Physiology, from Cell to Circulation 2nd ed. 277-300. 1991. New York, Raven Press.

Opie, L. H. (1998). *The Heart. Third edition ed. Philadelphia, New York: Lippincott-Raven Publishers*.

Orel, M., Lichnovska, R., Gwozdziewiczova, S., Zlamalova, N., Klementa, I., Merkunova, A., & Hrebicek, J. (2004). Gender differences in tumor necrosis factor alpha and leptin secretion from subcutaneous and visceral fat tissue. *Physiol Res.* **53**, 501-505.

Ornish, D., Brown, S. E., Scherwitz, L. W., Billings, J. H., Armstrong, W. T., Ports, T. A., McLanahan, S. M., Kirkeeide, R. L., Brand, R. J., & Gould, K. L. (1990). Can lifestyle changes reverse coronary heart disease? The Lifestyle Heart Trial. *Lancet* **336**, 129-133.

Ouwens, D. M., Boer, C., Fodor, M., de Galan, P., Heine, R. J., Maassen, J. A., & Diamant, M. (2005). Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. *Diabetologia* **48**, 1229-1237.

Padwal, R., Li, S. K., & Lau, D. C. (2003). Long-term pharmacotherapy for obesity and overweight. *Cochrane.Database.Syst.Rev.* CD004094.

Pascale, R. W., Wing, R. R., Butler, B. A., Mullen, M., & Bononi, P. (1995). Effects of a behavioral weight loss program stressing calorie restriction versus calorie plus fat restriction in obese individuals with NIDDM or a family history of diabetes. *Diabetes Care* **18**, 1241-1248.

Patlak, C. S. & Blasberg, R. G. (1985). Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. *J Cereb.Blood Flow Metab* **5**, 584-590.

Peltoniemi, P., Lönnroth, P., Laine, H., Oikonen, V., Tolvanen, T., Grönroos, T., Strindberg, L., Knuuti, J., & Nuutila, P. (2000). Lumped constant for [(18)F]fluorodeoxyglucose in skeletal muscles of obese and nonobese humans. *Am.J.Physiol Endocrinol.Metab* **279**, E1122-E1130.

Perseghin, G., Scifo, P., De Cobelli, F., Pagliato, E., Battezzati, A., Arcelloni, C., Vanzulli, A., Testolin, G., Pozza, G., Del Maschio, A., & Luzi, L. (1999). Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* **48**, 1600-1606.

Perusse, L., Chagnon, Y. C., Weisnagel, S. J., Rankinen, T., Snyder, E., Sands, J., & Bouchard, C. (2001). The human obesity gene map: the 2000 update. *Obes.Res.* **9**, 135-169.

Peterson, L. R., Herrero, P., Schechtman, K. B., Racette, S. B., Waggoner, A. D., Kisrieva-Ware, Z., Dence, C., Klein, S., Marsala, J., Meyer, T., & Gropler, R. J. (2004). Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. *Circulation* **109**, 2191-2196.

Pitkänen, O. P., Nuutila, P., Raitakari, O. T., Ronnemaa, T., Koskinen, P. J., Iida, H., Lehtimaki, T. J., Laine, H. K., Takala, T., Viikari, J. S., & Knuuti, J. (1998). Coronary flow reserve is reduced in young men with IDDM. *Diabetes* **47**, 248-254.

Popkin, B. M. (2002). The shift in stages of the nutrition transition in the developing world differs from past experiences! *Public Health Nutr.* **5**, 205-214.

Porte, D., Jr., Baskin, D. G., & Schwartz, M. W. (2002). Leptin and insulin action in the central nervous system. *Nutr.Rev.* **60**, S20-S29.

Pouliot, M. C., Despres, J. P., Lemieux, S., Moorjani, S., Bouchard, C., Tremblay, A., Nadeau, A., & Lupien, P. J. (1994). Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol.* **73**, 460-468.

Powell, E. E., Cooksley, W. G., Hanson, R., Searle, J., Halliday, J. W., & Powell, L. W. (1990). The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* **11**, 74-80.

Preiss-Landl, K., Zimmermann, R., Hammerle, G., & Zechner, R. (2002). Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. *Curr.Opin.Lipidol.* **13**, 471-481.

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson.Med.* **30**, 672-679.

Pyörälä, M., Miettinen, H., Laakso, M., & Pyörälä, K. (1998). Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men: the 22-year follow-up results of the Helsinki Policemen Study. *Circulation* **98**, 398-404.

Raitakari, M., Ilvonen, T., Ahotupa, M., Lehtimäki, T., Harmoinen, A., Suominen, P., Elo, J., Hartiala, J., & Raitakari, O. T. (2004). Weight reduction with very-low-caloric diet and endothelial function in overweight adults: role of plasma glucose. *Arterioscler.Thromb.Vasc.Biol* **24**, 124-128.

Randle, P. J., Garland, P. B., Hales, C. N., & Newsholme, E. A. (1963). The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1**, 785-789.

Ratib, O., Phelps, M. E., Huang, S. C., Henze, E., Selin, C. E., & Schelbert, H. R. (1982). Positron tomography with deoxyglucose for estimating local myocardial glucose metabolism. *J Nucl Med* **23**, 577-586.

Reaven, G. M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* **37**, 1595-1607.

Revicki, D. A. & Israel, R. G. (1986). Relationship between body mass indices and measures of body adiposity. *Am.J Public Health* **76**, 992-994.

Rinella, M. E., Elias, M. S., Smolak, R. R., Fu, T., Borensztajn, J., & Green, R. M. (2008). Mechanisms of hepatic steatosis in mice fed a lipogenic methionine choline-deficient diet. *J Lipid Res.* **49**, 1068-1076.

Roden, M., Stingl, H., Chandramouli, V., Schumann, W. C., Hofer, A., Landau, B. R., Nowotny, P., Waldhäusl, W., & Shulman, G. I.

(2000). Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. *Diabetes* **49**, 701-707.

Romanski, S. A., Nelson, R. M., & Jensen, M. D. (2000). Meal fatty acid uptake in adipose tissue: gender effects in nonobese humans. *Am.J.Physiol Endocrinol.Metab* **279**, E455-E462.

Rönnemaa, T., Koskenvuo, M., Marniemi, J., Koivunen, T., Sajantila, A., Rissanen, A., Kaitsaari, M., Bouchard, C., & Kaprio, J. (1997). Glucose metabolism in identical twins discordant for obesity. The critical role of visceral fat. *J Clin Endocrinol Metab* **82**, 383-387.

Ross, R., Freeman, J., Hudson, R., & Janssen, I. (2002). Abdominal obesity, muscle composition, and insulin resistance in premenopausal women. *J Clin Endocrinol Metab* **87**, 5044-5051.

Ross, R., Leger, L., Morris, D., de Guise, J., & Guardo, R. (1992). Quantification of adipose tissue by MRI: relationship with anthropometric variables. *J Appl.Physiol* **72**, 787-795.

Ross, R., Shaw, K. D., Martel, Y., de Guise, J., & Avruch, L. (1993). Adipose tissue distribution measured by magnetic resonance imaging in obese women. *Am J Clin Nutr.* **57**, 470-475.

Ruan, H. & Lodish, H. F. (2004). Regulation of insulin sensitivity by adipose tissue-derived hormones and inflammatory cytokines. *Curr.Opin.Lipidol.* **15**, 297-302.

Ruderman, N. B., Saha, A. K., Vavvas, D., & Witters, L. A. (1999). Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol* **276**, E1-E18.

Ryttig, K. R., Flaten, H., & Rossner, S. (1997). Long-term effects of a very low calorie diet (Nutrilett) in obesity treatment. A prospective, randomized, comparison between VLCD and a hypocaloric diet+behavior modification and their combination. *Int.J Obes.Relat Metab Disord.* **21**, 574-579.

Saddik, M. & Lopaschuk, G. D. (1991). Myocardial triglyceride turnover and contribution to energy substrate utilization in isolated working rat hearts. *J Biol Chem* **266**, 8162-8170.

Saha, G. B., MacIntyre, W. J., & Go, R. T. (1992). Cyclotrons and positron emission tomography radiopharmaceuticals for clinical imaging. *Semin.Nucl.Med.* **22**, 150-161.

Sanyal, A. J., Campbell-Sargent, C., Mirshahi, F., Rizzo, W. B., Contos, M. J., Sterling, R. K., Luketic, V. A., Shiffman, M. L., & Clore, J. N. (2001). Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* **120**, 1183-1192.

Sato, H., Terasaki, T., Mizuguchi, H., Okumura, K., & Tsuji, A. (1991). Receptor-recycling model of clearance and distribution of insulin in the perfused mouse liver. *Diabetologia* **34**, 613-621.

Schaap, F. G., van der Vusse, G. J., & Glatz, J. F. (1998). Fatty acid-binding proteins in the heart. *Mol Cell Biochem.* **180**, 43-51.

Schaffer, J. E. (2003). Lipotoxicity: when tissues overeat. *Curr.Opin.Lipidol.* **14**, 281-287.

Scherer, P. E., Williams, S., Fogliano, M., Baldini, G., & Lodish, H. F. (1995). A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol.Chem.* **270**, 26746-26749.

Schremmer, B. & Dhainaut, J. F. (1990). Regulation of myocardial oxygen delivery. *Intensive Care Med.* **16 Suppl 2**, S157-S163.

Schutz, Y., Kyle, U. U., & Pichard, C. (2002). Fat-free mass index and fat mass index percentiles in Caucasians aged 18-98 y. *Int.J Obes.Relat Metab Disord.* **26**, 953-960.

Seppälä-Lindroos, A., Vehkavaara, S., Häkkinen, A. M., Goto, T., Westerbacka, J., Sovijärvi, A., Halavaara, J., & Yki-Järvinen, H. (2002). Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J.Clin.Endocrinol.Metab* **87**, 3023-3028.

Shek, E. W., Brands, M. W., & Hall, J. E. (1998). Chronic leptin infusion increases arterial pressure. *Hypertension* **31**, 409-414.

Shirai, K. (2004). Obesity as the core of the metabolic syndrome and the management of coronary heart disease. *Curr.Med.Res.Opin.* **20**, 295-304.

Shulman, G. I., Barrett, E. J., & Sherwin, R. S. (1997). Integrated Fuel Metabolism. In *Ellenberg & Rifkin's Diabetes Mellitus*, eds. Porte, D. J. & Sherwin, R. S., pp. 1-17. Appleton & Lange, Stamford.

Sierra-Honigmann, M. R., Nath, A. K., Murakami, C., Garcia-Cardena, G., Papapetropoulos, A., Sessa, W. C., Madge, L. A., Schechner, J. S., Schwabb, M. B., Polverini, P. J., & Flores-

Riveros, J. R. (1998). Biological action of leptin as an angiogenic factor. *Science* **281**, 1683-1686.

Silventoinen, K., Jousilahti, P., Vartiainen, E., & Tuomilehto, J. (2003). Appropriateness of anthropometric obesity indicators in assessment of coronary heart disease risk among Finnish men and women. *Scand.J Public Health* **31**, 283-290.

Singh, R. B., Rastogi, S. S., Verma, R., Laxmi, B., Singh, R., Ghosh, S., & Niaz, M. A. (1992). Randomised controlled trial of cardioprotective diet in patients with recent acute myocardial infarction: results of one year follow up. *BMJ* **304**, 1015-1019.

Sjöström, C. D., Lissner, L., Wedel, H., & Sjöström, L. (1999). Reduction in incidence of diabetes, hypertension and lipid disturbances after intentional weight loss induced by bariatric surgery: the SOS Intervention Study. *Obes.Res.* **7**, 477-484.

Sjöström, L. (2000). Surgical intervention as a strategy for treatment of obesity. *Endocrine.* **13**, 213-230.

Sjöström, L. (2008). Bariatric surgery and reduction in morbidity and mortality: experiences from the SOS study. *Int.J Obes.(Lond)* **32 Suppl 7**, S93-S97.

Sjöström, L. & Björntorp, P. (1974). Body composition and adipose cellularity in human obesity. *Acta Med Scand.* **195**, 201-211.

Smith, T. P. & Canty, J. M. (1993). Modulation of coronary autoregulatory responses by nitric oxide. Evidence for flow-dependent resistance adjustments in conscious dogs. *Circ.Res* **73**, 232-240.

Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O., & Shinohara, M. (1977). The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem.* **28**, 897-916.

Stallknecht, B., Larsen, J. J., Mikines, K. J., Simonsen, L., Bulow, J., & Galbo, H. (2000). Effect of training on insulin sensitivity of glucose uptake and lipolysis in human adipose tissue. *Am.J.Physiol Endocrinol.Metab* **279**, E376-E385.

Stamler, R., Stamler, J., Gosch, F. C., Civinelli, J., Fishman, J., McKeever, P., McDonald, A., & Dyer, A. R. (1989). Primary prevention of hypertension by nutritional-hygienic means. Final report of a randomized, controlled trial. *JAMA* **262**, 1801-1807.

Stanley, W. C., Lopaschuk, G. D., Hall, J. L., & McCormack, J. G. (1997). Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions. *Cardiovasc Res* **33**, 243-257.

Stefanick, M. L., Mackey, S., Sheehan, M., Ellsworth, N., Haskell, W. L., & Wood, P. D. (1998). Effects of diet and exercise in men and postmenopausal women with low levels of HDL cholesterol and high levels of LDL cholesterol. *N.Engl.J Med.* **339**, 12-20.

Stevens, V. J., Obarzanek, E., Cook, N. R., Lee, I. M., Appel, L. J., Smith, W. D., Milas, N. C., Mattfeldt-Beman, M., Belden, L., Bragg, C., Millstone, M., Raczynski, J., Brewer, A., Singh, B., & Cohen, J. (2001). Long-term weight loss and changes in blood pressure: results of the Trials of Hypertension Prevention, phase II. *Ann.Intern.Med.* **134**, 1-11.

Stock, M. J. (1997). Sibutramine: a review of the pharmacology of a novel anti-obesity agent. *Int.J Obes.Relat Metab Disord.* **21 Suppl 1**, S25-S29.

Stolen, K. Q., Kemppainen, J., Kalliokoski, K. K., Luotolahti, M., Viljanen, T., Nuutila, P., & Knuuti, J. (2003). Exercise training improves insulin-stimulated myocardial glucose uptake in patients with dilated cardiomyopathy. *J.Nucl.Cardiol.* **10**, 447-455.

Summers, S. A. (2006). Ceramides in insulin resistance and lipotoxicity. *Prog.Lipid Res.* **45**, 42-72.

Sun, D., Nguyen, N., DeGrado, T. R., Schwaiger, M., & Brosius, F. C., III (1994). Ischemia induces translocation of the insulin-responsive glucose transporter GLUT4 to the plasma membrane of cardiac myocytes. *Circulation* **89**, 793-798.

Sundell, J., Laine, H., Luotolahti, M., Kalliokoski, K., Raitakari, O., Nuutila, P., & Knuuti, J. (2002). Obesity affects myocardial vasoreactivity and coronary flow response to insulin. *Obes.Res* **10**, 617-624.

Szczepaniak, L. S., Babcock, E. E., Schick, F., Dobbins, R. L., Garg, A., Burns, D. K., McGarry, J. D., & Stein, D. T. (1999). Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am.J.Physiol* **276**, E977-E989.

Taegtmeyer, H. (1994). Energy metabolism of the heart: from basic concepts to clinical applications. *Curr.Probl.Cardiol.* **19**, 59-113.

Takala, T. O., Nuutila, P., Pulkki, K., Oikonen, V., Grönroos, T., Savunen, T., Vähäsilta, T., Luotolahti, M., Kallajoki, M., Bergman, J., Forsback, S., & Knuuti, J. (2002). 14(R,S)-[18F]Fluoro-6-thia-heptadecanoic acid as a tracer of free fatty acid uptake and oxidation in myocardium and skeletal muscle. *Eur.J.Nucl.Med.Mol.Imaging* **29**, 1617-1622.

Tanko, L. B., Bagger, Y. Z., Qin, G., Alexandersen, P., Larsen, P. J., & Christiansen, C. (2005). Enlarged waist combined with elevated triglycerides is a strong predictor of accelerated atherogenesis and related cardiovascular mortality in postmenopausal women. *Circulation* **111**, 1883-1890.

Thomsen, C., Becker, U., Winkler, K., Christoffersen, P., Jensen, M., & Henriksen, O. (1994). Quantification of liver fat using magnetic resonance spectroscopy. *Magn Reson.Imaging* **12**, 487-495.

Troiano, R. P., Frongillo, E. A., Jr., Sobal, J., & Levitsky, D. A. (1996). The relationship between body weight and mortality: a quantitative analysis of combined information from existing studies. *Int.J Obes.Relat Metab Disord.* **20**, 63-75.

Tschritter, O., Fritsche, A., Thamer, C., Haap, M., Shirkavand, F., Rahe, S., Staiger, H., Maerker, E., Haring, H., & Stumvoll, M. (2003). Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes* **52**, 239-243.

Tuomilehto, J., Lindström, J., Eriksson, J. G., Valle, T. T., Hämäläinen, H., Ilanne-Parikka, P., Keinänen-Kiukaanniemi, S., Laakso, M., Louheranta, A., Rastas, M., Salminen, V., & Uusitupa, M. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N.Engl.J Med* **344**, 1343-1350.

Turpeinen, A. K., Takala, T. O., Nuutila, P., Axelin, T., Luotolahti, M., Haaparanta, M., Bergman, J., Hamalainen, H., Iida, H., Maki, M., Uusitupa, M. I., & Knuuti, J. (1999). Impaired free fatty acid uptake in skeletal muscle but not in myocardium in patients with impaired glucose tolerance: studies with PET and 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid. *Diabetes* **48**, 1245-1250.

Unger, R. H. (2002). Lipotoxic diseases. *Annu.Rev.Med.* **53**, 319-336.

Unger, R. H. (2003). Minireview: weapons of lean body mass destruction: the role of ectopic lipids in the metabolic syndrome. *Endocrinology* **144**, 5159-5165.

Uren, N. G., Melin, J. A., de Bruyne, B., Wijns, W., Baudhuin, T., & Camici, P. G. (1994). Relation between myocardial blood flow and the severity of coronary-artery stenosis. *N.Engl.J Med* **330**, 1782-1788.

Utriainen, T., Takala, T., Luotolahti, M., Rönnemaa, T., Laine, H., Ruotsalainen, U., Haaparanta, M., Nuutila, P., & Yki-Järvinen, H. (1998). Insulin resistance characterizes glucose uptake in skeletal muscle but not in the heart in NIDDM. *Diabetologia* **41**, 555-559.

Utzschneider, K. M. & Kahn, S. E. (2006). The Role of Insulin Resistance in Non-alcoholic Fatty Liver Disease. *J.Clin.Endocrinol.Metab*.

Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Kales, A., Tyson, K., & Chrousos, G. P. (1997). Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab* **82**, 1313-1316.

Viljanen, A. P., Virtanen, K. A., Järvisalo, M. J., Hällsten, K., Parkkola, R., Rönnemaa, T., Lönnqvist, F., Iozzo, P., Ferrannini, E., & Nuutila, P. (2005). Rosiglitazone treatment increases subcutaneous adipose tissue glucose uptake in parallel with perfusion in patients with type 2 diabetes: a double-blind, randomized study with metformin. *J Clin.Endocrinol.Metab* **90**, 6523-6528.

Virtanen, K. A., Iozzo, P., Hällsten, K., Huupponen, R., Parkkola, R., Janatuinen, T., Lönnqvist, F., Viljanen, T., Rönnemaa, T., Lönnroth, P., Knuuti, J., Ferrannini, E., & Nuutila, P. (2005). Increased fat mass compensates for insulin resistance in abdominal obesity and type 2 diabetes: a positron-emitting tomography study. *Diabetes* **54**, 2720-2726.

Virtanen, K. A., Lönnroth, P., Parkkola, R., Peltoniemi, P., Asola, M., Viljanen, T., Tolvanen, T., Knuuti, J., Rönnemaa, T., Huupponen, R., & Nuutila, P. (2002). Glucose Uptake and Perfusion in Subcutaneous and Visceral Adipose Tissue during Insulin Stimulation in Nonobese and Obese Humans. *J Clin Endocrinol Metab* **87**, 3902-3910.

Virtanen, K. A., Peltoniemi, P., Marjamäki, P., Asola, M., Strindberg, L., Parkkola, R., Huupponen, R., Knuuti, J., Lönnroth, P., & Nuutila, P. (2001). Human adipose tissue glucose uptake determined using [^{18}F]-fluoro-

deoxy-glucose ([^{18}F]FDG) and PET in combination with microdialysis. *Diabetologia* **44**, 2171-2179.

Vozarova, B., Weyer, C., Hanson, K., Tataranni, P. A., Bogardus, C., & Pratley, R. E. (2001). Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes.Res.* **9**, 414-417.

Wanless, I. R. & Lentz, J. S. (1990). Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* **12**, 1106-1110.

Wannamethee, S. G. & Shaper, A. G. (1999). Weight change and duration of overweight and obesity in the incidence of type 2 diabetes. *Diabetes Care* **22**, 1266-1272.

Wannamethee, S. G., Shaper, A. G., & Walker, M. (1998). Changes in physical activity, mortality, and incidence of coronary heart disease in older men. *Lancet* **351**, 1603-1608.

West, D. B., Prinz, W. A., & Greenwood, M. R. (1989). Regional changes in adipose tissue blood flow and metabolism in rats after a meal. *Am J Physiol* **257**, R711-R716.

Westerbacka, J., Corner, A., Tiikkainen, M., Tamminen, M., Vehkavaara, S., Häkkinen, A. M., Fredriksson, J., & Yki-Järvinen, H. (2004). Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia* **47**, 1360-1369.

Westerbacka, J., Kolak, M., Kiviluoto, T., Arkkila, P., Siren, J., Hamsten, A., Fisher, R. M., & Yki-Järvinen, H. (2007). Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes* **56**, 2759-2765.

Westerterp-Plantenga, M. S., Saris, W. H., Hukshorn, C. J., & Campfield, L. A. (2001). Effects of weekly administration of pegylated recombinant human OB protein on appetite profile and energy metabolism in obese men. *Am.J Clin.Nutr.* **74**, 426-434.

Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R. E., & Tataranni, P. A. (2001). Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin.Endocrinol.Metab* **86**, 1930-1935.

Whalley, G. A., Doughty, R. N., Gamble, G. D., Oxenham, H. C., Walsh, H. J., Reid, I. R., & Baldi, J. C. (2004). Association of fat-free mass and training status with left ventricular size and mass in endurance-trained athletes. *J Am Coll Cardiol* **44**, 892-896.

Whalley, G. A., Gamble, G. D., Doughty, R. N., Culpan, A., Plank, L., MacMahon, S., & Sharpe, N. (1999). Left ventricular mass correlates with fat-free mass but not fat mass in adults. *J Hypertens.* **17**, 569-574.

Williamson, D. F., Pamuk, E., Thun, M., Flanders, D., Byers, T., & Heath, C. (1995). Prospective study of intentional weight loss and mortality in never-smoking overweight US white women aged 40-64 years. *Am.J Epidemiol.* **141**, 1128-1141.

Williamson, D. F., Thompson, T. J., Thun, M., Flanders, D., Pamuk, E., & Byers, T. (2000). Intentional weight loss and mortality among overweight individuals with diabetes. *Diabetes Care* **23**, 1499-1504.

Williamson, J. R., Kreisberg, R. A., & Felts, P. W. (1966). Mechanism for the stimulation of gluconeogenesis by fatty acids in perfused rat liver. *Proc.Natl.Acad.Sci.U.S.A* **56**, 247-254.

Wilson, J. E. (2003). Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. *J Exp.Biol.* **206**, 2049-2057.

Wilson, P. W., D'Agostino, R. B., Sullivan, L., Parise, H., & Kannel, W. B. (2002). Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch.Intern.Med.* **162**, 1867-1872.

Wing, R. R., Blair, E., Marcus, M., Epstein, L. H., & Harvey, J. (1994a). Year-long weight loss treatment for obese patients with type II diabetes: does including an intermittent very-low-calorie diet improve outcome? *Am.J Med.* **97**, 354-362.

Wing, R. R., Blair, E. H., Bononi, P., Marcus, M. D., Watanabe, R., & Bergman, R. N. (1994b). Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care* **17**, 30-36.

Wing, R. R., Marcus, M. D., Salata, R., Epstein, L. H., Miaskiewicz, S., & Blair, E. H. (1991). Effects of a very-low-calorie diet on long-term glycemic control in obese type 2 diabetic subjects. *Arch.Intern.Med.* **151**, 1334-1340.

Wisneski, J. A., Gertz, E. W., Neese, R. A., Gruenke, L. D., Morris, D. L., & Craig, J. C. (1985). Metabolic fate of extracted glucose in normal human myocardium. *J Clin.Invest* **76**, 1819-1827.

Wisneski, J. A., Gertz, E. W., Neese, R. A., & Mayr, M. (1987). Myocardial metabolism of free fatty acids. Studies with 14C-labeled substrates in humans. *J Clin.Invest* **79**, 359-366.

Wood, P. D., Stefanick, M. L., Williams, P. T., & Haskell, W. L. (1991). The effects on plasma lipoproteins of a prudent weight-reducing diet, with or without exercise, in overweight men and women. *N.Engl.J Med.* **325**, 461-466.

World Health Organisation. Obesity: Preventing and managing the global epidemic. WHO Technical Report Series; 894:1-253. 2000.

World Health Organisation. Life course perspectives on coronary heart disease, stroke and diabetes: key issues and implications for policy and research. Genova, World Health Organisation, (WHO/NMH/NPH/01.4). 2001.

Xu, A., Wang, Y., Keshaw, H., Xu, L. Y., Lam, K. S., & Cooper, G. J. (2003). The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin.Invest* **112**, 91-100.

Yagyu, H., Chen, G., Yokoyama, M., Hirata, K., Augustus, A., Kako, Y., Seo, T., Hu, Y., Lutz, E. P., Merkel, M., Bensadoun, A., Homma, S., & Goldberg, I. J. (2003). Lipoprotein lipase (LpL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. *J Clin.Invest* **111**, 419-426.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., Tobe, K., Nagai, R., Kimura, S., Tomita, M., Froguel, P., & Kadowaki, T. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat.Med.* **7**, 941-946.

Yeh, M. M. & Brunt, E. M. (2007). Pathology of nonalcoholic fatty liver disease. *Am.J Clin.Pathol.* **128**, 837-847.

Yoshii, H., Lam, T. K., Gupta, N., Goh, T., Haber, C. A., Uchino, H., Kim, T. T., Chong, V. Z., Shah, K., Fantus, I. G., Mari, A., Kawamori, R., & Giacca, A. (2006). Effects of portal free fatty acid elevation on insulin clearance and hepatic glucose flux. *Am.J.Physiol Endocrinol.Metab* **290**, E1089-E1097.

Yu-Poth, S., Zhao, G., Etherton, T., Naglak, M., Jonnalagadda, S., & Kris-Etherton, P. M. (1999). Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. *Am.J Clin.Nutr.* **69**, 632-646.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425-432.

Zhi, J., Melia, A. T., Guerciolini, R., Chung, J., Kinberg, J., Hauptman, J. B., & Patel, I. H. (1994). Retrospective population-based analysis of the dose-response (fecal fat excretion) relationship of orlistat in normal and obese volunteers. *Clin.Pharmacol.Ther.* **56**, 82-85.

Zhou, Y. T., Grayburn, P., Karim, A., Shimabukuro, M., Higa, M., Baetens, D., Orci, L., & Unger, R. H. (2000). Lipotoxic heart disease in obese rats: implications for human obesity. *Proc.Natl.Acad.Sci.U.S.A* **97**, 1784-1789.

Zhu, S., Wang, Z., Heshka, S., Heo, M., Faith, M. S., & Heymsfield, S. B. (2002). Waist circumference and obesity-associated risk factors among whites in the third National Health and Nutrition Examination Survey: clinical action thresholds. *Am J Clin.Nutr.* **76**, 743-749.

Zimmet, P., Magliano, D., Matsuzawa, Y., Alberti, G., & Shaw, J. (2005). The metabolic syndrome: a global public health problem and a new definition. *J Atheroscler.Thromb.* **12**, 295-300.