Effects of nutrients and exercise on skeletal muscle and adipose tissue

Merethe Helene Rokling-Andersen

Dissertation for the degree of Philosophiae Doctor (Ph.D.)

Department of Nutrition
Institute of Basic Medical Sciences
Faculty of Medicine
University of Oslo
Norway

2009

© Merethe Helene Rokling-Andersen, 2009

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo
No. 855

ISBN 978-82-8072-370-3

All rights reserved. No part of this publication may be
reproduced or transmitted, in any form or by any means, without permission.

Cover: Inger Sandved Anfinsen.
Printed in Norway: AiT e-dit AS, Oslo, 2009.

Produced in co-operation with Unipub AS.
The thesis is produced by Unipub AS merely in connection with the
thesis defence. Kindly direct all inquiries regarding the thesis to the copyright
holder or the unit which grants the doctorate.

Unipub AS is owned by
The University Foundation for Student Life (SiO)

This work has been financially supported by the
Norwegian Health Association (Norwegian Council on Cardiovascular Diseases)

CONTENTS

Acknowledgements 2

List of original papers included 3

Abbreviations 4

Introduction 6

1. Overweight and obesity 6
2. Insulin resistance 7
3. Adipose tissue 8
   - 3.1 White adipose tissue 8
   - 3.2 Brown adipose tissue 10
   - 3.2 Depot variations 11
4. Adipocytes 13
5. Skeletal muscle cells 14
6. Adipokines 15
   - 6.1 Leptin 16
   - 6.2 Adiponectin 17
   - 6.3 Inflammatory adipokines 21
7. Lipids 21
   - 7.1 Fatty acids 21
   - 7.2 Marine n-3 fatty acids 23
   - 7.3 Tetradecylthioacetic acid 26
8. Lipid metabolism 27
   - 8.1 Hepatic lipogenesis and β-oxidation 27
   - 8.2 Triacylglycerol storage and lipolysis in adipose tissue 28
9. The Oslo Diet and Exercise Study (ODES) 29

Aims of present studies 31

Summary of papers 32

Discussion 35

1. Methodological considerations 35
2. Fatty acids and insulin sensitivity 37
3. Adipose tissue 37
4. Adipokines 38
5. Concluding remarks 39
6. Future prospects 40

References 41

Papers I-IV

ACKNOWLEDGEMENTS

The present work was carried out at the Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo. The primary financial support was a doctoral fellowship from the Norwegian Health Association (Norwegian Council on Cardiovascular Diseases).

I am truly indebted to my supervisor, Christian A. Drevon, for teaching me so much about science, medicine and nutrition. His immense knowledge and understanding constantly impress me. I am grateful to him for always keeping an open door, and for his encouragement and support throughout the years.

Thanks to Fred Haugen for all of our interesting talks, and for his encouragement and enthusiasm. I am also very thankful to Arild C. Rustan and Andreas J. Wensaas for excellent collaboration, and their positive involvement. Thanks to Anne Randi Enget for her help in the lab. She is always so supportive and encouraging, and it has been great working with her.

I want to express my gratitude to Marit B. Veierød and David R. Jacobs Jr. for their scholastic and patient statistical guidance. I also greatly acknowledge my other co-authors for their contributions.

Thanks are due to the rest of the Drevon-group, former and present office-mates and all others at the Department of Nutrition for creating a friendly and stimulating workplace. Thanks to my colleagues and friends Karianne, Ingrid, Anne Marte, Trude, Anja and Amrit for your support. Special thanks to Susanne Weedon-Fekjær, I truly value our friendship.

I am especially grateful to my parents for always believing in me and supporting me. Also thanks to the rest of my family and other friends for being there for me.

Thanks to my two remarkable children, Magnus and Kristiane, for filling my life with so much joy, laughter and love. Last but not least, thanks to my husband Henning. Thank you for being my biggest fan and best friend, and for all your love and support. I could not have done this without you.

Oslo, June 2009

Merethe H. Rokling-Andersen

LIST OF ORIGINAL PAPERS INCLUDED

This thesis is based on the following papers:

Paper I

Aas V, Rokling-Andersen MH, Kase ET, Thoresen H and Rustan AC. *Eicosapentaenoic acid (20:5 n-3) increases fatty acid and glucose uptake in cultured human skeletal muscle cells.* J Lipid Res. 2006 Feb;47(2):366-74.

Paper II

Rokling-Andersen MH, Rustan AC, Wensaas AJ, Kaalhus O, Wergedahl H, Røst TH, Jensen J, Graff BA, Caesar R, Drevon CA. *Marine n-3 fatty acids promote size-reduction of visceral adipose depots, without altering body weight and composition, in male Wistar rats fed a high-fat diet*. Br J Nutr. 2009 Apr 28; 1-12. (Epub ahead of print).

Paper III

Wensaas AJ, Rustan AC, Rokling-Andersen MH, Caesar R, Jensen J, Kaalhus O, Graff BA, Gudbrandsen OA, Berge RK, Drevon CA. *Dietary supplementation of tetradecylthioacetic acid increases feed intake but reduces body weight gain and adipose depot sizes in rats fed high-fat diets.* Diabetes Obes Metab. 2009, in press.

Paper IV

Rokling-Andersen MH, Reseland JE, Veierød MB, Anderssen SA, Jacobs DR Jr, Urdal P, Jansson JO, Drevon CA. *Effects of long-term exercise and diet intervention on plasma adipokine concentrations*. Am J Clin Nutr. 2007 Nov;86(5):1293-301.

ABBREVIATIONS

ACC	Acetyl-CoA carboxylase
ACO	Acyl-CoA oxidase
ACS	Acyl-CoA synthetase
AdipoR	Adiponectin receptor
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
AN	Adiponectin
ATGL	Adipose triglyceride lipase
BAT	Brown adipose tissue
BBB	Blood brain barrier
BMI	Body mass index
CoA	Coenzyme A
CPT	Carnitine palmitoyl transferase
CSF	Cerebrospinal fluid
DAG	Diacylglycerol
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acids
FFA	Free fatty acids
FAS	Fatty acid synthase
FFA	Free fatty acids
HDL	High-density lipoprotein
HMW	High molecular weight
HSL	Hormone sensitive lipase
ICV	Intracerebroventricular
IL	Interleukin
IMTG	Intramyocellular TAG
LC-PUFA	Long chain PUFAs
LCFA-CoA	Long chain fatty acyl-CoA
LPL	Lipoprotein lipase
MS	Metabolic syndrome

MUFA	Monounsaturated fatty acid
NEFA	Non-esterified fatty acid
OA	Oleic acid
ODES	Oslo Diet and Exercise Study
PAI	Plasminogen activator inhibitor
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acid
SCD	Stearoyl-CoA-desaturase
SFA	Saturated fatty acid
TAG	Triacylglycerol
TNF	Tumour necrosis factor
TTA	Tetradecylthioacetic acid
TZD	Thiazolidinedione
UCP	Uncoupling protein
VEGF	Vascular endothelial growth factor
VLC	Very long chain
VLDL	Very low density lipoprotein
WAT	White adipose tissue

INTRODUCTION

1. OVERWEIGHT AND OBESITY

The World Health Organisation (WHO) defines overweight and obesity as "abnormal or excessive fat accumulation that presents a risk to health" (1). In 2005, WHO projections indicated that globally there were approximately 1.6 billion overweight adults (age 15+) and at least 400 million obese adults. Obesity is an increasing health problem not only in Western industrialized nations but also in low- and middle-income countries, particularly in urban settings. Recent data from the Nord-Trøndelag Health Study (HUNT3) in Norway showed that more than 20 % of the participants were obese; this represented a three-fold increase for women and a doubling of the numbers for men since the 1980s (unpublished). The increase was highest in the younger age categories.

Overweight and obesity are major risk factors for a number of diseases, including type 2 diabetes, cardiovascular diseases, gall bladder disease and osteoarthritis. Also, according to the World Cancer Research Fund/American Institute of Cancer Research's Second Expert Report from 2007 there are convincing data indicating that obesity is a risk factor for development of cancer in the oesophagus, pancreas, colon, rectum, endometrium and kidneys, as well as for postmenopausal breast cancer (2).

According to the "thrifty gene hypothesis" proposed by Neel in 1962, we are evolutionarily programmed to maximize energy storage, so that these reserves can be tapped during times of food shortage (3). In an affluent society, an imbalance of the intake and consumption of energy often occurs and is probably the most common cause of overweight and obesity. Treatment of overweight and obesity largely relies on lifestyle intervention in the form of reduced energy consumption and increased physical activity. However, according to Wing and Phelan only approximately 20% of overweight individuals are successful at maintaining a 10% weight loss over one year (4), making prevention an important issue.

The pharmaceutical industry searches intently for pharmacological agents for the treatment of obesity. There are only two drugs on the Norwegian market today but both have limited effect and possibly harmful side effects. Orlistat (Xenical™ and Alli™) inhibits gastrointestinal lipases, thus reducing the uptake of fats from the diet. Sibutramin (Reductil™) is a selective serotonin-norepinephrine reuptake inhibitor and works by suppressing of appetite. Rimonabant (Acomplia™) works by selectively blocking the cannabinoid receptor 1 but was recently suspended from the European market due to serious

psychiatric side effects. Under development for the treatment of obesity and/or metabolic syndrome (MS) are dual peroxisome proliferator-activated receptors- (PPAR-) δ/γ agonists and pan-PPAR (α/δ/γ) agonists (5). Other future mechanisms of actions may involve targeting adenosine monophosphate- (AMP-) activated protein kinase (AMPK) or uncoupling proteins (UCP), inhibiting lipogenesis, regulating appetite, activating adiponectin receptors (6) or increasing the activity of brown adipose tissue (BAT).

2. INSULIN RESISTANCE

There is a close association between obesity and insulin resistance. Together with increased waist circumference, high blood pressure, elevated plasma triacylglycerol (TAG) and reduced high-density lipoprotein (HDL) cholesterol, these signs comprise the MS, which is associated with an increased risk of cardiovascular disease. Resistance to the actions of insulin is compensated by an increased secretion of insulin by the pancreatic beta-cells (hyperinsulinemia), and may lead to glucose intolerance and eventually to type 2 diabetes mellitus. Type 2 diabetes mellitus is on the rise in all age groups of the population and also among adolescents. Insulin resistance and diabetes lead to harmful alternations in plasma lipids, and are strong, independent risk factors for the development of cardiovascular diseases.

The mechanisms underlying insulin resistance are complex and not yet fully understood. Skeletal muscle, adipose tissue and the liver are the organs most affected by insulin resistance. Several theories for skeletal muscle insulin resistance have been proposed, including substrate competition between glucose and fatty acids, and a connection between intramyocellular TAG (IMTG) accumulation, insulin signalling and muscle oxidative capacity as reviewed elsewhere (7). There have been several reports on the correlation between IMTG content, insulin resistance and insulin signalling. However, this theory is in contradiction with the observation that well trained athletes have high IMTG and are insulin sensitive (8). Recent evidence does not suggest that IMTG itself causes insulin resistance but rather that lipid metabolites such as long-chain fatty acyl-CoA (LCFA-CoA), diacylglycerol (DAG) and ceramide are active players (7).

"Metabolic flexibility" is a new term that connotes the ability of skeletal muscle to switch between oxidation of lipids and glucose, burning lipids during fasting and glucose in insulin-stimulated conditions (9). Reduced metabolic flexibility has been associated with increased accumulation of intramyocellular TAG and insulin resistance, although the importance of this is controversial (9;10).

3. ADIPOSE TISSUE

Adipose tissue is a vital organ consisting of two main tissue types – white adipose tissue (WAT) and brown adipose tissue (BAT) - which collaborate in energy partitioning towards storage or thermogenesis, respectively.

3.1 White adipose tissue

WAT is the most flexible organ with regard to hyperplastic and hypertrophic expansion (11), and has several other functions in addition to the storage of excess energy (table 3.1).

Table 3.1 *Major functions of white adipose tissue.*

Major functions of white adipose tissue
Storage of energy in the form of triacylglycerol
Insulation (thermal, mechanical and electrical)
Depot of cholesterol, vitamins D and E
Synthesis and secretion of adipokines
Release of free fatty acids
Provide essential fatty acids
Source of metabolic water

In mammals, WAT is organized in distinct depots throughout the body (table 3.2), and also diffusely around or within organs. Adipocytes may also “infiltrate” organs such as the skin, skeletal muscles, liver, synovia, parathyroid and parotid glands, lymph nodes, bone marrow, pancreas and thymus (12).

Table 3.2 *Main white adipose tissue depots (12).*

Main white adipose tissue depots
Subcutaneous adipose tissue
Abdominal subcutaneous adipose tissue
Gluteo-femoral subcutaneous adipose tissue
Mammary subcutaneous adipose tissue
In rodents: anterior and posterior subcutaneous tissue
Visceral adipose tissue
Mediastinal
Mesenteric
Omental
Perirenal
Retroperitoneal
Perigonadal

White adipose tissue includes several cell types besides white adipocytes (table 3.3), all of which have different functions. Obesity is associated with increased infiltration of immune cells, particularly macrophages leading to local and systemic low-grade inflammation, contributing to the development of insulin resistance (13-16). Cinti *et al.* have shown in obese mice and humans that more than 90% of all macrophages in WAT are localized around dead adipocytes, suggesting that the scavenging of adipocyte remnants is an important function for WAT macrophages in obese individuals (13). One interesting theory that has received considerable focus in recent years suggests that with the expansion of adipose tissue comes the need for increased vascularisation and remodelling of the tissue (17;18). Hypoxic conditions may emerge if these processes lag, leading to the activation of the transcription factor hypoxia-inducible factor- (HIF-) 1 and to the induction of several genes involved in angiogenesis, inflammation and cellular stress.

Table 3.3 *Cell types in white adipose tissue and their functions (12;19;20).*

Cell type	Function in adipose tissue
Adipocytes	Main lipid-storing cells
Preadipocytes	Precursors of brown and white adipocytes
Macrophages	Scavengers of dead adipocyte remnants
Monocytes	Precursors of macrophages and dendritic cells Phagocyte tissue debris
Dendritic cells	Contribute to and/or reflect local inflammation Fight local infections
Mast cells	Unknown significance in white adipose tissue
Fibroblasts	Constitutive cells of the connective tissue Equivalency with preadipocytes and fibroblasts?
Nerve cells	Innervation of adipose tissue
Vascular endothelial cells	Line the inner walls of the capillaries Secrete factors that promote preadipocyte proliferation

3.2 Brown adipose tissue

The main functions of BAT are the generation of heat to maintain body temperature, arousal of hibernation and dissipation of excess food energy. Brown adipocytes are characterised by the presence of uncoupling protein 1 (UCP1), which uncouples the mitochondrial respiratory chain of brown adipocytes. The brown appearance is due to the high number of mitochondria containing enzymes with dark metals. BAT is most prominently found in the interscapular depot in rodents, and also in the perirenal and axillary depots. Human newborns have a substantial amount of BAT, enabling their maintenance of body temperature without shivering; however the amount gradually decreases during the first year of living. It has been a long-standing dogma that adult humans have little or no brown adipose tissue. However, advances in nuclear medicine have revealed that BAT is in fact present and

active in a significant proportion of the adult man (21). BAT can be found primarily in two depots in the neck and supraclavicular region and also in the mediastinum (para-aortic), paravertebral and suprarenal regions (21-23). Cypess *et al.* recently reported that females have more BAT than males, and that the amount of BAT correlates negatively with body mass index (BMI), especially in elderly people (22). Also, it has been reported that the activity of BAT in response to cold is reduced in obese or overweight men (23). Thus, it is of interest to explore the potential of certain types of food to activate BAT, and to consider it a potential drug target in the treatment of obesity.

Two homologues to UCP1 have been described - UCP2 and UCP3 - and their roles are not fully understood. There is a general consensus that the primary functions of UCP2 and UCP3 are not related to thermogenesis. Knock-out mice for UCP2 and UCP3 have normal responses to cold exposure and are not obese (24). However, there is also evidence that *in vivo* activation of UCP3 by physiological activators or pharmacological intervention might have the capacity to be significantly thermogenic as reviewed elsewhere (24). Other roles that have been proposed for UCP2 and UCP3 are attenuation of reactive oxygen species (ROS) production and protection against oxidative damage. UCP2 expression has a signalling role in pancreatic beta-cells and is involved in attenuation of insulin secretion (24;25).

3.3 Depot variations

Adipose tissue is a heterogeneous metabolic organ and there are several biological differences between the different adipose depots. Adipose tissue located viscerally is associated with increased risk of cardiovascular disease, hypertension and type 2 diabetes (26). However, increased hip circumference, mostly reflecting subcutaneous fat storage, has been associated with lower risk of myocardial infarction in the INTERHEART study (27;28). Visceral depots drain directly into the portal vein (29). This increases the exposure of free fatty acids (FFAs), as well as adipokines to the liver, which may lead to increased hepatic glucose production and very low density lipoprotein (VLDL) secretion (30;31). However, the idea that insulin resistance is partly caused by high rates of non-esterified FAs (NEFAs) release from visceral adipose tissue (the "portal theory") is controversial (32).

Visceral depots are more innervated and hence more sensitive to catecholamine-induced lipolysis, and less sensitive to insulin (33). There are several differences between adipose depots concerning steroid metabolism. Higher expressions of androgen (34) and glucocorticoid receptors (35) in visceral than subcutaneous adipocytes have been shown. Bujalska *et al.* (36) have reported that adipose stromal cells from omental fat but not

subcutaneous fat express 11β-hydroxysteroid dehydrogenase, which converts cortisone to the more potent hydrocortisone (cortisol). However, in papers II and III, we found the lowest expressions of *Hsd11b2* in the mesenteric and interscapular adipose depots. Differences in lipolytic, anti-lipolytic and biochemical pathways between subcutaneous and visceral adipocytes are reviewed in (37).

With regard to adipokine production, here also are several differences between the different adipose depots. There is an increased production of interleukin- (IL-) 6, IL-8, vascular endothelial growth factor (VEGF), resistin and plasminogen activator inhibitor 1 (PAI-1) in visceral adipose tissue as compared to subcutaneous adipose tissue (38-40). Fain (38) found no significant differences in the release of leptin from human subcutaneous and visceral adipose tissue explants. This was contrary to the previous reports from Russel *et al.* (41) and Van Harmelen *et al.* (42) who found higher leptin mRNA expression and secretion from subcutaneous fat as compared to visceral fat. Drolet *et al.* (43) observed no significant differences between the subcutaneous and omental release of adiponectin, contrary to Motoshima *et al.* (44) and Fain (38). However, they reported that omental (but not subcutaneous) adipocyte adiponectin release was reduced in overweight women as compared to lean women. Some authors found significantly lower adiponectin mRNA in visceral adipose tissue as compared to subcutaneous adipose tissue (45;46), whereas others reported no differences (47).

White adipose tissue is primarily innervated by fibres from the sympathetic nervous system (48). Interestingly, Kreier and co-workers have shown selective parasympathetic innervation of subcutaneous and intra-abdominal adipose depots in rats, enhancing lipogenesis and anabolically modulating insulin sensitivity and glucose metabolism (49).

The regional differences in adipose tissue function are caused by both intrinsic characteristics of the adipocytes or other cell types, and extrinsic, environmental factors. In a study by Tran *et al.*, subcutaneous and visceral adipose tissues were transplanted into the subcutaneous and visceral adipose depots of mice in a 2x2 design (50). Transplantation of subcutaneous fat into a visceral depot improved insulin sensitivity and lowered plasma glucose and insulin levels, demonstrating that intrinsic adipocyte factors may be central.

Sex hormones play a clear role in determining the adipose tissue distribution, with testosterone leading to accumulation of visceral fat and estrogen promoting storage of subcutaneous fat in the gluteo-femoral area. High levels of growth hormone and insulin also promote visceral fat deposition. We show in paper II of this thesis that the replacement of lard with marine n-3 FAs in the diets of rats fed a high-fat diet causes a redistribution of adipose

tissue away from the visceral depots. The PPARγ agonist drugs redistribute fat to the subcutaneous depot and away from the visceral depots (51-54), whereas glucocorticoid drugs (as well as high levels of endogenous glucocorticoids) promote visceral fat accumulation (55).

4. ADIPOCYTES

Adipocytes originate from mesenchymal stem cells, as do chondrocytes, myocytes, and osteoblasts. Lipid-filled white adipocytes are spherical cells with a diameter ranging from 15 to 150 μg and can typically store 1 μg of TAG. The unilocular lipid droplet can fill approximately 90% of the cell volume, pushing the nucleus and other organelles against the cell membrane. Brown adipocytes are rich in mitochondria and have multilocular lipid droplets.

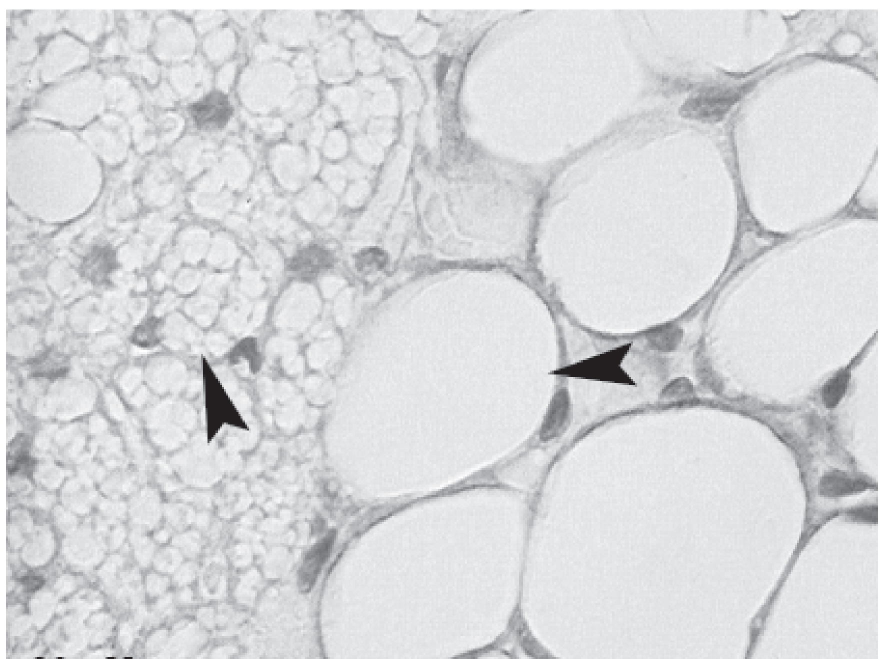
Adipogenesis, the formation of new adipocytes, is a highly regulated, multistep process. Three main classes of transcriptional regulators are central in the differentiation process: the PPARs, the CCAAT/enhancer-binding proteins (C/EBPs) and the sterol regulatory element-binding proteins (SREBPs).

There are three PPARs; PPARα, β and γ. PPARγ is a master regulator in adipocyte differentiation. Polyunsaturated fatty acids (PUFAs) are activators of PPARγ (see chapter 7). According to the "lipotoxicity hypothesis", insulin resistance develops when lipids are stored in organs other than adipose tissue, such as the skeletal muscles, liver and pancreas. This ectopic fat storage may be due to the high release of NEFAs from hypertrophic adipocytes. White adipocytes can be regarded as a lipid sink, storing esterified fatty acids in an inert manner, thereby preventing ectopic fat storage. Thus, adequate recruitment and differentiation of preadipocytes is important.

Impaired preadipocyte differentiation has been associated with abdominal obesity (56;57). Isakson *et al.* (58) recently reported that the differentiation capability correlates negatively with BMI. This could be due to fewer preadipocytes, or to an inability of the preadipocytes to differentiate into hypertrophic adipocytes. Also, Isakson *et al.* (58) found that TNFα prevented the differentiation of preadipocytes to adipocytes. Lacasa *et al.* have previously reported that TNFα released from macrophages promoted a partial trans-differentiation of the preadipocytes to assume a macrophage-like phenotype (59).

Trans-differentiation of white adipocytes to brown adipocytes, as well as to secretory epithelial cells in the mammary gland cells has been described in mice (60;61).

Figure 4.1 *Brown adipocytes (left): rich in mitochondria and with multilocular lipid droplets. White adipocytes (right): with one large unilocular lipid droplet. The nuclei are the dark round structures. Picture from "The Adipose Organ" by Cinti (62).*

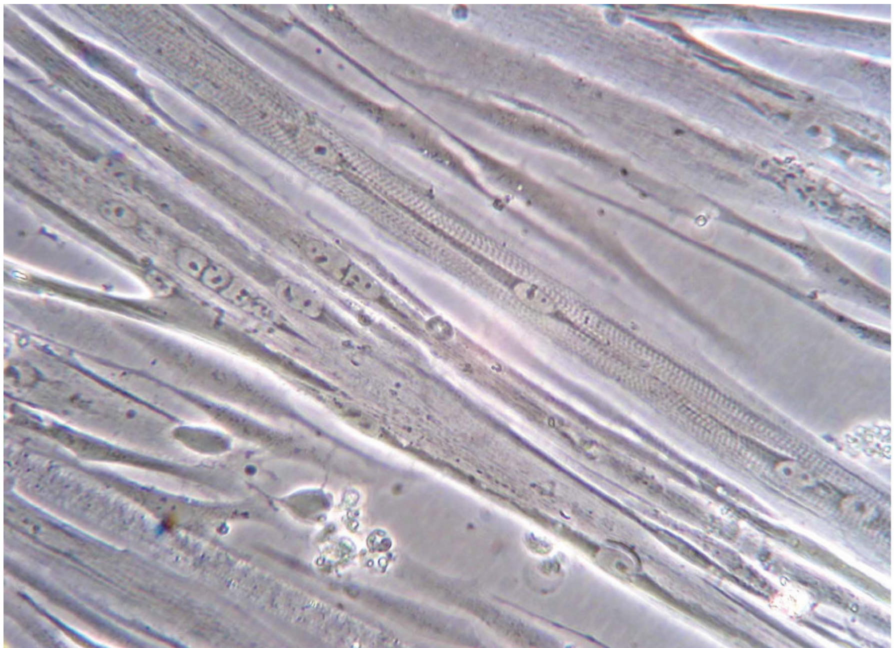


5. SKELETAL MUSCLE CELLS

Skeletal muscle is the major site of insulin-stimulated glucose uptake in the body. It accounts for approximately 70-80% of insulin-stimulated glucose disposal, and is therefore an important site for regulation of insulin sensitivity.

In paper I, an *in vitro* human skeletal muscle cell model (figure 5.1) was utilised for studying the effects of eicosapentaenoic acid (EPA) on glucose and lipid metabolism. The method for isolating satellite cells and differentiating to mature myotubes was established by Henry *et al.*, and later modified by Gaster *et al.* (63-65). Satellite cells were isolated from muscle biopsies of the *vastus lateralis* muscle of healthy volunteers, and differentiation and fusion of myoblasts to multinucleated myotubes was induced *in vitro*.

Figure 5.1 *Photograph of in vitro human myotubes, utilised in paper I (from A.C. Rustan).*



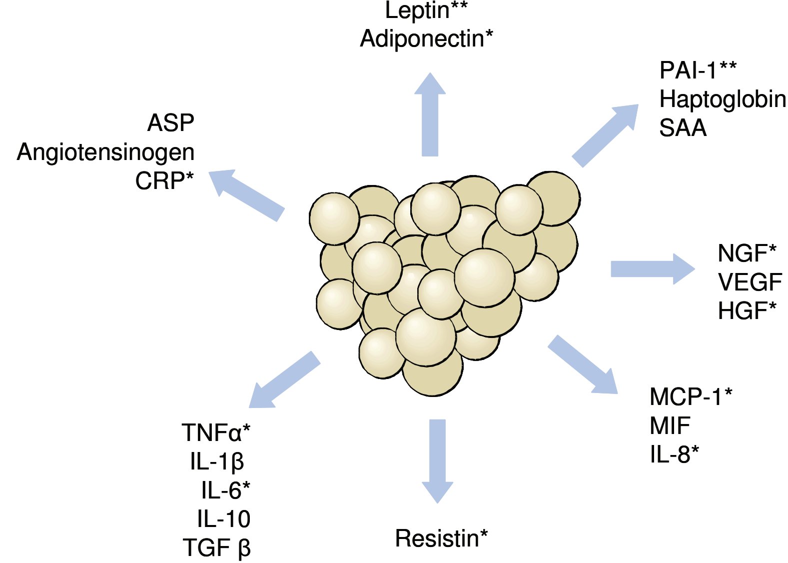
Human skeletal muscle fibres are composed of slow twitch (type I) and fast twitch (type II) fibres (66). Slow twitch fibres contract slowly, are predominantly oxidative and use mostly fatty acids as fuel. They are insulin sensitive and associated with high endurance. Fast twitch fibres are glycolytic (type IIx) or both glycolytic and oxidative (type IIa). They contract fast, are less insulin sensitive, and are associated with rapid contractions.

6. ADIPOKINES

In 1987, Flier and Spiegelman identified adipose tissue as a site for production of adipsin, an endocrine factor markedly down-regulated in obesity (67). It was, however, not until the discovery of leptin in 1994 that adipose tissue was firmly established as an endocrine organ (68). It is now well recognized that adipose tissue expresses and secretes a large number of biologically active proteins, known as adipokines. Adipokines are factors derived from all cells located in the adipose tissue, although some prefer to restrict the term to adipocyte-derived factors. Adipokines play important roles in the regulation of appetite,

insulin sensitivity, immune function, fibrinolysis, and hypertension. They can act locally in the adipose tissue (autocrine/paracrine function), or they can reach the systemic circulation and act on receptors in other organs (endocrine function).

Figure 6.1 *Some of the adipokines released from white adipose tissue. Abbreviations: ASP, acylating stimulation protein; CRP, C-reactive protein; HGF, hepatocyte growth factor; MCP, monocyte chemoattractant protein; MIF, macrophage migration inhibitory factor; NGF, nerve growth factor; SAA, serum amyloid A; TGF, transforming growth factor; TNF, tumour necrosis factor. *Analysed in paper IV in samples from the Oslo Diet and Exercise Study (ODES), **previously published on samples from ODES.*



6.1 Leptin

Leptin, a 16 kDa peptide of 167 amino acids, is the most intensely studied adipokine. Leptin is a multipotent adipokine, with several effects in different tissues. Peripheral effects have been observed on insulin signalling, reproduction, angiogenesis, haematopoiesis and

bone remodelling. Central effects include the regulation of food intake and regulation of body weight (69). Quantitatively, the key locus for leptin production is white adipocytes but it is also produced in brown adipocytes, the gastric epithelium, hair follicles, ovaries, the placenta and osteoblasts (70). Receptors for leptin are expressed in most tissues such as adipose tissue, liver, skeletal muscle, hypothalamus, pancreatic beta-cells, placenta and several fetal tissues (70;71). Leptin expression and secretion correlate positively with adipocyte size and number. Plasma levels of leptin correlate closely with body fat mass, and are thus elevated in obesity. However, leptin resistance may occur in parallel (72).

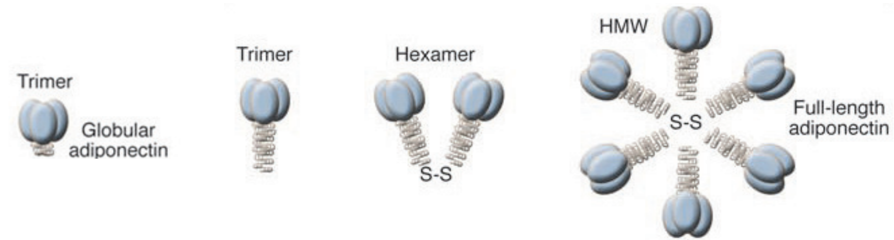
Actions of leptin on skeletal muscle include stimulation of fatty acid oxidation and reduction of intramyocelluar TAG content by activation of AMPK (73;74).

6.2 Adiponectin

Adiponectin was identified by four independent groups around 1995/96. Scherer and co-workers (75) discovered it in a subtractive hybridization screening comparing 3T3-L1 adipocytes with undifferentiated preadipocytes, and named it adipocyte complement-related protein of 30 kDa (Acrp30) due to its structural similarities to complement factor C1q. Hu *et al*. employed a similar approach and termed the protein adipoQ (76). Human adiponectin was first cloned by Maeda and co-workers, and named adipose most abundant gene transcript 1 (apM1) (77). Finally, Tomita *et al.* isolated adiponectin from human plasma by virtue of its affinity for gelatin, and named it gelatin-binding protein of 28 kDa (GBP28) (78). Today, adiponectin is the preferred name used by most researchers.

Human adiponectin is a 244 amino acid protein with MW of approximately 28 kDa, and it is an abundant plasma protein. Plasma concentrations range from 3-30 µg/mL, which is equivalent to approximately 0.01% of total plasma protein. It is produced primarily by adipocytes, although it is also synthesized by other cell types (38;79-84). Adiponectin is secreted in three forms: trimer, hexamer and high molecular weight forms (HMW; 12-36mer) as shown in figure 6.2. The ratio of circulating adiponectin complexes is regulated at the level of secretion (85), and once secreted, these forms are not interchangeable (86). Also, there is a smaller, proteolytic cleavage product present in serum containing the globular head of adiponectin, which circulates as a trimer (87-89).

Figure 6.2 *Different forms of adiponectin observed in serum. Adapted from (88).*



Adiponectin exhibits a sexual dimorphism, with higher levels in women, and Nishizawa *et al.* reported that androgens decrease plasma adiponectin (90). This could partially explain why women are more insulin sensitive than men.

In contrast to most other known adipokines, adiponectin levels are negatively correlated with BMI (91-94) although the correlations are relatively weak in most cross-sectional and longitudinal studies. Fain *et al.* have reported that the release of adiponectin from visceral adipose tissue as well as isolated adipocytes was lower in individuals with BMI 45 than with 32 (95). It has also been reported that adiponectin release correlates negatively with adipocyte size (96).

Adiponectin has been found to be negatively associated with fasting insulin levels (94;97), plasma TAG concentrations (94), as well as fasting, postprandial and 2-h plasma glucose concentrations (94;97). Adiponectin is a predictor of future insulin resistance (98) and risk of type 2 diabetes (99). Mice lacking adiponectin show decreased hepatic insulin sensitivity and a reduced response to PPARγ agonists (100). Activation of PPARγ by the antidiabetic TZD drugs enhances adiponectin gene expression and plasma levels in mice with diabetes, whereas adiponectin null mice show reduced responsiveness to TZDs (100;101). However, the effect of PPARγ on insulin resistance is not entirely dependent on adiponectin (102). There is evidence that HMW adiponectin is the most important form of adiponectin with regard to glucose tolerance (85;103) and that impaired multimerization is associated with diabetes (104).

Three receptors for adiponectin have been reported. The transmembrane receptors adiponectin receptors (AdipoR) 1 and 2, were discovered and characterized by Yamauchi *et*

al. (105). Later, T-cadherin was described as an extracellular receptor for adiponectin (106). AdipoR1 is found predominantly in skeletal muscle but also in the liver and other organs, and is a high-affinity receptor for globular adiponectin, as well as a low-affinity receptor for full-length adiponectin (105). AdipoR2 is most abundant in the liver and is an intermediate-affinity receptor for both globular and full-length adiponectin. Also, AdipoR1 and 2 are expressed in human and rat pancreatic beta-cells (107). Gu *et al.* (108) found that globular adiponectin enhanced insulin secretion from pancreatic beta-cells of rats, whereas Staiger *et al*. (109) found no effect of adiponectin on insulin secretion or beta-cell lipoapoptosis in humans. AdipoR1 is highly expressed in human adipose tissue and the expression is reduced in obese subjects (110). This suggests that adiponectin acts in a paracrine/autocrine manner and the low levels of receptor associated with obesity may further aggravate the negative effects of low adiponectin levels in obese subjects.

It has been shown that osmotin, a protein ubiquitous in fruits and vegetables, is a ligand for the yeast homolog of AdipoR, and may be a naturally occurring AdipoR agonist (111). It is a highly stable protein that is absorbed in the gastrointestinal tract. In mouse C2C12 myocytes, osmotin activated AMPK, and the suppression of AdipoR expression by siRNA markedly reduced the phosphorylation of AMPK induced by osmotin. This finding increases the likelihood that it will be feasible to develop AdipoR agonists that can be absorbed in the gastrointestinal tract.

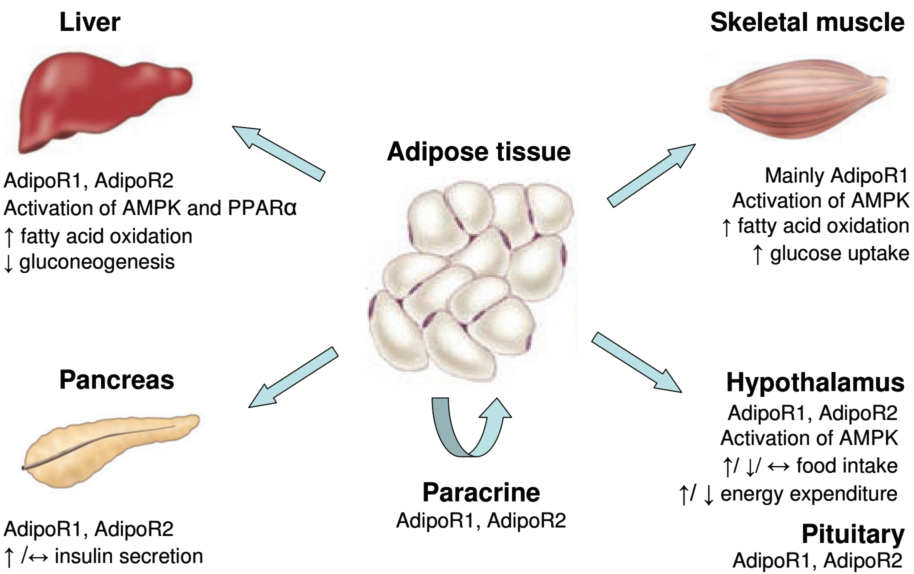
The binding of adiponectin to AdipoR1 results in the activation of AMPK, leading to increased glucose uptake in the skeletal muscle, decreased hepatic gluconeogenesis and increased fatty acid oxidation in the liver and skeletal muscle (112). The effects of AdipoR2 are mediated via induction of PPARα, which stimulates fatty acid oxidation (112). Knock-out models of AdipoR1 and 2 exhibited quite different phenotypes (113). The *AdipoR1*$^{-/-}$ mice showed increased adiposity and decreased glucose tolerance, whereas the *AdipoR2*$^{-/-}$ mice were lean and resistant to high-fat diet-induced obesity. Adiponectin reverses insulin resistance associated with both obesity and lipodystrophy by decreasing the content of TAG in skeletal muscle as well as the liver (89).

In 2004, it was reported by Qi *et al.* that intracerebroventricular (ICV) administration of adiponectin decreased body weight in mice. This was mainly due to increased energy expenditure, whereas the feed intake was unaltered. Coope *et al*. later showed that ICV administration of adiponectin reduced feed intake in Wistar rats and that the effect was mediated via AdipoR1 (114). Several groups have shown by immuno-histochemistry that both AdipoR1 and 2 are present in rat and human hypothalamus (114-117). ICV injection of

adiponectin induced AMPK phosphorylation in the hypothalamus (115). In the hypothalamus, AMPK activity is negatively correlated with malonyl-CoA, and accumulation of malonyl-CoA in the hypothalamus inhibits feed intake (115). The literature is equivocal regarding the central effects of adiponectin on feed intake and energy expenditure. Kadowaki's group has reported opposite findings to those of Qi *et al*. and Coope *et al*.: they showed that adiponectin injections stimulated feed intake and decreased energy expenditure in mice (116). Recently, interesting data were reported on the expression of adiponectin and its receptors in the human pituitary gland, suggesting the existence of a local system that may modulate this endocrine axis (80). Local production of adiponectin in the brain may be important as there is conflicting evidence concerning the ability of adiponectin to cross the blood-brain barrier.

Adiponectin also plays important roles in relation to inflammation, dyslipidemia, vascular function and atherosclerosis but this will not be discussed further here.

Figure 6.3 *Adiponectin is mainly released from adipose tissue and acts on adiponectin receptors in target organs and exerts its effects.*



6.3 Inflammatory adipokines

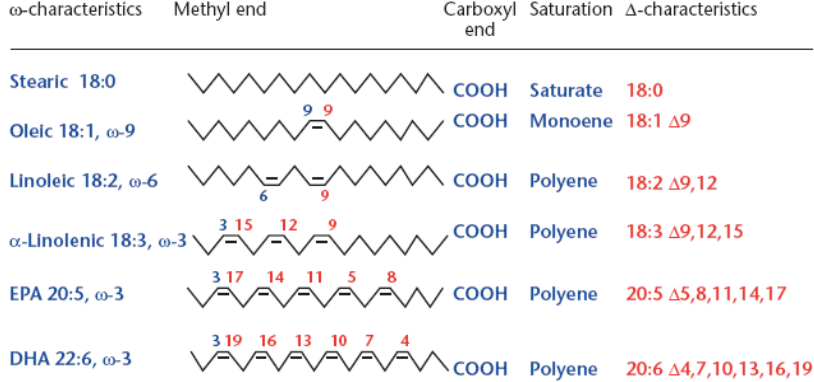
Obesity may be associated with elevated circulating levels of inflammation markers, pro-inflammatory cytokines and chemokines as reviewed in (118-120). This again may lead to a chronic low-grade inflammation and cause insulin resistance. Both adipocytes and other cell types in adipose tissue, in particular macrophages, express and release adipokines. Fain and co-workers have compared the release of many adipokines from adipocytes and non-adipocyte cells in adipose tissue (38). Several of the adipokines were predominantly produced by the non-adipocyte fraction. Interestingly, more adiponectin was released from non-fat cells than from adipocytes, and leptin was almost exclusively produced by the adipocyte fraction. It has been shown that large adipocytes exhibit the highest rate of production of several pro-inflammatory adipokines (121).

7. LIPIDS

7.1 Fatty acids

Fatty acids consist of a hydrocarbon chain with a carboxyl group at one end. Saturated FAs (SFAs) have only single bonds, whereas monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) have one or several double bonds, respectively (figure 7.1).

Figure 7.1 *Structures of some fatty acids (122).*



The α-position of the fatty acid is the carbon next to the carboxyl group. The methyl end is denoted ω or n. SFAs are derived from the diet or synthesized *de novo* in the human body. A double bond in the n-9 position can be introduced by the action of the enzyme stearoyl-coenzyme A-desaturase (SCD). The preferred substrates for SCD are palmitoyl- (16:0) and stearoyl-CoA (18:0) (123). However, the human body is incapable of introducing double bonds distal to n-7 position. Thus, n-6 and n-3 fatty acids are essential and must be provided in the diet. Linoleic acid (18:2, n-6) and α-linolenic acid (18:3, n-3) are produced by plants such as soy, corn, safflower, canola, and algae. Both n-6 and n-3 fatty acids can be chain-elongated and desaturated (figure 7.2). Fatty acids with 20 C-atoms or more are termed very long chain- (VLC-) FAs.

Figure 7.2 *In vivo elongation and desaturation of n-6 and n-3 fatty acids (122).*

ω-6 Fatty acids	Enzymes	ω-3 Fatty acids
Linoleic 18:2		α-Linolenic 18:3
⇩	Δ^6-desaturase	⇩
γ-Linolenic 18:3		Octadecatetraenoic 18:4
⇩	elongase	⇩
Dihomo-γ-linolenic 20:3		Eicosatetraenoic 20:4
⇩	Δ^5-desaturase	⇩
Arachidonic 20:4		Eicosapentaenoic 20:5
⇩	elongase	⇩
Adrenic 22:4		Docosapentaenoic 22:5
⇩	elongase	⇩
Tetracosatetraenoic 24:4		Tetracosapentaenoic 24:5
⇩	Δ^6-desaturase	⇩
Tetracosapentaenoic 24:5		Tetracosahexaenoic 24:6
⇩	β-oxidation	⇩
Docosapentaenoic 22:5		Docosahexaenoic 22:6

Most fatty acids are synthesized in the cytosolic compartment of hepatocytes. *De novo* synthesis of fatty acids in adipocytes and myotubes are generally of little importance on a mixed diet. Acetyl-CoA is generated from glucose catabolism in mitochondria. Acetyl-CoA is converted to malonyl-CoA via the action of acetyl-CoA carboxylase (ACC). The fatty acyl chain grows by two carbon units in a stepwise manner by the actions of the multienzyme

complex fatty acid synthase (FAS), and stops when the acyl chain is 16 carbon atoms long (palmitate) (124).

7.2 Marine n-3 fatty acids

The VLC-PUFAs EPA and DHA are obtained from fatty fish, fish oil and cod liver oil, or from *in vivo* elongation and desaturation of α-linolenic acid as shown in figure 7.2. N-6 and n-3 PUFAs of chain length 20 are precursors to the bioactive eicosanoids, which include prostaglandins, prostacyclins, leukotriens and thromboxans. Some of the effects of n-3 LC-PUFAs are mediated via eicosanoids.

There is strong evidence for the efficacy of n-3 long chain (LC)-PUFAs in the prevention of heart disease (table 7.1).

Table 7.1 *Effects of LC-PUFAs on dyslipidemia and cardiovascular disease (125;126).*

Dyslipidemia	Cardiovascular disease
Reduction of plasma NEFAs	Lower incidence of ischaemic heart disease
Reduction of plasma TAG	Anti-arrhythmic effects
Increase of plasma HDL	Blood-pressure lowering effects
	Slower progression of atherosclerosis
	Anti-thrombotic effects

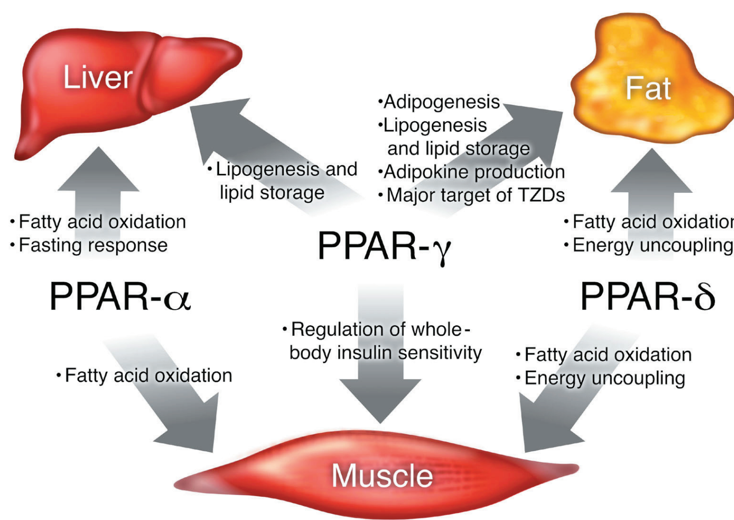
Studies in animals have shown that replacement of some of the dietary fat with VLC-n-3 PUFAs prevents the development of insulin resistance caused by high-fat feeding (127-129). In contrast, the evidence in humans with type 2 diabetes is equivocal. Some but not all studies have found reduced metabolic control. Puhakainen *et al*. found that n-3 FAs increased gluconeogenesis from glycerol but found no deterioration of metabolic control (130). Decreased insulin secretion has also been implicated (131-133). Mostad *et al*. reported a moderate increase in blood glucose and insulin sensitivity in type 2 diabetics in an intervention study with high doses of fish oil for nine weeks (134). The fish oil group showed increased fat utilization and reduced glucose utilization after 9 weeks. This switch in substrate

metabolism has also been observed by others (131;135;136). Type 2 diabetics can benefit from the TAG lowering effects of n-3 PUFAs but are generally not recommended to ingest in high doses.

Interestingly, there may be a connection between n-3 PUFA intake and type 1 diabetes. In a Norwegian case-control study, children with type 1 diabetes were less likely to have been given cod liver oil during infancy than children without diabetes (137). Dietary intake of n-3 PUFAs was associated with reduced risk of islet autoimmunity in children at increased genetic risk for type 1 diabetes (138).

Long chain n-3 PUFAs are natural ligands for all three PPAR nuclear receptors. PPAR heterodimerises with retinoid X receptor, a receptor for 9-cis-retinoic acid, and binds to PPAR response elements (PPREs) in the DNA. PPARα is predominantly expressed in tissues with high capacity for fatty acid oxidation, such as liver and skeletal muscle but also in adipocytes. It regulates genes involved in mitochondrial and peroxisomal FA oxidation (139;140). PPARγ is mainly present in adipose tissue where it plays a central role in adipocyte differentiation and fat storage. Activation of PPARδ in adipocytes may promote fatty acid oxidation opposing fat storage (141).

Figure 7.3 *The PPARs may use fatty acids as ligands and regulate metabolic processes in liver, skeletal muscle and adipose tissue (141).*



The potent lipid-lowering properties of VLC n-3 PUFAs are due to their ability to increase expression of genes involved in peroxisomal and mitochondrial fatty acid oxidation while decreasing expression of lipogenic enzymes (142;143). In liver, PUFAs alters expression lipogenic enzymes by interfering with expression (144;145) and proteolytic maturation of SREBP1 (146;147). The exact mechanisms behind the repression of lipogenic enzymes in adipose tissue are more uncertain. The increase of hepatic β-oxidation mediated by PUFAs is due to activation of PPARα, as demonstrated by studies in PPARα knock-out mice (148;149)

Part of the metabolic effect of n-3 LC-PUFAs occurs via stimulation of AMPK (150). AMPK is a sensor of the cellular metabolic status and is activated by physiological and pathological stresses that deplete cellular ATP, including hypoxia, exercise and muscle contraction. Also, leptin and adiponectin activate AMPK. It controls partitioning between lipid oxidation and lipogenesis by inhibiting lipogenesis while stimulating β-oxidation. This occurs via inhibition by AMPK of acetyl-CoA carboxylase, resulting in a decrease in malonyl-CoA. Malonyl-CoA is a key lipogenic intermediate and inhibits mitochondrial carnitine-palmitoyl transferase (CPT) -1. Moreover, AMPK inhibits gluconeogenesis in liver and stimulates glucose uptake in skeletal muscle (151). Activation of hypothalmic AMPK increases food intake and body weight (151). The much used anti-diabetic drug metformin is an activator of AMPK in skeletal muscle (151-153).

SCD is the rate-limiting enzyme in the biosynthesis of MUFAs, and it is well documented that SCD is down-regulated by PUFAs (123). Hepatic AMPK activity was increased in SCD1 knock-out mice, implicating SCD1 in the regulation of hepatic β-oxidation mice (154). SCD is involved in the formation of ceramide in oxidative myofibres, thus down-regulation of SCD may improve insulin signalling (155). Furthermore, SCD1 knock-out mice exhibit reduced adiposity and increased energy expenditure. Basal thermogenesis as well as lipolysis and fatty acid oxidation were increased (156).

N-3 LC-PUFAs may inhibit progression and relapse, and/or reduce severity of some inflammatory diseases such as rheumatoid arthritis, Systemic *lupus erythematosus* and inflammatory bowel disease (157). Prostaglandins and thromboxans produced from EPA instead of arachidonic acid (AA; 20:4, n-6) are generally less potent and less pro-inflammatory. Moreover, it was recently reported that actions of lipo-oxygenase on DHA generate resolvins and protectins, a novel family of lipid mediators which have anti-inflammatory pro-resolving effects, and protects against tissue damage (158).

7.3 Tetradecylthioacetic acid (TTA)

Tetradecylthioacetic acid is a synthetic, 3-thia substituted fatty acid analogue (CH_3-(CH_2)$_{13}$-S-CH_2-COOH). The sulphur atom makes TTA a poor substrate for β-oxidation, so TTA undergoes sulphur- and ω-oxidation instead. Otherwise it is metabolised as a normal saturated fatty acid; it is converted to its CoA-ester and incorporated into different cellular lipid classes (159;160).

TTA is a pan-PPAR activator in both rodents and humans, and activates all three subtypes in a cell and species specific manner (161-163). In a human keratinocyte cell line, TTA activated PPARs in the following order: PPARδ >> PPARα > PPARγ (162). In the murine embryonic fibroblast cell line NIH-3T3, however, the order was PPARα >> PPARδ > PPARγ (164). TTA prevents high fat diet-induced insulin resistance and adiposity (164). The pleiotrophic effects of TTA suggest that pan-PPAR agonists may have a potential in the treatment of lipid related diseases. The effects of TTA in rats are summarized in table 7.2.

Table 7.2 *Some effects of TTA in rats (165;166).*

Adipose tissue	**Liver**
Reduction in epididymal adipose tissue mass	Reduction in triacylglycerol synthesis rate
Reduction in retroperitoneal adipose tissue mass	Increased CD36 (FAT) mRNA
Plasma	Increase in mitochondrial β-oxidation
Reduction in free fatty acids	Increase in ketone body formation
Reduction in triacylglycerol	Increase in HMG-CoA synthase
Reduction in cholesterol	

8. LIPID METABOLISM

8.1 Hepatic lipogenesis and β-oxidation

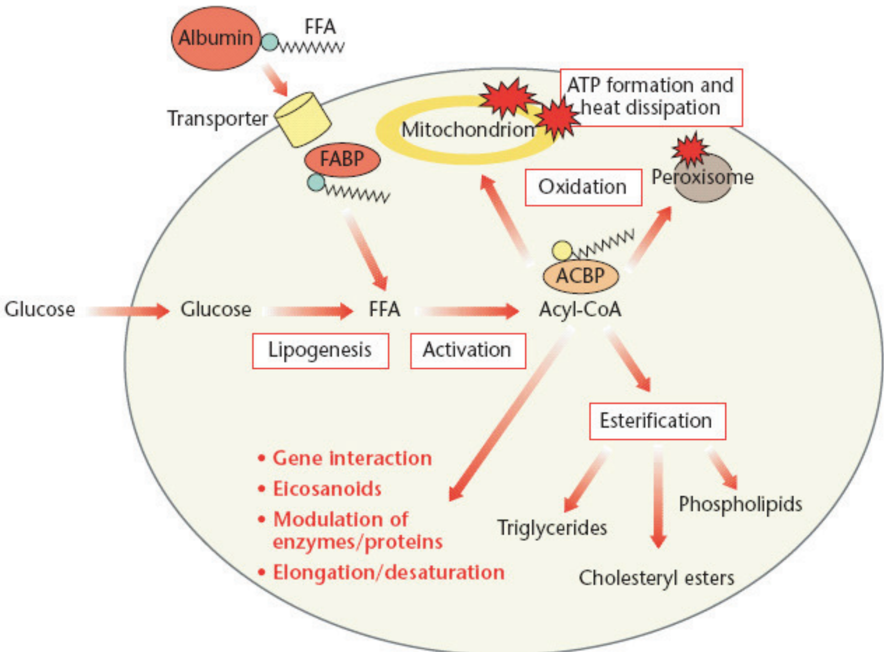
Fatty acids are activated by acyl-CoA synthetases (ACSs) present in the outer mitochondrial membrane, and can 1) enter the mitochondrion or peroxisome for oxidation or 2) be converted to complex lipids; TAG, cholesterol esters or other cellular lipids (figure 8.1).

For TAG synthesis, acyl-CoA enters the phosphatidic acid pathway and three acyl-chains are stepwise added to the glycerol backbone.

For oxidation, acyl-CoAs are converted to acyl-carnitine by the action of CPT-1 and transported across the inner mitochondrial membrane by facilitated diffusion, into the mitochondrion. By the action of CPT-2 on the inner surface of the inner mitochondrial membrane, carnitine is removed and acyl-CoA is available for metabolism. The first step of mitochondrial β-oxidation is catalyzed by acyl-CoA dehydrogenase, whereas in the peroxisome, β-oxidation is initialized by acyl-CoA oxidase (ACO) (167). Hepatic activities of ACS, CPT-2 and ACO were measured in paper II of this thesis.

The main role of peroxisomal β-oxidation is to shorten or otherwise convert fatty acids for further oxidation by the mitochondrial enzymes. VLC-FAs are primarily oxidised in the peroxisomes, as they are poor substrates for the mitochondrial ACSs and CPTs.

Figure 8.1 *Some aspects of fatty acid metabolism. Free fatty acids (FFAs) are taken up into the cells mainly by protein carriers in the plasma membrane and transported intracelluarly by fatty acid binding proteins (FABPs). Fatty acids can be activated to acyl-CoA, and shuttled to the mitochondria or peroxisomes for β-oxidation, or to the endoplasmatic reticulum for esterification to complex lipids. Acyl-CoA and certain FFAs may also bind to nuclear receptors and regulate gene expression, be converted to signal molecules, directly or indirectly modulate various proteins, or be chain-elongated or desaturated (122).*



8.2 Triacylglycerol storage and lipolysis in adipose tissue

Adipose tissue releases NEFAs to the extracellular space under conditions such as fasting and uncontrolled diabetes. NEFAs circulate in plasma bound to albumin and are taken up by energy-demanding organs such as skeletal muscle and liver. Lipolysis is in the fasting state initiated by adipose triglyceride lipase (ATGL), which cleaves the first of the three fatty

acids in TAG (168;169). The second fatty acid is cleaved off by hormone-sensitive lipase (HSL), and the third by monoacylglycerol lipase (170).

After a meal, insulin levels rise and activates lipoprotein lipase (LPL) in the endothelial cells of the capillaries in adipose tissue. LPL hydrolyzes TAG in the lipoprotein particles to NEFAs outside the adipocyte. The fatty acids are stored as TAG in the lipid droplets of adipocytes after esterification to glycerol-3-phosphate, by the actions of glycerol-3-phosphate acyltransferase (GPAT), phosphatidate phosphohydrolase (PAP) and diacylglycerol acyltransferase (DGAT) (124).

9. THE OSLO DIET AND EXERCISE STUDY (ODES)

Paper IV is based on the ODES study; a 1-year intervention study investigating the effects of dietary and/or exercise intervention. A detailed description of the study design and population and intervention principles and primary outcome variables is given by the ODES investigators (171). The primary aims of the study were to investigate the effects of the interventions on fibrinogen, fibrinolytic activity, coagulation factor VII and platelet volume (172). A series of secondary outcome variables have since been monitored (173-178)

The basis for recruitment to the ODES study was a screening among 40-year olds in Oslo which started in 1981 and lasted until 1999. In 1990, 660 women and men fulfilled the inclusion criteria (among until then 20,000 screened individuals) and were invited to participate in the study. 198 men and 21 women were included (aged 41-50), of which approximately 50% of the study subjects met Adult Treatment Panel III criteria for MS (179). The participants were randomly allocated to one of four groups (table 8.1) and received either dietary advice and/or participated in an exercise program for one year, or constituted the control group which received no intervention. Fasting blood samples were drawn at baseline and after 1 year of intervention, and were used for the analyses presented in paper IV. Participants in both groups receiving dietary counselling lost a healthy amount of weight during the year long intervention, and in all three intervention groups the mean waist-circumference was reduced. The interventions led to positive effects on parameters such as blood pressure, plasma lipids and haemostatic variables.

Table 8.1 *The 2x2 factorial design of ODES. The participants were randomly allocated to receive no intervention (control group), either diet or exercise intervention, or both interventions.*

Control	Diet
Exercise	Diet & Exercise

AIMS OF PRESENT STUDIES

The questions raised in the present thesis were:

1) How does incubation with the marine polyunsaturated n-3 fatty acid EPA influence glucose and lipid metabolism in a human skeletal muscle model?
2) What are the effects of partly substituting lard with marine polyunsaturated fatty acids or the synthetic fatty acid tetradecylthioacetic acid (TTA) on adipose tissue content, distribution and gene expression?
3) Do long term changes in diet and/or physical activity lead to altered plasma concentrations of adipokines?

SUMMARY OF PAPERS

Paper I: Eicosapentaenoic acid (20:5 n-3) increases fatty acid and glucose uptake in cultured human skeletal muscle cells

The aim was to study the effects of chronic (24 h) incubation of the marine polyunsaturated fatty acid EPA on glucose and lipid metabolism in an *in vitro* model of human myotubes. The effects of EPA were compared with those of oleic acid (OA; a common monounsaturated fatty acid) and a fatty acid-free control (containing BSA).

EPA promoted increased uptake and oxidation of glucose, despite markedly increased fatty acid uptake and synthesis of complex lipids. Fatty acid β-oxidation was unchanged, and complete oxidation (CO_2) decreased in EPA-incubated cells. The mechanisms may involve GLUT1 and CD36/FAT (fatty acid transporter), as mRNA levels of these transporters were increased in cells preincubated with EPA.

Paper II: Marine n-3 fatty acids promote size-reduction of visceral adipose depots, without altering body weight and composition, in male Wistar rats fed a high-fat diet

We investigated the effects of marine polyunsaturated n-3 FAs on adipose tissue content, distribution and gene expression.

By the use of MRI, we found that the volumes of all three visceral adipose depots analysed were lower in the n-3 FA group as compared to the lard group, while the percentage of total body fat was the same. Thus, our findings suggested that feeding with n-3 FAs led to a redistribution of adipose tissue away from the visceral compartment. We observed enhanced mRNA levels of several inflammatory cytokines and chemokines in the adipose tissues of the n-3 FA fed animals as compared with the lard-fed animals. The biological significance of these findings is however hard to interpret. Fasting insulin levels were markedly lower in the n-3 FA group as compared to the lard group, suggesting insulin resistance and hyperinsulinemia in the lard group. However, no differences between the two groups were found for *in vitro* glucose uptake in soleus muscle strips and epitrochlearis, or glycogen content of soleus and epitrochlearis muscle.

Paper III: Dietary supplementation of tetradecylthioacetic acid increases feed intake but reduces body weight gain and adipose depot sizes in rats fed high-fat diets

The synthetic fatty acid analogue tetradecylthioacetic acid (TTA) is a sulphur-substituted, saturated fatty acid, and resistant to β-oxidation. TTA may activate all PPAR, and is known to reduce plasma lipids and enhance lipid metabolism, as well as reduce adipose tissue sizes in rats fed high-fat diets.

We further explored the effects of TTA on weight gain, feed intake and adipose tissue distribution and gene expression. Despite a higher feed-intake, rats fed TTA gained less body weight than lard-fed rats, and had markedly decreased subcutaneous, epididymal, perirenal and mesenteric adipose depots. Plasma lipids were reduced and fatty acid β-oxidation in liver and heart were enhanced in the TTA-fed rats. Hepatic UCP3 was expressed ectopically at both protein and mRNA levels, whereas *Ucp1* mRNA was increased in epididymal and mesenteric adipose depots in the TTA group compared to the lard group. Our data support the hypothesis that TTA-feeding may increase hepatic fatty acid β-oxidation, thereby diminishing storage of fat in adipose tissues. The increased expression of *Ucp3* in liver and *Ucp1* in visceral adipose tissues may together promote enhanced energy dissipation and reduced weight gain in rats fed a high-fat diet.

Paper IV: Effects of long-term exercise and diet intervention on plasma adipokine concentrations

This paper is based on samples from the Oslo Diet and Exercise Study; a one-year long diet and exercise intervention study (2x2 factorial design). We tested the hypothesis that long-term lifestyle changes and moderate weight loss would reduce the plasma concentrations of adipokines involved in inflammation, angiogenesis, and chemotaxis and would increase adiponectin concentrations. We selected nine adipokines: adiponectin, IL-6, IL-8, MCP-1, TNF-α, HGF, NGF, CRP and resistin. Data on leptin and PAI-1 were published previously but were included in the paper for comparison.

We found that plasma adiponectin levels remained unchanged, whereas body mass index (BMI) and fat mass were reduced after improvements in dietary habits and an increase in physical activity. However, adiponectin concentrations were reduced in the control group. Minor changes were found for the other adipokines. We found no correlation between adiponectin concentrations and BMI at baseline but for the one-year changes, there was a significant negative correlation. Neither baseline nor changes in plasma adiponectin and PAI-1 concentrations were significantly correlated to the other adipokines. Concentrations of and

changes in the other plasma adipokines were significantly correlated, suggesting mutually related pathways.

DISCUSSION

1. Methodological considerations

The culture of human myotubes utilised in paper I is widely used and well characterized. We combined the use of radio-labelled tracer studies with gene expression analyses and Western blotting for protein. Analyses on three levels; gene transcripts and protein expression, and functional studies, describe different processes in the cells, thus providing a more complete picture than when only one level is monitored. The insulin responses on glucose uptake and glycogen synthesis are relatively modest in this cell model, leading us to suspect that they mostly differentiate to type II muscle fibres. The effect of EPA on glucose uptake appears to be mediated by GLUT1, as the mRNA level of this glucose transporter was increased. The mechanisms behind this effect of EPA on GLUT1 expression and basal glucose uptake remain to be elucidated. Furthermore, it would have been informative to establish whether the increased incorporation of [1-^{14}C]OA into TAG following preincubation with EPA led to increased mass of cellular TAG.

In papers II and III, rats were fed a high-fat diet for seven weeks, and the main focus of the studies were to evaluate the effects of marine PUFAs and TTA on adipose tissue distribution and gene expression. As our main focus was on adipose tissues, we selected genes expression assays most relevant for this tissue. Gene expression levels of nuclear receptors, adipokines, uncoupling proteins, AMPK, lipid droplet associated proteins and genes involved in lipid metabolism were analysed. Since fatty acid oxidation is not prominent in adipose tissue in the case of a mixed diet, only few genes related to that were included.

We observed increased mRNA levels of several cytokines and chemokines in the visceral adipose depots of the n-3 FA-fed animals in paper II. This may suggest altered cell composition and it could have been informative to have included macrophage markers, e.g. CD68, f4/80 or MIP-1α. However, these markers may not be dependable markers of macrophage infiltration but rather markers of inflammation (58).

In paper III, we speculated that the observed ectopic expression of both gene and protein UCP3 in the liver and markedly increased *Ucp1* mRNA in the visceral adipose depots, promoted enhanced energy dissipation and thereby reduced weight gain in the TTA-fed animals. In order to claim this with certainty, indirect calorimetry would be necessary. Histological evaluation of the visceral adipose tissues in order to evaluate if TTA induced BAT formation would also have been informative.

We utilized MRI in addition to dissections and weighing to estimate the sizes of the distinct adipose depots in papers II and III. We observed high correlations between the estimates by the two methods for all depots, except the mesenteric. Dissecting out the mesenteric adipose can be difficult due to the anatomical nature of the depot and we believe MRI is a reliable alternative. A higher strength for detecting differences in subcutaneous adipose tissue depot sizes could have been obtained if we had dissected and analysed with MRI a larger portion of depot. That would have enabled us to claim with more certainty whether there was redistribution of adipose tissue to the subcutaneous depot in the n-3 FA-group.

The ODES was a well-designed and well-controlled intervention study, with beneficial results on weight, waist circumference and other cardiovascular risk factors. The interventions were moderate and long-term compared to others studies in the literature. We knew from a previous report on the ODES-study that there were significant effects of the interventions on plasma leptin concentrations (176), and tested our hypothesis that plasma levels of other adipokines would also be altered in paper IV. We found a significant effect of diet intervention of total adiponectin. It would have been interesting to know if the fall in plasma concentration of adiponectin during the one-year study in the control group, was due to a reduction of LMW or HMW adiponectin. At the time of the study, no satisfactory method for determination of HMW adiponectin was available.

The ODES study had a 2x2 factorial design. With a 2x2 factorial design, the separate effects as well as the interaction effects of the two interventions can be studied. In this context, interaction is the modification of the effect of one intervention by the influence of the other intervention. We found a significant interaction effect between the diet and exercise interventions of TNFα. Both interventions alone increased TNFα plasma concentrations, but when the interventions were combined there was no significant effect. With a 2x2 factorial design, a higher strength for detecting effects of the single interventions is obtained. E.g. for studying the effect of diet intervention, both the diet group and the diet+exercise group can be tested against the control group, thereby increasing *n*.

Statistical power analyses were performed when designing the ODES study, and were not performed prior to adipokines analyses. Power calculations were made with the primary end-points euglobin clot lysis time, fibrinogen, coagulation factor VII, and platelet volume (as a measure of platelet activity).

2. Fatty acids and insulin sensitivity

In paper I, we reported effects of incubation with EPA on glucose and fatty acid metabolism in human myotubes. EPA increased glucose uptake and oxidation in human myotubes after 24 h incubation with EPA. This enhanced glucose metabolism occurred despite increased uptake of OA and incorporation to TAG, which occurred contrary to our expectations. Increased IMTG content is associated with insulin resistance. However, because IMTG content is also increased in athletes with high sensitivity to insulin (8), it may not be IMTG storage *per se* that is detrimental to insulin signalling. Sequestration of cytosolic FFAs in IMTG stores may represent a cytoprotective mechanism.

In paper II, the only indication of improved insulin sensitivity in the n-3 FA-group was lower fasting plasma insulin levels than the lard group. We found no differences in glucose uptake and glycogen uptake in epitrochlearis muscles and soleus muscle strips, and hepatic and adipose tissue insulin sensitivity was not evaluated. However, we observed a 4-fold increase in *Adipoq* (adiponectin) mRNA in the mesenteric adipose depot of n-3 FA-fed animals, but this was not reflected in plasma or extract concentrations of adiponectin. In a study by Phillips *et al.*, SAT, not VAT, was the major contributor to increased circulating adiponectin levels in response to treatment with the PPARγ agonist pioglitazone (180). There may be local autocrine or paracrine effects of enhanced adiponectin in the mesenteric adipose depot, as adiponectin receptors are expressed in adipocytes.

3. Adipose tissue

In paper II, there were no differences between the two groups in body composition after 7 weeks of feeding. The volumes of the visceral adipose depots, as estimated by MRI, were reduced in the n-3 FA-group, thus redistribution of fat away from the visceral compartment took place. Redistribution from subcutaneous to visceral fat has been observed in both animals and humans treated with TZDs, which are PPARγ-agonists. Because n-3 FAs are also PPARγ-agonists, the redistribution effect observed in paper II can probably be attributed to PPARγ activation. This may be a favourable effect of n-3 FAs, as visceral fat storage can be regarded as "ectopic".

We observed that *Scd1* was significantly reduced in the epididymal, perirenal and interscapular depots, as well in the liver of the n-3 FA-fed rats. This is in accordance with previous observations that PUFAs repress the SCD gene expression (123;181). In the recent years, other functions besides desaturation of fatty acids have been shown for SCD. SCD deficiency in mice has been reported to increase AMPK activity and beta-oxidation in liver

AMPK (154), and enhance thermogenesis (182). Also, SCD deficiency reduced ceramide production in skeletal muscle (155). Hence, it is possible that some of the effects of n-3 FA-feeding are due to down-regulation of *Scd1*.

Our finding of increased expression of several cytokines and chemokines in visceral depots of n-3 animals was opposite of what we expected, considering n-3 PUFAs anti-inflammatory properties. Because gene expression analyses have limitations, further elucidation of the mechanisms causing this local increase is needed.

In paper III, we showed that 7 weeks of dietary supplementation with TTA increased feed intake but reduced weight gain compared to lard-feeding. Transdifferentiation of white adipocytes to brown adipocytes has previously been described (61) and may occur with TTA-feeding. Because there are no other markers for BAT than UCP1, histological evaluation of the adipose tissue is warranted.

4. Adipokines

Attie and Scherer have proposed that leptin and adiponectin have evolved to counteract the "thrifty genes" (183). However, leptin may not be very effective since leptin resistance develops during early stages of obesity. Also, with starvation and low leptin levels, the inhibitory effect of leptin on appetite and feed intake is lifted, promoting reduced loss of adipose tissue.

The evolutionary role of adiponectin is somewhat puzzling. The peripheral effects of leptin and adiponectin have several similarities, such as increasing insulin sensitivity and fatty acid oxidation via activation of AMPK. However, when fat mass increases, adiponectin levels fall, while leptin levels increase. Centrally, leptin and adiponectin have opposite effects on AMPK. Leptin inhibit hypothalamic AMPK, whereas adiponectin activates it (184). Kadowaki argues that adiponectin is a starvation gene (185), promoting fat storage when facing starvation and loss of fat mass, based on their finding that adiponectin stimulates appetite. Conversely, other groups have report increased energy expenditure and reduced or unaltered feed intake in response to ICV injections of adiponectin. It is still unknown if and how adiponectin crosses the blood brain barrier (BBB) in humans, and plasma and cerebrospinal fluid (CSF) adiponectin may not even be correlated. Adiponectin is detectable at very low concentrations in the CSF (~1/1,000 of serum concentration) (117;186), and Spranger *et al.* have shown that adiponectin does not cross the BBB in mice (187). Hence, the small amounts of adiponectin detectable in the CSF may stem from local production, given

the recent evidence of pituitary expression of adiponectin. Further elucidation of the central actions of adiponectin is necessary.

We investigated the effects of long-term diet and exercise intervention on plasma concentrations of 9 adipokines in paper IV. We knew from previous publications that that the interventions had been effective in reducing fat mass as well plasma leptin levels. The negative correlation between BMI and circulating adiponectin is generally weak in most cross-sectional and longitudinal studies, and in paper IV we found no significant correlation at baseline. However, changes in adiponectin and BMI during the one year intervention period, correlated negatively. Given the large interindividual variations in adiponectin, plasma levels are undoubtedly determined to a large degree by other factors than fat mass alone, in contrast to leptin which correlates more closely. We found a positive effect of diet intervention on adiponectin concentrations. After adjustment for change in body fat %, the effect remained positive but lost statistical significance. It was puzzling that adiponectin levels fell so much (28.5%) during one year only in a high risk population like the control group of the ODES. Our finding was in accordance with other investigators (98;188). The exact mechanisms regulating adiponectin production in adipose tissue remain unclear, but it has been shown that large adipocytes produce less adiponectin than smaller cells (96). We may speculate that adipocytes reach a threshold related to degree of lipid-loading or physical size, where adiponectin production decreases.

5. Concluding remarks

The main conclusions from the present study can be summarized as follows:

- Preincubation of EPA with cultured human myotubes increased glucose uptake and oxidation, despite markedly increased fatty acid uptake and synthesis of complex lipids. The mechanism may involve increased expression of CD36/FAT and GLUT1. Despite the enhanced fatty acid uptake and synthesis of complex lipids, the insulin responses after EPA preincubation were maintained for glucose uptake and oxidation, and increased for oleate uptake and distribution to complex lipids.
- Partly substituting lard with marine polyunsaturated fatty acids in rats fed a high-fat diet lead to a redistribution of adipose tissue away from the visceral compartment, without altering body weight and composition. The enhanced gene expression of *Il6* and *Tnf* in visceral adipose depots of the n-3 FA-fed animals was not reflected by altered plasma levels, suggesting that the effect was local in the adipose tissue. Fasting plasma insulin concentrations were lower in the n-3 FA-group, which may indicate

better insulin sensitivity. However, as no differences in glucose uptake were observed in skeletal muscle, improvement in insulin sensitivity may lie in other insulin sensitive organs.

- Supplementation of TTA increased feed intake but reduced body weight gain and adipose depot sizes in rats fed high-fat diets. The increased expressions of *Ucp3* in liver and *Ucp1* in visceral adipose depots may suggest that TTA has promoted enhanced energy dissipation, resulting in reduced weight gain in the TTA-group.
- Diet intervention had a significant positive effect on adiponectin concentrations, and was largely explained by a reduction in fat mass. Both baseline concentrations and changes in plasma concentrations of adiponectin and PAI-1 were not correlated with other adipokines, suggesting unrelated pathways.

6. Future prospects

Adipose tissue has received much attention by researchers over the last two decades and the field is evolving rapidly.

- Further characterization of the different adipose depots and elucidation of intrinsic and environmental factors causing these differences is needed. Moreover, studies on inflammation in relation to expanding adipose tissue and further exploration of the hypoxia-hypothesis are warranted.
- The mechanisms underlying the effects of VLC n-3 PUFAs in adipose tissue are far from clear and should be examined in new studies on rodents as well as humans.
- The possibility for the use of pan-PPAR agonists and selective PPAR modulators in the treatment of lipid related-diseases is undoubtedly a current focus of the pharmaceutical industry.
- The biology of BAT in adult humans is still relatively unexplored. Some but not all the knowledge from rodents can be transferred to the human situation. With increased understanding of the role of BAT, we will know if it has potential as a therapeutic target in obesity and other lipid-related diseases.
- The mechanisms linking adiposity, increased adipocyte cell size and adiponectin release remain unsolved and should be focused on. Also, the central effects of adiponectin, as well as the functions of adiponectin receptors in organs like the pancreas and adipose tissue, need to be clarified.
- TTA-induced increase in feed intake and reduction of body fat deserve more attention by monitoring whole body energy expenditure by calorimetry.

REFERENCES

1. WHO World Health Organization. 2009. http://www.who.int
   Ref Type: Online Source

2. WCRF (World Cancer Reaserch Fund) og IARC (American Institute of Cancer Research) (2007). Food, Nutrition, Physical activity and the prevention of cancer: a global perspective. Second expert report. The American Institute of Cancer Research, Washington DC. 2009.
   Ref Type: Online Source

3. Neel JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? 1962. Bull World Health Organ 1999;77:694-703.

4. Wing RR, Phelan S. Long-term weight loss maintenance. Am J Clin Nutr 2005;82:222S-5S.

5. Gross B, Staels B. PPAR agonists: multimodal drugs for the treatment of type-2 diabetes. Best Pract Res Clin Endocrinol Metab 2007;21:687-710.

6. Clapham JC, Arch JR. Thermogenic and metabolic antiobesity drugs: rationale and opportunities. Diabetes Obes Metab 2007;9:259-75.

7. Phielix E, Mensink M. Type 2 diabetes mellitus and skeletal muscle metabolic function. Physiol Behav 2008;94:252-8.

8. van Loon LJ. Intramyocellular triacylglycerol as a substrate source during exercise. Proc Nutr Soc 2004;63:301-7.

9. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 2000;49:677-83.

10. Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. Am J Physiol Endocrinol Metab 2008;295:E1009-E1017.

11. DiGirolamo M, Fine JB, Tagra K, Rossmanith R. Qualitative regional differences in adipose tissue growth and cellularity in male Wistar rats fed ad libitum. Am J Physiol 1998;274:R1460-R1467.

12. Klaus S, Cinti S, Ailhaud G, et al. *Adipose tissues*. Georgetown, Texas, USA: Eurekah.com / Landes Bioscience, 2001.

13. Cinti S, Mitchell G, Barbatelli G et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 2005;46:2347-55.

14. Curat CA, Miranville A, Sengenes C et al. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. Diabetes 2004;53:1285-92.

15. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796-808.

16. Xu H, Barnes GT, Yang Q et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112:1821-30.

17. Trayhurn P, Wang B, Wood IS. Hypoxia and the endocrine and signalling role of white adipose tissue. Arch Physiol Biochem 2008;114:267-76.

18. Trayhurn P, Wang B, Wood IS. Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? Br J Nutr 2008;100:227-35.

19. Bedford PA, Todorovic V, Westcott ED et al. Adipose tissue of human omentum is a major source of dendritic cells, which lose MHC Class II and stimulatory function in Crohn's disease. J Leukoc Biol 2006;80:546-54.

20. Hutley LJ, Herington AC, Shurety W et al. Human adipose tissue endothelial cells promote preadipocyte proliferation. Am J Physiol Endocrinol Metab 2001;281:E1037-E1044.

21. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2007;293:E444-E452.

22. Cypess AM, Lehman S, Williams G et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009;360:1509-17.

23. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med 2009;360:1500-8.

24. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell Metab 2005;2:85-93.

25. Joseph JW, Koshkin V, Saleh MC et al. Free fatty acid-induced beta-cell defects are dependent on uncoupling protein 2 expression. J Biol Chem 2004;279:51049-56.

26. Carr MC, Brunzell JD. Abdominal obesity and dyslipidemia in the metabolic syndrome: importance of type 2 diabetes and familial combined hyperlipidemia in coronary artery disease risk. J Clin Endocrinol Metab 2004;89:2601-7.

27. Yusuf S, Hawken S, Ounpuu S et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-52.

28. Yusuf S, Hawken S, Ounpuu S et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet 2005;366:1640-9.

29. Bjorntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis 1990;10:493-6.

30. Lam TK, Carpentier A, Lewis GF, van de Werve G, Fantus IG, Giacca A. Mechanisms of the free fatty acid-induced increase in hepatic glucose production. Am J Physiol Endocrinol Metab 2003;284:E863-E873.

31. Lewis GF, Vranic M, Harley P, Giacca A. Fatty acids mediate the acute extrahepatic effects of insulin on hepatic glucose production in humans. Diabetes 1997;46:1111-9.

32. Frayn KN. Visceral fat and insulin resistance--causative or correlative? Br J Nutr 2000;83 Suppl 1:S71-S77.

33. Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000;106:473-81.

34. Dieudonne MN, Pecquery R, Boumediene A, Leneveu MC, Giudicelli Y. Androgen receptors in human preadipocytes and adipocytes: regional specificities and regulation by sex steroids. Am J Physiol 1998;274:C1645-C1652.

35. Rebuffe-Scrive M, Bronnegard M, Nilsson A, Eldh J, Gustafsson JA, Bjorntorp P. Steroid hormone receptors in human adipose tissues. J Clin Endocrinol Metab 1990;71:1215-9.

36. Bujalska IJ, Kumar S, Stewart PM. Does central obesity reflect "Cushing's disease of the omentum"? Lancet 1997;349:1210-3.

37. Lafontan M, Berlan M. Do regional differences in adipocyte biology provide new pathophysiological insights? Trends Pharmacol Sci 2003;24:276-83.

38. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm 2006;74:443-77.

39. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 1998;83:847-50.

40. Bruun JM, Lihn AS, Madan AK et al. Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of nonadipose cells in adipose tissue. Am J Physiol Endocrinol Metab 2004;286:E8-13.

41. Russell CD, Petersen RN, Rao SP et al. Leptin expression in adipose tissue from obese humans: depot-specific regulation by insulin and dexamethasone. Am J Physiol 1998;275:E507-E515.

42. van H, V, Reynisdottir S, Eriksson P et al. Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes 1998;47:913-7.

43. Drolet R, Belanger C, Fortier M et al. Fat depot-specific impact of visceral obesity on adipocyte adiponectin release in women. Obesity (Silver Spring) 2009;17:424-30.

44. Motoshima H, Wu X, Sinha MK et al. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. J Clin Endocrinol Metab 2002;87:5662-7.

45. Lihn AS, Bruun JM, He G, Pedersen SB, Jensen PF, Richelsen B. Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. Mol Cell Endocrinol 2004;219:9-15.

46. Fisher FM, McTernan PG, Valsamakis G et al. Differences in adiponectin protein expression: effect of fat depots and type 2 diabetic status. Horm Metab Res 2002;34:650-4.

47. Yang WS, Chen MH, Lee WJ et al. Adiponectin mRNA levels in the abdominal adipose depots of nondiabetic women. Int J Obes Relat Metab Disord 2003;27:896-900.

48. Fliers E, Kreier F, Voshol PJ et al. White adipose tissue: getting nervous. J Neuroendocrinol 2003;15:1005-10.

49. Kreier F, Fliers E, Voshol PJ et al. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat--functional implications. J Clin Invest 2002;110:1243-50.

50. Tran TT, Yamamoto Y, Gesta S, Kahn CR. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab 2008;7:410-20.

51. Laplante M, Festuccia WT, Soucy G et al. Mechanisms of the depot specificity of peroxisome proliferator-activated receptor gamma action on adipose tissue metabolism. Diabetes 2006;55:2771-8.

52. Mori Y, Murakawa Y, Okada K et al. Effect of troglitazone on body fat distribution in type 2 diabetic patients. Diabetes Care 1999;22:908-12.

53. Miyazaki Y, Mahankali A, Matsuda M et al. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab 2002;87:2784-91.

54. Smith SR, de JL, Volaufova J, Li Y, Xie H, Bray GA. Effect of pioglitazone on body composition and energy expenditure: a randomized controlled trial. Metabolism 2005;54:24-32.

55. Rang, Dale, Ritter. Pharmacology. Churchill Livingstone, 1999.

56. Permana PA, Nair S, Lee YH, Luczy-Bachman G, Vozarova De Court, Tataranni PA. Subcutaneous abdominal preadipocyte differentiation in vitro inversely correlates with central obesity. Am J Physiol Endocrinol Metab 2004;286:E958-E962.

57. Tchoukalova Y, Koutsari C, Jensen M. Committed subcutaneous preadipocytes are reduced in human obesity. Diabetologia 2007;50:151-7.

58. Isakson P, Hammarstedt A, Gustafson B, Smith U. Impaired preadipocyte differentiation in human abdominal obesity -- role of Wnt, TNF{alpha} and inflammation. Diabetes 2009.

59. Lacasa D, Taleb S, Keophiphath M, Miranville A, Clement K. Macrophage-secreted factors impair human adipogenesis: involvement of proinflammatory state in preadipocytes. Endocrinology 2007;148:868-77.

60. Morroni M, Giordano A, Zingaretti MC et al. Reversible transdifferentiation of secretory epithelial cells into adipocytes in the mammary gland. Proc Natl Acad Sci U S A 2004;101:16801-6.

61. Murano I, Zingaretti MC, Cinti S. The adipose organ of SV129 mice contains a prevalence of brown adipocytes and shows plasticity after cold exposure. Adipocytes. 2005:121-30.

62. Cinti S. *The Adipose Organ*. Editrice Kurtis, Milano, Italy, 1999.

63. Gaster M, Kristensen SR, Beck-Nielsen H, Schroder HD. A cellular model system of differentiated human myotubes. APMIS 2001;109:735-44.

64. Gaster M, Beck-Nielsen H, Schroder HD. Proliferation conditions for human satellite cells. The fractional content of satellite cells. APMIS 2001;109:726-34.

65. Henry RR, Abrams L, Nikoulina S, Ciaraldi TP. Insulin action and glucose metabolism in nondiabetic control and NIDDM subjects. Comparison using human skeletal muscle cell cultures. Diabetes 1995;44:936-46.

66. Spangenburg EE, Booth FW. Molecular regulation of individual skeletal muscle fibre types. Acta Physiol Scand 2003;178:413-24.

67. Flier JS, Lowell B, Napolitano A et al. Adipsin: regulation and dysregulation in obesity and other metabolic states. Recent Prog Horm Res 1989;45:567-80.

68. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425-32.

69. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995;269:546-9.

70. Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. Int J Obes Relat Metab Disord 2002;26:1407-33.

71. Wang P, Mariman E, Renes J, Keijer J. The secretory function of adipocytes in the physiology of white adipose tissue. J Cell Physiol 2008;216:3-13.

72. Jequier E. Leptin signaling, adiposity, and energy balance. Ann N Y Acad Sci 2002;967:379-88.

73. Minokoshi Y, Kim YB, Peroni OD et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415:339-43.

74. Minokoshi Y, Kahn BB. Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. Biochem Soc Trans 2003;31:196-201.

75. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746-9.

76. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996;271:10697-703.

77. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 1996;221:286-9.

78. Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem 1996;120:803-12.

79. Berner HS, Lyngstadaas SP, Spahr A et al. Adiponectin and its receptors are expressed in bone-forming cells. Bone 2004;35:842-9.

80. Psilopanagioti A, Papadaki H, Kranioti EF, Alexandrides TK, Varakis JN. Expression of adiponectin and adiponectin receptors in human pituitary gland and brain. Neuroendocrinology 2009;89:38-47.

81. Pineiro R, Iglesias MJ, Gallego R et al. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. FEBS Lett 2005;579:5163-9.

82. Delaigle AM, Jonas JC, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. Endocrinology 2004;145:5589-97.

83. Caminos JE, Nogueiras R, Gaytan F et al. Novel expression and direct effects of adiponectin in the rat testis. Endocrinology 2008;149:3390-402.

84. Caminos JE, Nogueiras R, Gallego R et al. Expression and regulation of adiponectin and receptor in human and rat placenta. J Clin Endocrinol Metab 2005;90:4276-86.

85. Pajvani UB, Hawkins M, Combs TP et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 2004;279:12152-62.

86. Pajvani UB, Du X, Combs TP et al. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. J Biol Chem 2003;278:9073-85.

87. Fruebis J, Tsao TS, Javorschi S et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci U S A 2001;98:2005-10.

88. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784-92.

89. Yamauchi T, Kamon J, Waki H et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941-6.

90. Nishizawa H, Shimomura I, Kishida K et al. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes 2002;51:2734-41.

91. Arita Y, Kihara S, Ouchi N et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79-83.

92. Yamamoto Y, Hirose H, Saito I et al. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. Clin Sci (Lond) 2002;103:137-42.

93. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur J Endocrinol 2002;147:173-80.

94. Hotta K, Funahashi T, Arita Y et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595-9.

95. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004;145:2273-82.

96. Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? J Endocrinol Invest 2007;30:210-4.

97. Weyer C, Funahashi T, Tanaka S et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930-5.

98. Yamamoto Y, Hirose H, Saito I, Nishikai K, Saruta T. Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: two-year follow-up study in Japanese population. J Clin Endocrinol Metab 2004;89:87-90.

99. Tabak AG, Brunner EJ, Miller MA et al. Low Serum Adiponectin Predicts 10-Year Risk of Type 2 Diabetes and HbA1c Independently of Obesity, Lipids, and Inflammation: Whitehall II Study. Horm Metab Res 2009.

100. Nawrocki AR, Rajala MW, Tomas E et al. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. J Biol Chem 2006;281:2654-60.

101. Combs TP, Wagner JA, Berger J et al. Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. Endocrinology 2002;143:998-1007.

102. Kubota N, Terauchi Y, Kubota T et al. Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin dependent and independent pathway. J Biol Chem 2006.

103. Fisher FF, Trujillo ME, Hanif W et al. Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. Diabetologia 2005;48:1084-7.

104. Waki H, Yamauchi T, Kamon J et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem 2003;278:40352-63.

105. Yamauchi T, Kamon J, Ito Y et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762-9.

106. Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proc Natl Acad Sci U S A 2004;101:10308-13.

107. Kharroubi I, Rasschaert J, Eizirik DL, Cnop M. Expression of adiponectin receptors in pancreatic beta cells. Biochem Biophys Res Commun 2003;312:1118-22.

108. Gu W, Li X, Liu C et al. Globular adiponectin augments insulin secretion from pancreatic islet beta cells at high glucose concentrations. Endocrine 2006;30:217-21.

109. Staiger K, Stefan N, Staiger H et al. Adiponectin is functionally active in human islets but does not affect insulin secretory function or beta-cell lipoapoptosis. J Clin Endocrinol Metab 2005;90:6707-13.

110. Rasmussen MS, Lihn AS, Pedersen SB, Bruun JM, Rasmussen M, Richelsen B. Adiponectin receptors in human adipose tissue: effects of obesity, weight loss, and fat depots. Obesity (Silver Spring) 2006;14:28-35.

111. Narasimhan ML, Coca MA, Jin J et al. Osmotin is a homolog of mammalian adiponectin and controls apoptosis in yeast through a homolog of mammalian adiponectin receptor. Mol Cell 2005;17:171-80.

112. Yamauchi T, Nio Y, Maki T et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 2007;13:332-9.

113. Bjursell M, Ahnmark A, Bohlooly Y et al. Opposing effects of adiponectin receptors 1 and 2 on energy metabolism. Diabetes 2007;56:583-93.

114. Coope A, Milanski M, Araujo EP et al. AdipoR1 mediates the anorexigenic and insulin/leptin-like actions of adiponectin in the hypothalamus. FEBS Lett 2008;582:1471-6.

115. Guillod-Maximin E, Roy AF, Vacher CM et al. Adiponectin receptors are expressed in hypothalamus and colocalized with proopiomelanocortin and neuropeptide Y in rodent arcuate neurons. J Endocrinol 2009;200:93-105.

116. Kubota N, Yano W, Kubota T et al. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. Cell Metab 2007;6:55-68.

117. Kos K, Harte AL, da Silva NF et al. Adiponectin and resistin in human cerebrospinal fluid and expression of adiponectin receptors in the human hypothalamus. J Clin Endocrinol Metab 2007;92:1129-36.

118. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548-56.

119. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005;115:911-9.

120. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 2004;92:347-55.

121. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 2007;92:1023-33.

122. Rustan A.C and Drevon, C. A. Fatty Acids: Structures and Properties. Encyclopedia of Life Sciences. 2005. John Wiley & Sons, Inc.
Ref Type: Online Source

123. Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res 1999;40:1549-58.

124. Lehninger, Nelson, Cox. Principles of Biochemistry. Worth Publishers, 1993.

125. Connor WE. n-3 Fatty acids from fish and fish oil: panacea or nostrum? Am J Clin Nutr 2001;74:415-6.

126. Flachs P, Rossmeisl M, Bryhn M, Kopecky J. Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. Clin Sci (Lond) 2009;116:1-16.

127. Rustan AC, Hustvedt BE, Drevon CA. Dietary supplementation of very long-chain n-3 fatty acids decreases whole body lipid utilization in the rat. J Lipid Res 1993;34:1299-309.

128. Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS. Fish oil prevents insulin resistance induced by high-fat feeding in rats. Science 1987;237:885-8.

129. Ukropec J, Reseland JE, Gasperikova D et al. The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression. Lipids 2003;38:1023-9.

130. Puhakainen I, Ahola I, Yki-Jarvinen H. Dietary supplementation with n-3 fatty acids increases gluconeogenesis from glycerol but not hepatic glucose production in patients with non-insulin-dependent diabetes mellitus. Am J Clin Nutr 1995;61:121-6.

131. Delarue J, Couet C, Cohen R, Brechot JF, Antoine JM, Lamisse F. Effects of fish oil on metabolic responses to oral fructose and glucose loads in healthy humans. Am J Physiol 1996;270:E353-E362.

132. Borkman M, Chisholm DJ, Furler SM et al. Effects of fish oil supplementation on glucose and lipid metabolism in NIDDM. Diabetes 1989;38:1314-9.

133. Hendra TJ, Britton ME, Roper DR et al. Effects of fish oil supplements in NIDDM subjects. Controlled study. Diabetes Care 1990;13:821-9.

134. Mostad IL, Bjerve KS, Bjorgaas MR, Lydersen S, Grill V. Effects of n-3 fatty acids in subjects with type 2 diabetes: reduction of insulin sensitivity and time-dependent alteration from carbohydrate to fat oxidation. Am J Clin Nutr 2006;84:540-50.

135. Delarue J, Labarthe F, Cohen R. Fish-oil supplementation reduces stimulation of plasma glucose fluxes during exercise in untrained males. Br J Nutr 2003;90:777-86.

136. Couet C, Delarue J, Ritz P, Antoine JM, Lamisse F. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. Int J Obes Relat Metab Disord 1997;21:637-43.

137. Stene LC, Ulriksen J, Magnus P, Joner G. Use of cod liver oil during pregnancy associated with lower risk of Type I diabetes in the offspring. Diabetologia 2000;43:1093-8.

138. Norris JM, Yin X, Lamb MM et al. Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. JAMA 2007;298:1420-8.

139. Madsen L, Petersen RK, Kristiansen K. Regulation of adipocyte differentiation and function by polyunsaturated fatty acids. Biochim Biophys Acta 2005;1740:266-86.

140. Dreyer C, Keller H, Mahfoudi A, Laudet V, Krey G, Wahli W. Positive regulation of the peroxisomal beta-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). Biol Cell 1993;77:67-76.

141. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med 2004;10:355-61.

142. Raclot T, Groscolas R, Langin D, Ferre P. Site-specific regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues. J Lipid Res 1997;38:1963-72.

143. Shillabeer G, Hornford J, Forden JM, Wong NC, Lau DC. Hepatic and adipose tissue lipogenic enzyme mRNA levels are suppressed by high fat diets in the rat. J Lipid Res 1990;31:623-31.

144. Mater MK, Thelen AP, Pan DA, Jump DB. Sterol response element-binding protein 1c (SREBP1c) is involved in the polyunsaturated fatty acid suppression of hepatic S14 gene transcription. J Biol Chem 1999;274:32725-32.

145. Xu J, Nakamura MT, Cho HP, Clarke SD. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. J Biol Chem 1999;274:23577-83.

146. Nakatani T, Kim HJ, Kaburagi Y, Yasuda K, Ezaki O. A low fish oil inhibits SREBP-1 proteolytic cascade, while a high-fish-oil feeding decreases SREBP-1 mRNA in mice liver: relationship to anti-obesity. J Lipid Res 2003;44:369-79.

147. Yahagi N, Shimano H, Hasty AH et al. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 1999;274:35840-4.

148. Dallongeville J, Bauge E, Tailleux A et al. Peroxisome proliferator-activated receptor alpha is not rate-limiting for the lipoprotein-lowering action of fish oil. J Biol Chem 2001;276:4634-9.

149. Ren B, Thelen AP, Peters JM, Gonzalez FJ, Jump DB. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alpha. J Biol Chem 1997;272:26827-32.

150. Suchankova G, Tekle M, Saha AK, Ruderman NB, Clarke SD, Gettys TW. Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. Biochem Biophys Res Commun 2005;326:851-8.

151. Xue B, Kahn BB. AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. J Physiol 2006;574:73-83.

152. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. Endocrinology 2003;144:5179-83.

153. Wong AK, Howie J, Petrie JR, Lang CC. AMP-activated protein kinase pathway: a potential therapeutic target in cardiometabolic disease. Clin Sci (Lond) 2009;116:607-20.

154. Dobrzyn P, Dobrzyn A, Miyazaki M et al. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. Proc Natl Acad Sci U S A 2004;101:6409-14.

155. Dobrzyn A, Dobrzyn P, Lee SH et al. Stearoyl-CoA desaturase-1 deficiency reduces ceramide synthesis by downregulating serine palmitoyltransferase and increasing beta-oxidation in skeletal muscle. Am J Physiol Endocrinol Metab 2005;288:E599-E607.

156. Lee SH, Dobrzyn A, Dobrzyn P, Rahman SM, Miyazaki M, Ntambi JM. Lack of stearoyl-CoA desaturase 1 upregulates basal thermogenesis but causes hypothermia in a cold environment. J Lipid Res 2004;45:1674-82.

157. Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of gene expression. Nutr Rev 2004;62:333-9.

158. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 2007;447:869-74.

159. Aarsland A, Berge RK. Peroxisome proliferating sulphur- and oxy-substituted fatty acid analogues are activated to acyl coenzyme A thioesters. Biochem Pharmacol 1991;41:53-61.

160. Grav HJ, Asiedu DK, Berge RK. Gas chromatographic measurement of 3- and 4-thia fatty acids incorporated into various classes of rat liver lipids during feeding experiments. J Chromatogr B Biomed Appl 1994;658:1-10.

161. Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci U S A 1997;94:4312-7.

162. Westergaard M, Henningsen J, Svendsen ML et al. Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid. J Invest Dermatol 2001;116:702-12.

163. Berge K, Tronstad KJ, Flindt EN et al. Tetradecylthioacetic acid inhibits growth of rat glioma cells ex vivo and in vivo via PPAR-dependent and PPAR-independent pathways. Carcinogenesis 2001;22:1747-55.

164. Madsen L, Guerre-Millo M, Flindt EN et al. Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance. J Lipid Res 2002;43:742-50.

165. Berge RK, Tronstad KJ, Berge K et al. The metabolic syndrome and the hepatic fatty acid drainage hypothesis. Biochimie 2005;87:15-20.

166. Berge RK, Skorve J, Tronstad KJ, Berge K, Gudbrandsen OA, Grav H. Metabolic effects of thia fatty acids. Curr Opin Lipidol 2002;13:295-304.

167. Nakajima Y, Miyahara I, Hirotsu K et al. Three-dimensional structure of the flavoenzyme acyl-CoA oxidase-II from rat liver, the peroxisomal counterpart of mitochondrial acyl-CoA dehydrogenase. J Biochem 2002;131:365-74.

168. Zimmermann R, Strauss JG, Haemmerle G et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 2004;306:1383-6.

169. Arner P. Human fat cell lipolysis: biochemistry, regulation and clinical role. Best Pract Res Clin Endocrinol Metab 2005;19:471-82.

170. Frayn KN. Metabolic Regulation. A human perspective. Blackwell Publishing, Blackwell Science Ltd., 2003.

171. The Oslo Diet and Exercise Study (ODES): design and objectives. Control Clin Trials 1993;14:229-43.

172. Anderssen, S. A., Haaland, A., Hjermann, I., Urdal, P., Gjesdal, K, and Holme, I. Oslo Diet and Exercise Study: a one-year randomized intervention trial. Effect on hemostatic variables and other coronary risk factors. Nutr Metab Cardiovasc Dis 5, 189-200. 1995.

Ref Type: Generic

173. Anderssen S, Holme I, Urdal P, Hjermann I. Diet and exercise intervention have favourable effects on blood pressure in mild hypertensives: the Oslo Diet and Exercise Study (ODES). Blood Press 1995;4:343-9.

174. Anderssen SA, Hjermann I, Urdal P, Torjesen PA, Holme I. Improved carbohydrate metabolism after physical training and dietary intervention in individuals with the "atherothrombogenic syndrome'. Oslo Diet and Exercise Study (ODES). A randomized trial. J Intern Med 1996;240:203-9.

175. Anderssen SA, Holme I, Urdal P, Hjermann I. Associations between central obesity and indexes of hemostatic, carbohydrate and lipid metabolism. Results of a 1-year intervention from the Oslo Diet and Exercise Study. Scand J Med Sci Sports 1998;8:109-15.

176. Reseland JE, Anderssen SA, Solvoll K et al. Effect of long-term changes in diet and exercise on plasma leptin concentrations. Am J Clin Nutr 2001;73:240-5.

177. Torjesen PA, Birkeland KI, Anderssen SA, Hjermann I, Holme I, Urdal P. Lifestyle changes may reverse development of the insulin resistance syndrome. The Oslo Diet and Exercise Study: a randomized trial. Diabetes Care 1997;20:26-31.

178. Urdal P, Anderssen SA, Holme I et al. Monday and non-Monday concentrations of lifestyle-related blood components in the Oslo Diet and Exercise Study. J Intern Med 1998;244:507-13.

179. Jacobs DR, Jr., Sluik D, Rokling-Andersen MH, Anderssen SA, Drevon CA. Association of 1-y changes in diet pattern with cardiovascular disease risk factors and adipokines: results from the 1-y randomized Oslo Diet and Exercise Study. Am J Clin Nutr 2009;89:509-17.

180. Phillips SA, Ciaraldi TP, Oh DK, Savu MK, Henry RR. Adiponectin secretion and response to pioglitazone is depot dependent in cultured human adipose tissue. Am J Physiol Endocrinol Metab 2008;295:E842-E850.

181. Sessler AM, Kaur N, Palta JP, Ntambi JM. Regulation of stearoyl-CoA desaturase 1 mRNA stability by polyunsaturated fatty acids in 3T3-L1 adipocytes. J Biol Chem 1996;271:29854-8.

182. Mainieri D, Summermatter S, Seydoux J et al. A role for skeletal muscle stearoyl-CoA desaturase 1 in control of thermogenesis. FASEB J 2006;20:1751-3.

183. Attie AD, Scherer PE. Adipocyte metabolism and obesity. J Lipid Res 2008.

184. Minokoshi Y, Shiuchi T, Lee S, Suzuki A, Okamoto S. Role of hypothalamic AMP-kinase in food intake regulation. Nutrition 2008;24:786-90.

185. Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett 2008;582:74-80.

186. Kusminski CM, McTernan PG, Schraw T et al. Adiponectin complexes in human cerebrospinal fluid: distinct complex distribution from serum. Diabetologia 2007;50:634-42.

187. Spranger J, Verma S, Gohring I et al. Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 2006;55:141-7.

188. Weiss EP, Racette SB, Villareal DT et al. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am J Clin Nutr 2006;84:1033-42.

Paper I

This article is removed.

Paper II

Rokling-Andersen MH, Rustan AC, Wensaas AJ, Kaalhus O, Wergedahl H, Røst TH, Jensen J, Graff BA, Caesar R, Drevon CA. Marine n-3 fatty acids promote size-reduction of visceral adipose depots, without altering body weight and composition, in male Wistar rats fed a highfat diet. Br J Nutr. 2009 Apr 28; 1-12. (Epub ahead of print).

doi:10.1017/S0007114509353210

The original publication is available at Cambridge University Press

Access to the published version may require journal subscription.

British Journal of Nutrition (2009), page 1 of 12
doi:10.1017/S0007114509353210
© The Authors 2009

Marine *n*-3 fatty acids promote size reduction of visceral adipose depots, without altering body weight and composition, in male Wistar rats fed a high-fat diet

Merethe H. Rokling-Andersen[1], Arild C. Rustan[2], Andreas J. Wensaas[1], Olav Kaalhus[3], Hege Wergedahl[4], Therese H. Røst[4,5], Jørgen Jensen[6], Bjørn A. Graff[7], Robert Caesar[1] and Christian A. Drevon[1]*

[1]*Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Blindern, 0316 Oslo, Norway*
[2]*Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway*
[3]*Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway*
[4]*Institute of Medicine, University of Bergen, Bergen, Norway*
[5]*Department of Medicine, Haukeland University Hospital, Bergen, Norway*
[6]*National Institute of Occupational Health, Oslo, Norway*
[7]*Norwegian Knowledge Centre for the Health Services, Oslo, Norway*

(Received 8 December 2008 – Revised 2 March 2009 – Accepted 12 March 2009)

We evaluated the effects of partly substituting lard with marine *n*-3 fatty acids (FA) on body composition and weight, adipose tissue distribution and gene expression in five adipose depots of male Wistar rats fed a high-fat diet. Rats were fed diets including lard (19·5 % lard) or *n*-3 FA (9·1 % lard and 10·4 % Triomar™) for 7 weeks. Feed consumption and weight gain were similar, whereas plasma lipid concentrations were lower in the *n*-3 FA group. Magnetic resonance imaging revealed smaller visceral (mesenteric, perirenal and epididymal) adipose depots in the *n*-3 FA-fed animals (35, 44 and 32 % reductions, respectively). *n*-3 FA feeding increased mRNA expression of cytokines as well as chemokines in several adipose depots. Expression of *Adipoq* and *Pparg* was enhanced in the mesenteric adipose depots of the *n*-3 FA-fed rats, and fasting plasma insulin levels were lowered. Expression of the lipogenic enzymes *Acaca* and *Fasn* was increased in the visceral adipose depots, whereas *Dgat1* was reduced in the perirenal and epididymal depots. *Cpt2* mRNA expression was almost doubled in the mesenteric depot and liver. Carcass analyses showed similar body fat (%) in the two feeding groups, indicating that *n*-3 FA feeding led to redistribution of fat away from the visceral compartment.

Marine *n*-3 fatty acids: Body composition: Visceral adipose depots: Gene expression

Numerous studies in animals, populations and clinical trials have revealed beneficial effects of *n*-3 very-long-chain PUFA in health and disease. Marine oils contain high proportions of the *n*-3 very-long-chain PUFA EPA and DHA. Dietary intake of these fatty acids (FA) may delay the development of atherosclerosis and reduce the risk of CVD. Moreover, dietary intake of *n*-3 FA decreases postprandial concentrations of NEFA and plasma VLDL concentrations[1–3]. Experiments in cell models have elucidated the mechanisms behind the lipid-lowering effects. Incubation of cultured rat hepatocytes with EPA reduces cholesterol and TAG esterification by inhibiting acyl coenzyme A:cholesterol acyltransferase and acyl coenzyme A:1,2 diacylglycerol acyltransferase, respectively[4,5]. This, in turn, inhibits synthesis and secretion of VLDL[5,6]. The TAG-lowering effect of marine *n*-3 FA is also mediated *via* stimulation of FA oxidation in liver and to a smaller extent in skeletal muscle[7]. Replacing dietary saturated fat with *n*-3 FA has been shown to promote decreased whole-body lipid utilisation and increased carbohydrate utilisation in rats[8].

The risk of developing type 2 diabetes mellitus and CVD is markedly enhanced with visceral adiposity as compared with subcutaneous distribution of fat[9]. There are regional differences between adipose tissue depots with respect to expression of enzymes in lipolytic and anti-lipolytic pathways, uptake and release of NEFA, as well as adipokine production[10]. Several studies have shown that *n*-3 FA feeding reduces the size of perirenal and epididymal white adipose depots[1,11–13]. Rustan *et al.* showed that this effect was associated with a reduction in adipocyte size in these depots[1]. Belzung *et al.* showed in high-fat-fed rats that *n*-3 FA selectively limited the hypertrophy of retroperitoneal and epididymal adipose depots, with no effect on other major depots and no hyperplasia in the retroperitoneal depot[11].

Abbreviations: AOAC, Association of Official Analytical Chemists; CRP, C-reactive protein; FA, fatty acid; I, intensity; MR, magnetic resonance; MRI, magnetic resonance imaging; ROI, region of interest; TZD, thiazolidinedione.

* **Corresponding author:** Professor Christian A. Drevon, fax +47 22851393, email c.a.drevon@medisin.uio.no

More knowledge is needed about the effect of dietary fat on whole-body fat distribution and adipose tissue depot functions.

In the present study we report results from a feeding experiment with rats, where we aimed to elucidate the effects of long-term dietary supply of marine *n*-3 FA on whole-body composition, as well as sizes and functions of adipose tissues evaluated by gene expression analysis. The reference group was fed a lard-enriched high-fat diet, whereas the *n*-3 FA group had one-third of the lard substituted with concentrated EPA and DHA. We also provide gene expression analyses for forty-four genes involved in energy metabolism and inflammation for five different adipose depots (subcutaneous, mesenteric, perirenal, epididymal and interscapular), as well as the liver. Interscapular adipose tissue may under certain conditions contain a high proportion of brown adipose tissue[14], whereas the other depots primarily consist of white adipose tissue. We also performed some metabolic assays and plasma analyses of lipids and adipokines, and carcass analyses to evaluate the effect of *n*-3 FA feeding on whole-body composition. To our knowledge, we are the first to report a comprehensive study of genes involved in lipogenesis and lipid metabolism, as well as adipokines, in an *n*-3 FA feeding study.

Materials and methods

Animals

Male rats of the Wistar strain (SPF, Mol) were purchased from Møllegaard Breeding Centre (Ejby, Denmark). The rats were fed *ad libitum* a low-fat reference diet (chow) for 1 week, before a high-fat feeding regimen with two semi-synthetic diets (see below) for 49 d. The body weights of the animals were within the range 211–265 g at the start of the experimental feeding, approximately aged 7 weeks. The rats were randomly divided into two groups with ten animals in each group and housed in individual cages. The temperature in the animal quarters was 21 ± 1°C, the humidity was 55 ± 10 % and the dark period was from 19.00 to 07.00 hours. The rats were given free access to tap water. The protocol was approved by the National Animal Research Authority.

Diets

Each animal group was offered one of two semi-synthetic diets: lard (19·5 % lard, Erica Lard; Ten Kate Vetten BV, Musselkanaal, The Netherlands) or *n*-3 FA (9·1 % lard and 10·4 % Triomar™ (EPAX5500); Pronova Biocare, Lysaker, Norway). Triomar contained > 55 % of total *n*-3 FA as TAG: EPA, 300 mg/g; DHA, 190 mg/g; total *n*-3 FA, 580 mg/g (total *n*-3: EPA, DHA, 18 : 3, 18 : 4, 20 : 4, 21 : 5, 22 : 5). This dose represents about 3·6 % of total energy intake of the rats and is comparable with traditional Inuit intakes of marine FA[15]. In addition, 1·5 % of soyabean oil (Mills Soyaolje; Denofa Lilleborg, Fredrikstad, Norway) was provided to both dietary groups to avoid essential FA deficiency. The dietary composition (g/100 g) was: maize starch, 31·5; fat, 21·5; sucrose, 20; casein, 20; salt mixture, 5; vitamin mixture, 1·5; cellulose, 1. The diets provided approximately 40 % of the energy from fat. The diets were kept at −20°C and given to the rats in portions sufficient for 1 d supply.

The FA composition of the experimental diets is given in Table 1. The *n*-3 FA diet included 17·4 % EPA and 10·1 % DHA, whereas the lard diet included 0·03 % or less of these very-long-chain *n*-3 PUFA. The lard diet was particularly rich in the MUFA oleic acid (18 : 1*n*-9; 36·2 % of the total FA), and also consisted of a high amount of the SFA palmitic acid (16 : 0; 25·3 %) and stearic acid (18 : 0; 13·1 %). For determination of FA composition, lipids were extracted by a mixture of chloroform and methanol[16]. The extracts were added heneicosanoic acid (21 : 0) as internal standard. To remove neutral sterols and non-saponifiable material, the extracts were heated in 0·5 M-KOH in an ethanol–water solution. Recovered FA were re-esterified using BF_3–methanol. The methyl esters were quantified by GLC as previously described[17].

Experimental protocol

The rats were offered 20 g/d of the experimental diets in a tray that allowed no spilling of the pasty diet, and individual daily feed intake was recorded. The intake of *n*-3 FA was on average 1·25 g/d in the *n*-3 FA group calculated from analysis of FA composition of the diet (Table 1) and an average feed intake of 18 g/animal per d. Body weight was registered twice weekly. At the end of the feeding period, five animals in each group were used for the estimation of adipose depot volumes by magnetic resonance imaging (MRI), dissection

Table 1. Fatty acid composition of the experimental diets (% total fatty acids)*

Fatty acid	Lard	*n*-3
14 : 0	1·6	0·9
15 : 0	0·07	0·06
16 : 0	25·3	14·5
16 : 1*n*-7	1·9	1·5
16 : 1*n*-9	0·2	0·1
17 : 0	0·3	0·4
18 : 0	13·1	8·8
18 : 1*n*-7	2·6	2·8
18 : 1*n*-9	36·2	22·4
18 : 2*n*-6	15	10·1
18 : 3*n*-3	1·3	1·3
18 : 3*n*-6	0·02	0·1
18 : 4*n*-3	0	1·4
20 : 0	0·2	0·2
20 : 1*n*-7	0·04	0·1
20 : 1*n*-9	0·6	0·9
20 : 1*n*-11	0·02	0·07
20 : 2*n*-6	0·3	0·3
20 : 3*n*-6	0·09	0·1
20 : 3*n*-9	0·03	0·06
20 : 4*n*-3	0	0·6
20 : 4*n*-6	0·2	1·0
20 : 5*n*-3	0·01	17·4
22 : 0	0·05	0·1
22 : 1	0	0·4
22 : 4*n*-6	0·08	0·1
22 : 5*n*-3	0·9	1·6
22 : 5*n*-6	0	0·3
22 : 6*n*-3	0·03	10·1
24 : 1*n*-9	0	0·8

* Data are presented as the average of three measurements.

and carcass analysis, whereas the other five rats were used for other analyses of plasma and several tissues.

Plasma analysis

The rats were anaesthetised with 20 mg pentobarbital intraperitoneally (50 mg/ml). Blood was collected by aortic puncture, mixed with 0·1 % EDTA and immediately chilled on ice. Plasma was prepared and stored at −70°C before analyses. Plasma lipids were measured enzymically on the Technicon Axon system (Miles, Tarrytown, NY, USA) using the following kits: TAG (Bayer, Tarrytown, NY, USA), phospholipids (PAP150; BioMerieux, Lyon, France), total cholesterol (Bayer) and NEFA (NEFA C; Wako Chemicals, Dalton, OH, USA). Plasma glucose was measured enzymically on the Technicon Axon system (Miles, NY) using the Gluco-quant kit (Roche, Mannheim, Germany). Plasma levels of TNFα, IL-6, IL-10, C-reactive protein (CRP) and insulin were measured using commercial ELISA. Samples were analysed in duplicates, and the intra-assay CV were as follows: TNFα (Bender MedSystems, Vienna, Austria), 12·4 %; IL-6 (Bender MedSystems), 8·1 %; CRP (Alpha Diagnostic International, San Antonio, TX, USA), 1·8 %; insulin (Linco Research, St Charles, MO, USA), 3·5 %. Plasma levels of IL-10 (BioSource International, Camarillo, CA, USA) were below the detection limit of the assay. Leptin and adiponectin were measured by competitive RIA (Linco Research) with the use of [^{125}I]leptin and [^{125}I]adiponectin, respectively, as tracers. The intra-assay CV were 4·4 % for leptin and 7·7 % for adiponectin.

Dissection

From killed rats, mesenteric adipose tissue was obtained by stripping out the whole mesenterium from the duodenum to the appendix. Subcutaneous fat was dissected from the lower abdominal part on the left side in an area of about 2 × 2 cm. Epididymal fat was taken from the region around the testis and epididymis on the right-hand side. Perirenal fat included the depot located around the right kidney and suprarenal gland in addition to the abdominal pelvic depot as described by Murano *et al.* [18]. The interscapular adipose depots were obtained by dissecting the white superficial and the deeper brown fat between the shoulder blades.

Magnetic resonance imaging

The rats were killed with pentobarbital intraperitoneal injections and mounted in a supine position in a plastic bed in a home-built, solenoid-type double Cu sheet induction coil, 30 cm long and 100 mm diameter with an unloaded Q-factor of 435. The coil was positioned transversely in the middle of the coil of a General Electric SIGNA 1.5 T clinical magnetic resonance (MR) scanner (General Electric Medical Systems, Milwaukee, WI, USA). An external attenuator was used in addition to the internal attenuation to reduce the transmission signal amplitude to a suitable value.

The rats were scanned in sagittal, coronal and axial planes with a fast spin echo (FSE) T_1 sequence, TE/TR = 13/100 ms (where TE is the echo time after excitation and TR the MR sequence repetition time). To enhance the signals from fat, the frequency was centred on the fat peak, f_{fat} about 63 880 220 Hz. The forty sagittal and coronal slices were interleaved with a thickness of 2 mm, an image acquisition matrix of 256 × 160 and a field of view (FOV) of 250 × 156 mm. The sixty-four axial image slices were interleaved with a thickness of 4 mm, an acquisition matrix of 256 × 256 and a FOV of 80 × 80 mm. Only one excitation (per specific MR sequence; number of excitations (NEX) = 1) was used, giving a total scan time of approximately 10 min per rat.

Magnetic resonance imaging analysis

Interactive data language (IDL) software (RSI International (UK) Ltd, Crowthorne, Berkshire, UK) was used to develop a program where calculations were carried out over voxels satisfying certain inclusion criteria within specified regions of interest (ROI). The calculations involved counting and averaging over the voxels (the three-dimensional analogue of a pixel), and the inclusion criteria were usually values above certain thresholds. The voxels satisfying the criteria were depicted through a coloured overlay region over the original MR image within the present ROI. Preliminary measurements over regions with essentially no fat or pure fat established an intensity (I) scale for fat content in the different fat depots. The width of the intensity distribution in the fat depot ROI was much larger than the width measured in homogeneous adipose tissues, the latter giving a quasi-Gaussian high-intensity peak with a width of only 3–4 % of the peak intensity value. We assumed the low-intensity tail above the fat threshold to be due mainly to partial volume effects, and evaluated the fat content by linear interpolation of the established intensity scale. We used a threshold, I_{25}, corresponding to approximately 25 % fat on this scale, to evaluate the number of voxels (N_{vox}) with intensity larger than I_{25} in the present ROI, each multiplied by the fat content, I/I_{fat}, in this low-intensity region. The fat threshold value I_{25} produced overlay images that seemed to coincide with regions characterised as fat by visual inspection. The total tissue (fat and non-fat) threshold was chosen to be the voxel intensity value giving an overlay image coinciding maximally with the outline of the MR image of the animal. Sagittal and coronal slices were imaged with identical MRI settings and threshold values. Because axial slices were imaged with different resolution and thickness as compared with the sagittal and coronal slices, we used different threshold values providing similar results for total tissue and total body fat as the evaluation in the other two planes.

In addition to the total fat content, MRI volumes of the following fat depots were evaluated: interscapular, perirenal, mesenteric, subcutaneous abdominal, and epididymal. The sagittal, coronal or axial plane images were chosen for evaluation depending on in which plane the boundaries of the fat depot were most clearly depicted. In some cases images from two, or even all three planes were analysed for comparison.

Carcass analysis

After MRI analysis, the five rats from each feeding group were separately autoclaved at 121°C for about 30 min and

transferred to a custom-made homogeniser. Water containing a foam-reducing agent (Antifoam E100 conc.; Bayer Chemicals AG, Leverkusen, Germany) was added (1:1) before starting the homogeniser. After 2 min in the blender, the constituents were completely homogenised. Total fat percentage was determined by extracting the lipids from a part of the homogenate with petroleum ether at 100°C, and weighing the extracted material (Tecator™ application note AN 77/85 1985.03.15, Association of Official Analytical Chemists (AOAC) method 960.39 and AOAC method 945.16)[19]. Protein was determined by the Kjeldahl method (Tecator™ application note: Determination of Kjeldahl Protein in Fish and Fish-products using the Kjeltec Auto system 1983.02.01 ASN 56/83 (Cu catalyst), AOAC method 981.10[19]). Water was determined by desiccation of a freeze-dried part of each homogenate, and ash was determined by heating the dried material to 550°C for 18–20 h totally and weighing the remains.

Glucose transport and glycogen content in soleus muscle

Glucose uptake and glycogen content were measured in epitrochlearis muscles and in soleus muscle strips as described by Jensen *et al.* [20].

Hepatic enzyme activities

The livers were homogenised and fractionated[21], and the activities of acyl-CoA synthetase[22], carnitine palmitoyltransferase-II[23] and acyl-CoA oxidase[24] were determined in the post-nuclear fractions.

Adipose tissue and gene expression analyses

Mesenteric, subcutaneous, perirenal, epididymal and interscapular adipose tissue depots and liver were collected from each rat and snap-frozen in liquid N_2 before storage at -70°C. The tissues were pulverised with an ice-cold steel pestle and mortar. Total RNA was isolated from 100 mg tissue using the RNeasy Lipid Tissue Mini Kit from Qiagen (Venlo, The Netherlands). RNA was quantified by spectrophotometry (NanoDrop 1000; NanoDrop Technologies, Waltham, MA, USA), and the integrity was evaluated by capillary electrophoresis (Agilent 2100 Bioanalyser; Agilent Technologies, Inc., Santa Clara, CA, USA). Total RNA (400 ng) was reversely transcribed in 20 μl reactions using the High Capacity cDNA Reverse Transcription Kit with RNase inhibitor (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's directions. Real-time PCR was performed with custom-made 384-well microfluidic cards (TaqMan Low Density Arrays; Applied Biosystems). Forty-four genes of interest were selected, as well as four endogenous controls, and analysed in duplicates. Official symbols and full names of the genes, as well as Applied BioSystems' product codes, are given in Table 2. The expression values of each gene in all samples were normalised against the average of the endogenous controls. *18S* and *Arbp* varied significantly between the lard and *n*-3 groups in mesenteric fat, and were therefore excluded as endogenous controls in this depot.

Adipose tissue extraction and adipokine protein analyses

Approximately 0·1 g frozen, comminuted adipose tissue was mixed with 0·4 ml lysis buffer (1 M-2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)-HCl, 1 M-NaCl, 85 % glycerol), 0·5 M-EDTA (pH 8) and Complete protease inhibitor cocktail (Roche, Basel, Switzerland), and immediately homogenised for 1 min using an Ultra-Turrax device. Samples were centrifuged for 15 min at 3000 ***g***, the floating fat layers were discarded and the aqueous portions of the samples were centrifuged for another 15 min at 15 000 ***g***. Total protein concentrations were measured using a Multiskan Plus reader (Titertek, Labsystem, Helsinki, Finland). All samples were diluted to a total protein concentration of 0·5 μg/μl. Concentrations of adiponectin, monocyte chemoattractant protein 1, leptin, IL-1β, IL-6, TNFα and plasminogen activator inhibitor-1 (total) were measured using a rat adipocyte LINCO*plex* kit (RADPCYT-82K; Linco Research) according to the manufacturer's protocol. The samples were analysed in tetra- or pentaplicates using a Bio-Plex 200 instrument (Bio-Rad, Richmond, CA, USA).

Statistics

Values are reported as mean values and standard deviations for ten animals per group in Fig. 1, and mean values with their standard errors from four or five animals per group in the remaining Figs. 2–6 and Tables 2 and 4. Independent-samples *t* tests were used to compare the lard and *n*-3 FA groups. Significant differences in MRI volumes between the two diet groups were found by *t* tests. Correlation coefficients were calculated between dissection weights and volumes (determined by MRI) of adipose depots (Table 3). A 5 % level of significance was applied in all analyses.

Results

Animals and diets

Both experimental diets contained the same amount of energy (per g), and the rats were individually offered 20 g/d of the respective diets throughout the 7-week feeding period. There were no differences in average weight gain in the two groups of animals (Fig. 1(a)). The average amount of feed consumed by the rats in the *n*-3 FA group and the reference lard group was also indistinguishable (Fig. 1(b)). To determine if there were differences in body composition in the two dietary groups, we performed carcass analyses with no significant differences in the content of fat, protein, ash or water between the two groups after 7 weeks of feeding (Fig. 1(c)).

Plasma analyses

Plasma concentrations of TAG, phospholipids and cholesterol were decreased by 56, 41 and 40 %, respectively, after 7 weeks of feeding in the *n*-3 FA-fed as compared with the lard-fed animals (Fig. 2(a)). Plasma NEFA were reduced non-significantly in the *n*-3 FA group. There were no significant differences in plasma levels of adiponectin, leptin, CRP, TNFα, IL-6 or IL-10 between the groups (data not shown). Plasma insulin concentrations were markedly lower (72 %) in the *n*-3 FA-fed animals (Fig. 2(b)), whereas plasma glucose concentrations were similar in both groups (data not shown).

Glucose uptake and glycogen content in skeletal muscle

To investigate the insulin response of skeletal muscle after the experimental feeding, glucose uptake was measured *in vitro* in epitrochlearis muscles and in soleus muscle strips. There were no significant differences in either basal or insulin-stimulated glucose uptake in soleus muscle strips (Fig. 3) and epitrochlearis (data not shown) between the two dietary groups. The amount of glycogen in the epitrochlearis muscle was measured as 155 (SEM 9) mmol/kg dry weight in the *n*-3 FA group and 173 (SEM 8) mmol/kg in the lard group. In soleus, the glycogen content was 122 (SEM 16) and 130 (SEM 12) mmol/kg dry weight in the *n*-3 FA-fed group and lard-fed group, respectively, with no significant differences in muscle glycogen content between the two groups.

Hepatic enzyme activity

The hepatic enzyme activities of acyl-CoA synthetase, acyl-CoA oxidase and carnitine palmitoyltransferase-II were significantly increased in the *n*-3 FA group as compared with the lard group, by 92, 17 and 68 %, respectively (Fig. 4).

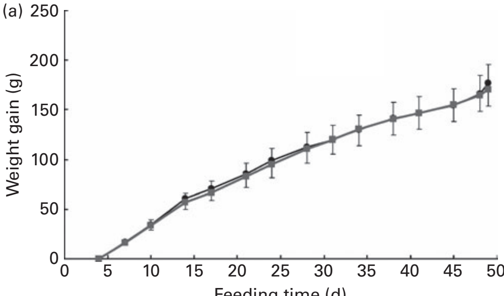
Table 2. Official symbol, official full name and Applied Biosystems' product code for the forty-four genes selected, as well as the four endogenous controls

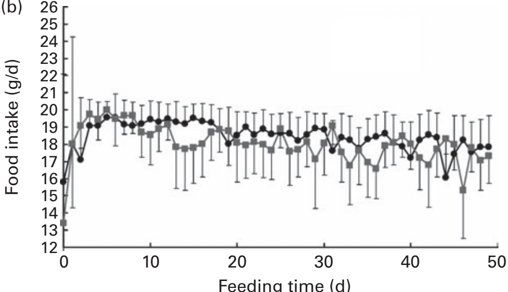
Official gene symbol	Official full name	Applied BioSystems' product code*
Acaca	Acetyl-coenzyme A carboxylase α	Rn00573474_m1 Acaca
Acbd3	Acyl-coenzyme A binding domain containing 3	Rn00788231_m1 Acbd3
Ace	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	Rn00561094_m1 Ace
Acsl1	Acyl-CoA synthetase long-chain family member 1	Rn00563137_m1 Acsl1
Adfp	Adipose differentiation related protein	Rn01472318_m1 Adfp
Adipoq	Adiponectin	Rn00595250_m1 Adipoq
Apln	Apelin, AGTRL1 ligand	Rn00581093_m1 Apln
Ccl2	Chemokine (C-C motif) ligand 2; also named MCP-1	Rn00580555_m1 Ccl2
Cpt1a	Carnitine palmitoyltransferase 1a, liver	Rn00580702_m1 Cpt1a
Cpt2	Carnitine palmitoyltransferase 2	Rn00563995_m1 Cpt2
Cxcl1	Chemokine (C-X-C motif) ligand 1; also named CINC-1; GRO1	Rn00578225_m1 Cxcl1
Dgat1	Diacylglycerol *O*-acyltransferase 1	Rn00584870_m1 Dgat1
Fabp4	Fatty acid binding protein 4, adipocyte	Rn00670361_m1 Fabp4
Fabp5	Fatty acid binding protein 5, epidermal	Rn00821817_g1 Fabp5
Fasn	Fatty acid synthase	Rn00569117_m1 Fasn
Hgf	Hepatocyte growth factor	Rn00566673_m1 Hgf
Hsd11b2	Hydroxysteroid 11-β dehydrogenase 2	Rn00492539_m1 Hsd11b2
Il10	Interleukin 10	Rn00563409_m1 Il10
Il1b	Interleukin 1β	Rn00580432_m1 Il1b
Il6	Interleukin 6	Rn00561420_m1 Il6
Lep	Leptin	Rn00565158_m1 Lep
Lipe	Lipase, hormone sensitive	Rn00563444_m1 Lipe
Lpl	Lipoprotein lipase	Rn00561482_m1 Lpl
Mt1a	Metallothionein 1a	Rn00821759_g1 Mt1a
Nr1h3	Nuclear receptor subfamily 1, group H, member 3; also named LXR α	Rn00581185_m1 Nr1h3
Pbef1	Pre-B-cell colony enhancing factor 1; also named visfatin	Rn00822046_m1 Pbef1
Pklr	Pyruvate kinase, liver and red blood cell	Rn00561764_m1 Pklr
Plin	Perilipin	Rn00558672_m1 Plin
Ppara	Peroxisome proliferator activated receptor α	Rn00566193_m1 Ppara
Pparg	Peroxisome proliferator activated receptor γ	Rn00440945_m1 Pparg
Prkaa1	Protein kinase, AMP-activated, α 1 catalytic subunit	Rn00569558_m1 Prkaa1
Prkaa2	Protein kinase, AMP-activated, α 2 catalytic subunit; also named AMPK	Rn00576935_m1 Prkaa2
Rbp4	Retinol binding protein 4, plasma	Rn01451318_m1 Rbp4
Retn	Resistin	Rn00595224_m1 Retn
RGD1652323	Similar to fatty acid translocase/CD36	Rn00580728_m1 RGD1562323_predicted Cd36
Scd1	Stearoyl-coenzyme A desaturase 1	Rn00594894_g1 Scd1
Serpine1	Serine (or cysteine) peptidase inhibitor, clade E, member 1; also named PAI-1	Rn00561717_m1 Serpine1
Slc27a1	Solute carrier family 27 (fatty acid transporter), member 1; also named FATP-1	Rn00585821_m1 Slc27a1
Slc2a4	Solute carrier family 2 (facilitated glucose transporter), member 4; also named GLUT-4	Rn00562597_m1 Slc2a4
Tgfb1	Transforming growth factor, β 1	Rn00572010_m1 Tgfb1
Tnf	Tumour necrosis factor	Rn99999017_m1 Tnf
Ucp1	Uncoupling protein 1	Rn00562126_m1 Ucp1
Ucp2	Uncoupling protein 2	Rn00571166_m1 Ucp2
Ucp3	Uncoupling protein 3	Rn00565874_m1 Ucp3
Arbp†	Acidic ribosomal phosphoprotein P0	Rn00821065_g1 Arbp
Gapdh†	Glyceraldehyde-3-phosphate dehydrogenase	Rn99999916_s1 Gapdh
Ppif†	Peptidylprolyl isomerase F (cyclophilin F)	Rn00597197_m1 Ppif
18S†		Hs99999901_s1

AGTRL1, angiotensin receptor-like 1; CINC-1; cytokine-induced neutrophil chemoattractant 1; GRO1, growth-related oncogene 1; MCP-1, monocyte chemoattractant protein 1; LXR, liver X receptor; AMPK, AMP-activated protein kinase; PAI-1, plasminogen activator inhibitor-1.

* Applied BioSystems, Foster City, CA, USA.

† Endogenous controls.





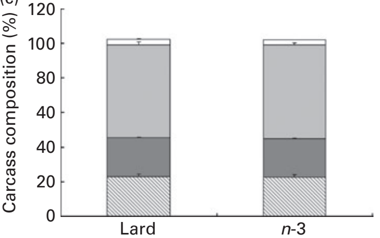


Fig. 1. (a) Body-weight gain in the two dietary groups (–●–, lard; –■–, *n*-3 fatty acids) during the 7 weeks of feeding. Values are means for ten rats per group, with standard deviations represented by vertical bars. The average start weight was 240 g. (b) Daily feed intake in the two dietary groups (–●–, lard; –■–, *n*-3 fatty acids) during the 7 weeks of feeding. Values are means for ten rats per group, with standard deviations represented by vertical bars. (c) Body composition in the two dietary groups determined by carcass analysis, shown as percentage of ash (□), water (▒), protein (■) and fat (▧) in autoclaved rats. Values are means for five rats per group, with standard errors represented by vertical bars.

Adipose tissue depots

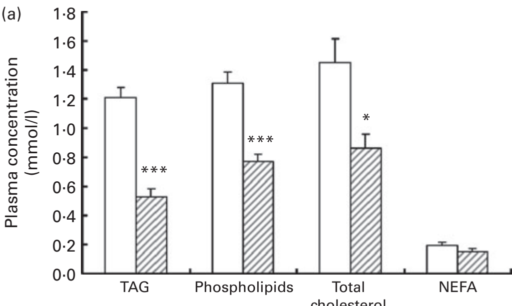
Five adipose tissue depots were dissected and weighed, and volumes were estimated using MRI on killed whole animals (Fig. 5). Dissection weights of the perirenal and epididymal depots were significantly reduced by 51 and 31 % after *n*-3 FA feeding as compared with the lard feeding, respectively, whereas the volume estimated by MRI was reduced by 43 and 32 % in these depots. There was no significant difference in dissection weights of the mesenteric adipose depots. Estimated MRI volume of the mesenteric depot was, however, significantly reduced by 35 %. There were no significant differences between the two feeding groups in weight or volume of interscapular and subcutaneous adipose depots. The correlation coefficients between estimated volume and dissection weight of the fat depots were significant and in the range 0·67–0·84 for subcutaneous, perirenal, epididymal and interscapular fat, whereas it was 0·39 and non-significant for the mesenteric depot (Table 3). Representative MR images of the different adipose depots are shown in Fig. 6.

Table 3. Correlation coefficients between depot volume estimated by magnetic resonance imaging and depot weight obtained by dissection

Fat depot	Correlation coefficient	*P*
Subcutaneous	0·81	$<0·001$
Mesenteric	0·39	0·15
Perirenal	0·84	$<0·001$
Epidymal	0·82	$<0·001$
Interscapular	0·67	0·006

Adipose tissue extracts

The concentrations of several adipokines (adiponectin, monocyte chemoattractant protein 1, leptin, IL-1β, IL-6, TNFα and plasminogen activator inhibitor-1 (total)) were determined in aqueous extracts from the five different adipose tissue depots. No statistically significant differences were observed between the two dietary groups (data not shown).



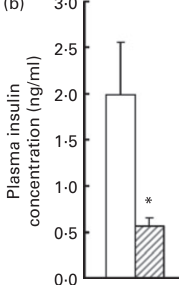


Fig. 2. Plasma concentrations of TAG, phospholipids, total cholesterol and NEFA (a) and insulin (b) in the two dietary groups (□, lard; ▨, *n*-3 fatty acids) after 7 weeks of feeding. Values are means for five rats per group, with standard errors represented by vertical bars. Mean value was significantly different from that of the lard group: * $P<0·05$, *** $P\leq0·001$ (*t* test).

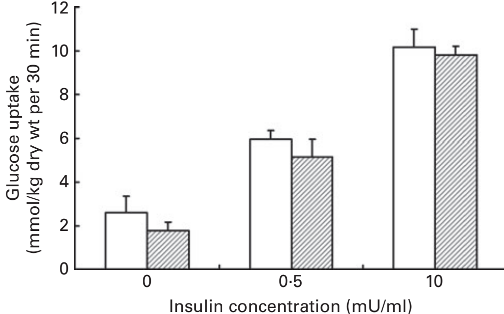


Fig. 3. Glucose uptake in dissected soleus skeletal muscle strips in the two dietary groups (□, lard; ▨, *n*-3 fatty acids) after 7 weeks of feeding. The muscle strips were incubated without insulin or with 0·5 and 10 mU insulin/ml, and glucose uptake was determined and calculated from the intracellular accumulation of 2-deoxy-D-[^{3}H]glucose. Values are means for five rats per group, with standard errors represented by vertical bars.

Gene expression analysis

The effects of *n*-3 FA feeding on mRNA expression of forty-four selected genes in five adipose depots and liver are presented in Table 4. Several cytokines and chemokines (*Il10*, *Il1b*, *Il6*, *Cxcl1*, *Ccl2*, *Mt1a*, *Retn*, *Tnf*) were significantly increased (1·5- to 13-fold) in the five depots in the *n*-3 FA-fed as compared with the lard-fed animals. The adipogenic transcription factor *Pparg* was increased 4-fold in the mesenteric depot in the *n*-3 FA group. The lipogenic enzymes *Acaca* and *Fasn*, as well as *Fabp5*, were enhanced in the mesenteric, perirenal (*Fasn* not significantly) and epididymal depots, whereas they were reduced in the interscapular depot containing significant amounts of brown adipose tissue. *Dgat1* was reduced in the perirenal and epididymal depots. *Scd1* was significantly reduced in the epididymal, perirenal and interscapular depots, as well in the liver of the *n*-3 FA-fed rats. *Ucp2* mRNA expression was doubled in the subcutaneous depots of *n*-3 FA-fed rats, whereas *Ucp3* was reduced in the perirenal adipose depots. mRNA expression of *Cpt2*, which is involved in FA transport and β-oxidation in mitochondria, was almost doubled in the mesenteric depot and liver, and reduced in the epididymal depot. Expression of *Adfp*, which is a lipid droplet-associated protein, was 2- to 3-fold increased in the epididymal and perirenal depots. The insulin-sensitising adipokine *Adipoq* (adiponectin) was 4-fold increased in the mesenteric depot. We observed that the RNA yield was lower, and the quality higher, from the mesenteric depots of the *n*-3 FA-fed rats compared with the lard-fed animals. This may reflect some contamination by pancreatic tissue in the lard-fed rats because we observed expression of the pancreatic markers *Ela1* and *Prss1* in some mesenteric adipose tissue samples[25].

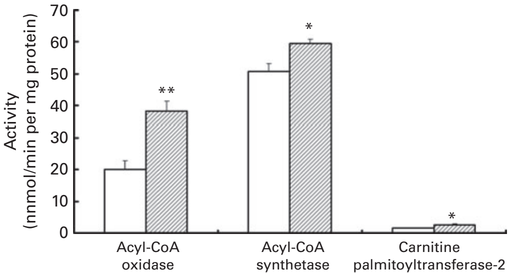


Fig. 4. Activities of acyl-CoA synthetase, acyl-CoA oxidase and carnitine palmitoyltransferase-II in livers from lard-fed (□) and *n*-3 fatty acid-fed (▨) rats after 7 weeks of feeding. The livers were homogenised and fractionated, and the enzyme activities were measured in post-nuclear fractions. Acyl-CoA oxidase was measured by a spectrophotometric assay, whereas acyl-CoA synthetase and carnitine palmitoyltransferase-II activities were measured utilising radioactive labelled substrates. Values are means for five rats per group, with standard errors represented by vertical bars. Mean value was significantly different from that of the lard group: * $P<0·05$, ** $P<0·01$ (*t* test).

Discussion

By using MRI, we showed that the mesenteric adipose depots are significantly smaller in the *n*-3 FA-fed animals as compared with lard-fed animals. Also epididymal and perirenal adipose depots were reduced in *n*-3 FA-fed animals in agreement with previous reports[1,12,13]. The mesenteric, perirenal and epididymal depots are all located in the visceral compartment inside the peritoneal cavity. A reduction in size of these depots is important because visceral adiposity is associated with the metabolic syndrome and is a risk factor for developing CVD and type 2 diabetes mellitus[9,26–28].

Waist:hip ratio or waist circumference is emerging as a better risk marker for CVD than BMI because the latter does not take into account the distribution of body fat. Results from the INTERHEART study show a protective effect of an increased hip circumference (reflecting subcutaneous storage of fat on the hips and thighs) related to risk of myocardial infarction[9,26]. Dietary marine oils limit the TAG accumulation in perirenal and epididymal adipose tissue, reducing hypertrophy of the adipocytes[11,13]. Rustan *et al.* [1] have previously shown that *n*-3 FA feeding of rats reduced adipocyte cell volume in perirenal and epididymal adipose depots, whereas the cell volume was unaltered in the mesenteric and

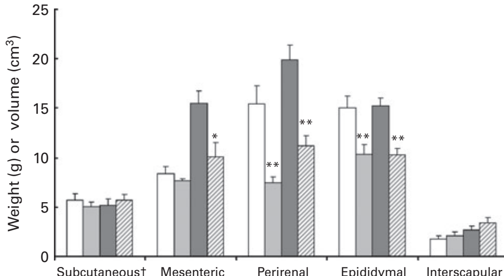


Fig. 5. Size of adipose tissue depots determined by dissection and weighing, and volume estimated by magnetic resonance imaging (MRI) of five depots in lard- and *n*-3 fatty acid-fed rats after 7 weeks of feeding. (□), Dissection weight, lard group; (▨), dissection weight, *n*-3 fatty acid group; (■), MRI volume, lard group; (▨), MRI volume, *n*-3 fatty acid group. Values are means, with standard errors represented by vertical bars. Mean value was significantly different from that of the lard group: * $P<0·05$, ** $P<0·01$ (*t* test). † The lower abdominal part on the left side of the subcutaneous adipose depot in an area of about 2 × 2 cm.

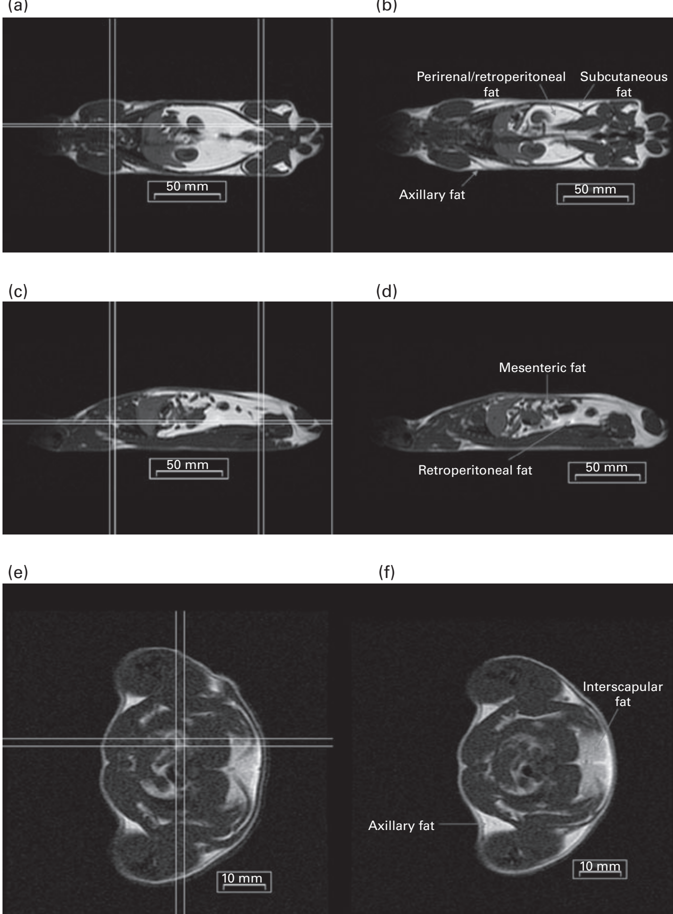


Fig. 6. Magnetic resonance (MR) images of adipose depots of representative rats in different planes after 7 weeks of feeding. Coronal MR images from the lard (a) and *n*-3 fatty acid (b) groups, showing perirenal/retroperitoneal, axillary and subcutaneous adipose depots. Sagittal MR images of left sections of the lard (c) and *n*-3 fatty acid (d) groups showing mesenteric and retroperitoneal adipose tissue depots. Axial MR images of the lard (e) and *n*-3 fatty acid (f) groups showing axillary and interscapular fat. Sections corresponding to the sagittal (2 mm) and axial (4 mm) slices are shown in (a), sections corresponding to the coronal (2 mm) and axial (4 mm) slices are shown in (c) and sections corresponding to the coronal (2 mm) and sagittal (2 mm) slices are shown in (e).

subcutaneous depots. Although we found the visceral adipose depots to be reduced in the *n*-3 FA-fed rats, we found no differences between the two groups in total weight gain, which is in agreement with Kusunoki *et al.* [29] and Pérez-Matute *et al.* [30]. Moreover, there were no differences between the two groups in body composition as determined by our whole-body carcass analysis.

By dissecting out and weighing the mesenteric, perirenal, interscapular and epididymal adipose depots, as well as subcutaneous adipose tissue on the left side of the lower abdominal part, we accounted for approximately 28 % of total body fat in the rats. Carcass analyses showed similar body fat percentage in the two groups. Thus, our finding of smaller mesenteric, perirenal and epididymal adipose depots in the *n*-3 FA-fed group suggests that *n*-3 FA feeding promoted a redistribution of adipose tissue, rather than a reduction in the total amount of fat. Both the dissection and MRI analysis included only the lower abdominal part on the left side of the subcutaneous depots, thereby providing low sensitivity for detecting differences in the two dietary groups in this depot. It is therefore possible that the animals in the *n*-3 FA group had more subcutaneous fat in total than the lard-fed group, because the MRI-estimated volume of the left abdominal subcutaneous adipose depot was higher (not statistically significant) in the *n*-3 FA-fed animals. In addition, expression of the lipolytic enzyme *Lipe* was reduced by 60 % in the subcutaneous depot. Studies on thiazolidinediones (TZD) have shown that the weight gain following treatment is due primarily to enhanced subcutaneous adiposity, accompanied by reduced visceral adiposity and intrahepatic TAG accumulation[31,32].

Table 4. Effects of 7 weeks of *n*-3 fatty acid (FA) feeding on mRNA expression levels in five adipose depots and liver†
(Mean values with their standard errors for four to five rats per group)

		Subcutaneous		Mesenteric		Perirenal		Epididymal		Interscapular		Liver	
Gene	Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Acaca	Lard	1·00	0·05	1·00	0·11	1·00	0·15	1·00	0·09	1·00	0·08	1·00	0·18
	n-3 FA	1·72***	0·15	3·60*	0·91	1·97*	0·30	1·78*	0·31	0·55**	0·11	0·56	0·04
Acbd3	Lard	1·00	0·12	1·00	0·06	1·00	0·16	1·00	0·06	1·00	0·08	1·00	0·05
	n-3 FA	0·92	0·12	1·22	0·14	1·07	0·06	0·81*	0·05	0·75*	0·04	0·88	0·05
Ace	Lard	1·00	0·12	1·00	0·61	1·00	0·18	1·00	0·24	1·00	0·09	1·00‡	0·01
	n-3 FA	1·11	0·47	1·24	0·48	0·62	0·08	0·52	0·02	0·69*	0·05	1·69	0·33
Acsl1	Lard	1·00	0·23	1·00	0·22	1·00	0·19	1·00	0·11	1·00	0·05	1·00	0·08
	n-3 FA	0·71	0·09	3·41*	1·05	0·72	0·06	0·70*	0·03	1·00	0·10	1·16	0·05
Adfp	Lard	1·00	0·24	1·00	0·20	1·00	0·16	1·00	0·08	1·00	0·15	1·00	0·19
	n-3 FA	2·55	0·71	2·39	0·64	2·52**	0·27	2·27***	0·19	1·08	0·17	0·76	0·17
Adipoq	Lard	1·00	0·21	1·00	0·20	1·00	0·13	1·00	0·09	1·00	0·06	1·00‡	0·07
	n-3 FA	0·53	0·14	4·11*	1·30	0·83	0·08	0·85	0·03	1·05	0·06	2·09	1·11
Apln	Lard	1·00	0·17	1·00	0·24	1·00	0·15	1·00	0·24	1·00	0·26	1·00‡	0·06
	n-3 FA	0·75	0·14	2·40*	0·48	0·51*	0·03	0·52	0·09	1·32	0·16	1·03	0·08
Ccl2	Lard	1·00	0·48	1·00	0·22	1·00	0·05	1·00	0·15	1·00	0·36	1·00‡	0·19
	n-3 FA	0·58	0·16	7·33*	2·67	2·49**	0·39	1·69*	0·18	3·62	1·89	0·70	0·07
Cd36§	Lard	1·00	0·19	1·00	0·26	1·00	0·15	1·00	0·07	1·00	0·10	1·00	0·27
	n-3 FA	0·46*	0·09	2·91	0·85	0·92	0·04	0·93	0·10	1·08	0·13	1·64	0·10
Cpt1a	Lard	1·00	0·17	1·00	0·08	1·00	0·16	1·00	0·17	1·00	0·27	1·00	0·20
	n-3 FA	1·73	0·63	0·96	0·05	1·04	0·06	0·84	0·05	0·85	0·12	1·28	0·06
Cpt2	Lard	1·00	0·13	1·00	0·09	1·00	0·06	1·00	0·06	1·00	0·10	1·00	0·04
	n-3 FA	1·34	0·37	1·83*	0·34	0·83	0·12	0·80*	0·03	0·89	0·12	1·78***	0·06
Cxcl1	Lard	1·00	0·43	1·00	0·17	1·00	0·23	1·00	0·22	1·00‡	0·14	1·00	0·63
	n-3 FA	0·91	0·66	11·56*	4·39	1·46	0·47	1·14	0·15	1·34	0·20	0·38	0·17
Dgat1	Lard	1·00	0·15	1·00	0·05	1·00	0·11	1·00	0·07	1·00	0·08	1·00	0·05
	n-3 FA	0·70	0·07	3·22	1·08	0·71*	0·03	0·80*	0·03	1·03	0·13	1·16	0·05
Fabp4	Lard	1·00	0·20	1·00	0·19	1·00	0·16	1·00	0·10	1·00	0·04	1·00	0·09
	n-3 FA	0·53	0·11	3·89	1·33	0·94	0·07	0·87	0·05	0·97	0·13	1·21	0·39
Fabp5	Lard	1·00	0·17	1·00	0·24	1·00	0·16	1·00	0·13	1·00	0·11	1·00	0·24
	n-3 FA	8·47	7·06	1·82*	0·28	1·51*	0·07	1·66**	0·14	0·60**	0·03	0·46	0·05
Fasn	Lard	1·00	0·30	1·00	0·19	1·00	0·20	1·00	0·14	1·00	0·06	1·00	0·46
	n-3 FA	2·68	0·98	3·77*	1·13	1·98	0·40	2·10*	0·43	0·40***	0·08	0·15	0·03
Hgf	Lard	1·00	0·21	1·00	0·23	1·00	0·13	1·00	0·10	1·00‡	0·26	1·00	0·15
	n-3 FA	0·40*	0·10	1·67	0·25	1·16	0·09	1·30	0·10	1·23	0·16	1·01	0·04
Hsd11b2	Lard	1·00	0·22	1·00‡	0·12	1·00	0·27	1·00	0·09	1·00‡	0·12	1·00	0·15
	n-3 FA	0·54	0·22	3·68*	1·05	0·69	0·09	0·99	0·10	1·98	1·24	0·77	0·23
Il10	Lard	1·00	0·32	1·00‡	0·18	1·00	0·19	1·00	0·22	1·00‡	0·41	1·00‡	0·22
	n-3 FA	0·38	0·05	13·55	9·98	1·78	0·44	1·73*	0·20	0·90	0·46	1·07	0·15
Il1b	Lard	1·00	0·34	1·00	0·26	1·00	0·24	1·00	0·28	1·00‡	0·12	1·00	0·14
	n-3 FA	0·65	0·25	2·23*	0·35	4·89***	0·71	2·02	0·40	1·08	0·06	1·45*	0·04
Il6	Lard	1·00‡	0·81	1·00	0·32	1·00	0·26	1·00	0·22	1·00‡	0·28	1·00‡	0·26
	n-3 FA	0·57	0·48	13·13*	4·36	3·55	1·33	1·96*	0·24	3·28	1·08	1·63	0·96
Lep	Lard	1·00	0·22	1·00	0·27	1·00	0·09	1·00	0·13	1·00	0·14	1·00‡	0·25
	n-3 FA	0·50	0·12	3·35	1·23	0·70	0·07*	0·65	0·09	0·67	0·16	7·25**	0·20
Lipe	Lard	1·00‡	0·11	1·00‡	0·10	1·00	0·18	1·00	0·24	1·00‡	0·25	NQ‡	
	n-3 FA	0·40**	0·05	3·30*	1·00	0·93	0·12	0·84	0·09	0·63	0·06	NQ	
Lpl	Lard	1·00	0·12	1·00	0·22	1·00	0·13	1·00	0·09	1·00	0·13	1·00	0·18
	n-3 FA	0·97	0·09	3·31*	1·00	0·89	0·10	0·77*	0·05	1·01	0·08	0·87	0·17
Mt1a	Lard	1·00	0·16	1·00	0·34	1·00	0·16	1·00	0·14	1·00	0·49	1·00	0·28
	n-3 FA	1·70	0·34	1·29	0·35	2·28**	0·26	1·52*	0·17	0·81	0·15	2·66	0·70
Nr1h3	Lard	1·00	0·17	1·00	0·13	1·00	0·12	1·00	0·08	1·00	0·29	1·00	0·12
	n-3 FA	0·46*	0·06	2·31	0·58	1·03	0·07	0·95	0·04	0·52	0·04	0·90	0·07
Pbef	Lard	1·00	0·12	1·00	0·24	1·00	0·06	1·00	0·07	1·00	0·12	1·00	0·08
	n-3 FA	1·96	0·57	2·58	0·68	0·92	0·03	1·02	0·10	0·82	0·16	1·99	0·33
Pklr	Lard	1·00‡	0·39	1·00‡	0·45	1·00‡	0·25	1·00‡	0·55	1·00‡	0·30	1·00	0·05
	n-3 FA	0·33	0·07	0·24	0·08	1·72	0·77	0·04	0·02	1·82	1·13	0·47***	0·01
Plin	Lard	1·00	0·21	1·00	0·17	1·00	0·17	1·00	0·10	1·00	0·03	1·00‡	0·12
	n-3 FA	0·50	0·13	3·73	1·32	0·70	0·05	0·73*	0·03	1·04	0·12	1·97	1·14
Ppara	Lard	1·00	0·09	1·00	0·16	1·00	0·10	1·00	0·06	1·00	0·19	1·00	0·13
	n-3 FA	0·91	0·13	1·24	0·39	0·93	0·30	0·70**	0·06	0·98	0·10	0·81	0·08
Pparg	Lard	1·00	0·27	1·00	0·14	1·00	0·17	1·00	0·11	1·00	0·17	1·00	0·28
	n-3 FA	0·36	0·10	3·42*	1·05	0·77	0·02	0·83	0·03	0·89	0·08	1·94	0·28
Prkaa1	Lard	1·00	0·13	1·00	0·17	1·00	0·10	1·00	0·08	1·00	0·07	1·00	0·15
	n-3 FA	0·71	0·03	1·70	0·31	0·89	0·05	0·89	0·03	0·94	0·16	1·18	0·11
Prkaa2	Lard	1·00	0·19	1·00	0·27	1·00	0·19	1·00	0·10	1·00	0·24	1·00	0·04
	n-3 FA	3·53*	1·19	1·14	0·33	0·48*	0·03	0·72*	0·04	0·58	0·07	1·37*	0·10

Table 4. *Continued*

Gene	Diet	Subcutaneous		Mesenteric		Perirenal		Epididymal		Interscapular		Liver	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Rbp4	Lard	1·00	0·21	1·00	0·22	1·00	0·13	1·00	0·13	1·00	0·07	1·00	0·09
	n-3 FA	0·40	0·12	3·74*	1·16	0·89	0·10	0·83	0·05	0·92	0·16	0·95	0·02
Retn	Lard	1·00	0·49	1·00	0·29	1·00	0·20	1·00	0·13	1·00	0·06	1·00‡	0·28
	n-3 FA	0·92	0·38	2·97*	0·81	0·95	0·09	0·96	0·11	0·63**	0·08	0·97	0·37
Scd1	Lard	1·00	0·27	1·00	0·19	1·00	0·11	1·00	0·08	1·00	0·20	1·00	0·15
	n-3 FA	0·54	0·10	0·87	0·38	0·18***	0·05	0·17***	0·07	0·20**	0·04	0·28**	0·08
Serpine1	Lard	1·00	0·26	1·00	0·21	1·00	0·26	1·00	0·15	1·00	0·15	1·00	0·17
	n-3 FA	1·11	0·42	6·99	2·94	0·91	0·16	0·85	0·11	0·59	0·19	1·49	0·48
Slc27a1	Lard	1·00	0·08	1·00	0·12	1·00	0·13	1·00	0·10	1·00	0·11	1·00	0·07
	n-3 FA	0·77	0·14	2·17*	0·36	1·16	0·14	1·25	0·11	0·48**	0·08	0·91	0·03
Slc2a4	Lard	1·00	0·21	1·00	0·16	1·00	0·12	1·00	0·09	1·00	0·03	1·00‡	0·12
	n-3 FA	0·45*	0·05	4·08	1·42	0·95	0·07	1·05	0·08	0·52***	0·04	0·69	0·10
Tgfb1	Lard	1·00	0·18	1·00	0·33	1·00	0·10	1·00	0·10	1·00	0·11	1·00	0·08
	n-3 FA	0·56	0·04	1·16	0·18	2·04***	0·05	1·74***	0·06	0·93	0·05	1·05	0·03
Tnf	Lard	1·00‡	0·10	1·00	0·27	1·00	0·13	1·00	0·11	1·00‡	0·14	1·00‡	0·13
	n-3 FA	1·23	0·13	1·57	0·43	1·86**	0·16	1·27	0·09	1·18	0·19	1·87	0·44
Ucp1	Lard	1·00‡	0·43	1·00‡	0·30	1·00	0·64	1·00‡	0·54	1·00	0·09	1·00‡	0·07
	n-3 FA	0·09	0·03	2·25	0·88	4·30	3·92	1·43	0·70	1·03	0·25	0·09	0·00
Ucp2	Lard	1·00	0·15	1·00	0·35	1·00	0·15	1·00	0·06	1·00	0·19	1·00	0·07
	n-3 FA	2·13*	0·52	1·17	0·19	1·28	0·06	1·08	0·06	1·20	0·18	0·94	0·04
Ucp3	Lard	1·00	0·28	1·00	0·29	1·00	0·11	1·00	0·12	1·00	0·15	NQ‡	
	n-3 FA	1·39	0·44	2·74	1·17	0·50**	0·04	0·75	0·11	0·96	0·10	NQ	

NQ, gene expression level not quantifiable.
Mean value was significantly different from that of the lard group: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (*t* test).
† The fold increase or reduction in the *n*-3 FA group as compared with the lard group is shown. Expression levels of target genes were normalised against the endogenous controls *18S*, *Arbp*, *Ppif* and *Gapdh*. In the mesenteric adipose depot, *18S* and *Arbp* varied significantly between the animals fed lard and *n*-3 FA, and were therefore excluded as endogenous controls. For explanation of gene symbols, see Table 2.
‡ Genes expressed at very low levels (Ct > 30).
§ The official gene symbol for *Cd36* is *RGD1652323*.

Because *n*-3 FA also activate *Pparg*, we suggest that there might be redistribution of adipose tissue from the visceral depots to the subcutaneous depot in the *n*-3 FA-fed animals.

The lower correlation coefficients between dissection weight and volume estimated by MRI for the mesenteric fat as compared with the other adipose tissue depots may reflect the difficulty of dissecting out this depot precisely. Fissoune *et al.* reported MRI measurements of two adipose tissue depots in mice, but did not validate against dissection weights as we have done[33]. MRI might represent a reliable non-invasive method and a more precise alternative to dissection in certain situations.

Expression of the adipogenic transcription factor *Pparg* was increased 3·4-fold in the mesenteric depot of *n*-3 FA-fed animals. As observed for the TZD, the *n*-3 FA EPA and DHA are good agonists for PPARγ[34,35], contrary to SFA predominantly found in lard. Activation of PPARγ is important for adipocyte differentiation[36]. Our finding that *Pparg* expression is increased in response to dietary *n*-3 FA is supported by Chambrier *et al.* who showed that EPA induced PPARγ gene expression in isolated human adipocytes[37]. This has also been shown in human skeletal muscle cells[38]. PPARγ activation may promote fat accumulation in subcutaneous depots, with reduced or unchanged visceral storage[39]. Also, some *ex vivo* preadipocyte studies have shown that abdominal subcutaneous preadipocytes differentiate in response to TZD more readily than cells from visceral depots of the same subjects[39]. A point to consider for all genes, and nuclear receptors in particular, is that gene expression levels provide limited information on their activities. The presence of cofactors, heterodimerisation, ligand availability and translocation to the nucleus are also of importance.

It is unexpected that *Acaca* (encoding acetyl-coenzyme A carboxylase α) expression, was increased in all four white adipose depots in the *n*-3 FA-fed animals, and *Fasn* (encoding FA synthase) was increased in the mesenteric and epididymal adipose depots. This may suggest enhanced synthesis of FA in these depots. However, *Dgat1* expression (encoding diacylglycerol acyltransferase) was reduced in the perirenal and epididymal adipose depots of the *n*-3 FA-fed animals. This is in line with decreased fat accumulation in these depots. It is possible that the simultaneous increase in the expression of the lipogenic enzymes *Acaca* and *Fasn*, with reduced *Dgat1* expression, reflects increased turnover with futile cycling of FA. Guan *et al.* have shown that glycerol kinase, which is normally not expressed in adipocytes, was induced by TZD in adipocytes, and propose a futile fuel cycle as a mechanism for TZD action[40] although this is controversial[41].

The hepatic activities of carnitine palmitoyltransferase-II and acyl-CoA oxidase were increased in the animals fed the *n*-3 FA diet, which might suggest that FA oxidation was elevated in these animals as compared with lard-fed rats. In addition, hepatic *Cpt2* mRNA was increased in the *n*-3 FA group in accordance with Halvorsen *et al.* [42]. Increased hepatic mitochondrial oxidation of FA may partially explain the reduction in plasma TAG observed in the *n*-3 FA group[7,43].

We observed that the mRNA levels of several cytokines and chemokines such as *Il1b*, *Tnfa*, *Tgfb1*, *Il6*, *Cxcl1*, *Ccl2* and *Retn*, and *Il10*, were increased in the mesenteric, perirenal

and/or epididymal adipose depots of the *n*-3 FA-fed animals as compared with the lard-fed animals. The metabolism of cytokines and chemokines is complex, and several of these proteins have both pro- and anti-inflammatory properties[44]. The biological effect of these findings is therefore difficult to interpret. For example, we do not know if cytokines secreted from skeletal muscle during exercise, such as IL-6, are beneficial or harmful[45,46]. The effect of *n*-3 FA feeding appears to be autocrine or paracrine in adipose tissue because we did not observe altered concentrations in plasma of TNFα, IL-6, IL-10, nor of the acute-phase protein CRP, after *n*-3 FA feeding. This could also be due to a low contribution by adipose tissue to the plasma pool of these factors. Moreover, it is possible that the increased expression of cytokines and chemokines reflects a lower proportion of adipocytes relative to leucocytes located in the adipose tissue[47,48], as well as a dilution of nuclear material in hypertrophic adipose tissue.

The effects observed in the present study may be due to an increased proportion of EPA and/or DHA, or to the reduction in content of SFA, although it is most likely that the effects are due to *n*-3 FA.

In conclusion, by substituting some dietary lard with very-long-chain *n*-3 FA, the volumes of the visceral adipose depots (mesenteric, perirenal and epididymal) in rats were markedly reduced. This occurred without affecting total body weight and body composition, suggesting that *n*-3 FA feeding redistributed fat within the body. The gene expression of several cytokines and chemokines was increased in different adipose depots, with unaltered plasma concentrations of the corresponding proteins.

Acknowledgements

We thank Anne Randi Enget, Mari-Ann Baltzersen and Oddrun Gudbrandsen for excellent technical assistance. We also thank Karsten Eilertsen, The Norwegian Radium Hospital, for help in developing the IDL program for fat depot analysis.

The contributions of the authors were as follows: M. H. R.-A. conducted the plasma analyses of adipokines and insulin, isolated and quality checked total RNA, conducted gene expression analyses and wrote the manuscript. A. C. R. planned and carried out the feeding experiment. A. J. W. isolated and quality checked total RNA, conducted gene expression analyses and carcass analyses. O. K. and B. A. G. performed the MRI analysis. H. W. and T. H. R. conducted the enzyme activity assays and plasma analyses of lipids and glucose. J. J. conducted the glucose uptake and glycogen content assays. R. C. conducted the adipose tissue extraction and adipokine analysis in tissue extracts. C. A. D. planned and carried out the feeding experiment, and coordinated the project. All authors contributed to revision of the manuscript.

The present study was supported by grants from the Freia Chocolade Fabriks Medical Foundation, Direktør Johan Throne Holst Foundation for Nutrition Research, Norwegian Health Association (The Norwegian Council on Cardiovascular Diseases), Lipgene (Integrated Project 6th Framework Programme, Food Quality & Safety; FOOD-CT-2003-505944) and NuGO (Nutrigenomics, a Network of Excellence, CT2004-505944).

None of the authors has any conflicts of interest.

References

1. Rustan AC, Hustvedt BE & Drevon CA (1998) Postprandial decrease in plasma unesterified fatty acids during *n*-3 fatty acid feeding is not caused by accumulation of fatty acids in adipose tissue. *Biochim Biophys Acta* **1390**, 245–257.
2. Harris WS (1997) *n*-3 Fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* **65**, Suppl., 1645S–1654S.
3. Harris WS (1997) *n*-3 Fatty acids and serum lipoproteins: animal studies. *Am J Clin Nutr* **65**, Suppl., 1611S–1616S.
4. Rustan AC, Nossen JO, Osmundsen H, *et al.* (1988) Eicosapentaenoic acid inhibits cholesterol esterification in cultured parenchymal cells and isolated microsomes from rat liver. *J Biol Chem* **263**, 8126–8132.
5. Nossen JO, Rustan AC, Gloppestad SH, *et al.* (1986) Eicosapentaenoic acid inhibits synthesis and secretion of triacylglycerols by cultured rat hepatocytes. *Biochim Biophys Acta* **879**, 56–65.
6. Rustan AC, Nossen JO, Christiansen EN, *et al.* (1988) Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A:1,2-diacylglycerol acyltransferase. *J Lipid Res* **29**, 1417–1426.
7. Ukropec J, Reseland JE, Gasperikova D, *et al.* (2003) The hypotriglyceridemic effect of dietary *n*-3 FA is associated with increased β-oxidation and reduced leptin expression. *Lipids* **38**, 1023–1029.
8. Rustan AC, Hustvedt BE & Drevon CA (1993) Dietary supplementation of very long-chain *n*-3 fatty acids decreases whole body lipid utilization in the rat. *J Lipid Res* **34**, 1299–1309.
9. Yusuf S, Hawken S, Ounpuu S, *et al.* (2005) Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case–control study. *Lancet* **366**, 1640–1649.
10. Lafontan M & Berlan M (2003) Do regional differences in adipocyte biology provide new pathophysiological insights? *Trends Pharmacol Sci* **24**, 276–283.
11. Belzung F, Raclot T & Groscolas R (1993) Fish oil *n*-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. *Am J Physiol* **264**, R1111–R1118.
12. Hill JO, Peters JC, Lin D, *et al.* (1993) Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. *Int J Obes Relat Metab Disord* **17**, 223–236.
13. Parrish CC, Pathy DA & Angel A (1990) Dietary fish oils limit adipose tissue hypertrophy in rats. *Metabolism* **39**, 217–219.
14. Cannon B & Nedergaard J (2004) Brown adipose tissue: function and physiological significance. *Physiol Rev* **84**, 277–359.
15. Dyerberg J (1989) Coronary heart disease in Greenland Inuit: a paradox. Implications for Western diet patterns. *Arctic Med Res* **48**, 47–54.
16. Bligh EG & Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
17. Wergedahl H, Liaset B, Gudbrandsen OA, *et al.* (2004) Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol, and lowers acyl-CoA:cholesterol acyltransferase activity in liver of Zucker rats. *J Nutr* **134**, 1320–1327.
18. Murano I, Zingaretti MC & Cinti S (2005) The adipose organ of SV129 mice contains a prevalence of brown adipocytes and shows plasticity after cold exposure. *Adipocytes* **2**, 121–130.
19. Association of Official Analytical Chemists (1990) *Official Methods of Analysis*, 15th ed. Arlington,VA: AOAC.
20. Jensen J, Jebens E, Brennesvik EO, *et al.* (2006) Muscle glycogen inharmoniously regulates glycogen synthase activity, glucose uptake, and proximal insulin signaling. *Am J Physiol Endocrinol Metab* **290**, E154–E162.

21. Berge RK, Flatmark T & Osmundsen H (1984) Enhancement of long-chain acyl-CoA hydrolase activity in peroxisomes and mitochondria of rat liver by peroxisomal proliferators. *Eur J Biochem* **141**, 637–644.
22. Gudbrandsen OA, Wergedahl H, Liaset B, *et al.* (2005) Dietary proteins with high isoflavone content or low methionine-glycine and lysine-arginine ratios are hypocholesterolaemic and lower the plasma homocysteine level in male Zucker fa/fa rats. *Br J Nutr* **94**, 321–330.
23. Madsen L, Froyland L, Dyroy E, *et al.* (1998) Docosahexaenoic and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation. *J Lipid Res* **39**, 583–593.
24. Small GM, Burdett K & Connock MJ (1985) A sensitive spectrophotometric assay for peroxisomal acyl-CoA oxidase. *Biochem J* **227**, 205–210.
25. Caesar R & Drevon CA (2008) Pancreatic contamination of mesenteric adipose tissue samples can be avoided by adjusted dissection procedures. *J Lipid Res* **49**, 1588–1594.
26. Yusuf S, Hawken S, Ounpuu S, *et al.* (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case–control study. *Lancet* **364**, 937–952.
27. Pais P, Pogue J, Gerstein H, *et al.* (1996) Risk factors for acute myocardial infarction in Indians: a case–control study. *Lancet* **348**, 358–363.
28. Dagenais GR, Yi Q, Mann JF, *et al.* (2005) Prognostic impact of body weight and abdominal obesity in women and men with cardiovascular disease. *Am Heart J* **149**, 54–60.
29. Kusunoki M, Tsutsumi K, Hara T, *et al.* (2003) Ethyl icosapentate (omega-3 fatty acid) causes accumulation of lipids in skeletal muscle but suppresses insulin resistance in OLETF rats. *Metabolism* **52**, 30–34.
30. Pérez-Matute P, Perez-Echarri N, Martinez JA, *et al.* (2007) Eicosapentaenoic acid actions on adiposity and insulin resistance in control and high-fat-fed rats: role of apoptosis, adiponectin and tumour necrosis factor-α. *Br J Nutr* **97**, 389–398.
31. Kelly IE, Han TS, Walsh K, *et al.* (1999) Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. *Diabetes Care* **22**, 288–293.
32. Carey DG, Cowin GJ, Galloway GJ, *et al.* (2002) Effect of rosiglitazone on insulin sensitivity and body composition in type 2 diabetic patients [corrected]. *Obes Res* **10**, 1008–1015.
33. Fissoune R, Pellet N, Chaabane L, *et al.* (2004) Evaluation of adipose tissue distribution in obese fa/fa Zucker rats by *in vivo* MR imaging: effects of peroxisome proliferator-activated receptor agonists. *MAGMA* **17**, 229–235.
34. Xu HE, Lambert MH, Montana VG, *et al.* (1999) Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. *Mol Cell* **3**, 397–403.
35. Forman BM, Chen J & Evans RM (1997) Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc Natl Acad Sci U S A* **94**, 4312–4317.
36. de Souza CJ, Eckhardt M, Gagen K, *et al.* (2001) Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. *Diabetes* **50**, 1863–1871.
37. Chambrier C, Bastard JP, Rieusset J, *et al.* (2002) Eicosapentaenoic acid induces mRNA expression of peroxisome proliferator-activated receptor γ. *Obes Res* **10**, 518–525.
38. Aas V, Rokling-Andersen MH, Kase ET, *et al.* (2005) Eicosapentaenoic acid (20 : 5 *n*-3) increases fatty acid and glucose uptake in cultured human skeletal muscle cells. *J Lipid Res* **47**, 366–374.
39. Semple RK, Chatterjee VK & O'Rahilly S (2006) PPARγ and human metabolic disease. *J Clin Invest* **116**, 581–589.
40. Guan HP, Li Y, Jensen MV, *et al.* (2002) A futile metabolic cycle activated in adipocytes by antidiabetic agents. *Nat Med* **8**, 1122–1128.
41. Tan GD, Debard C, Tiraby C, *et al.* (2003) A 'futile cycle' induced by thiazolidinediones in human adipose tissue? *Nat Med* **9**, 811–812.
42. Halvorsen B, Rustan AC, Madsen L, *et al.* (2001) Effects of long-chain monounsaturated and *n*-3 fatty acids on fatty acid oxidation and lipid composition in rats. *Ann Nutr Metab* **45**, 30–37.
43. Froyland L, Madsen L, Vaagenes H, *et al.* (1997) Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. *J Lipid Res* **38**, 1851–1858.
44. Opal SM & DePalo VA (2000) Anti-inflammatory cytokines. *Chest* **117**, 1162–1172.
45. Nielsen AR & Pedersen BK (2007) The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. *Appl Physiol Nutr Metab* **32**, 833–839.
46. Pedersen BK, Akerstrom TC, Nielsen AR, *et al.* (2007) Role of myokines in exercise and metabolism. *J Appl Physiol* **103**, 1093–1098.
47. Einstein FH, Atzmon G, Yang XM, *et al.* (2005) Differential responses of visceral and subcutaneous fat depots to nutrients. *Diabetes* **54**, 672–678.
48. Fain JN (2006) Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. *Vitam Horm* **74**, 443–477.

Paper III

This article is removed.

Paper IV

This article is removed.