HEALTH SCIENCES

Maija Vaittinen

The Role of Weight Reduction Sensitive Genes on Adipose Tissue Biology and Adipocyte Function

Special Emphasis on the Expression of MFAP5 and CPPED1

Publications of the University of Eastern Finland
Dissertations in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

MAIJA VAITTINEN

THE ROLE OF WEIGHT REDUCTION SENSITIVE GENES IN ADIPOSE TISSUE BIOLOGY AND ADIPOCYTE FUNCTION

Special emphasis on the expression of MFAP5 and CPPED1

To be presented by permission of the Faculty of Health Sciences, University of Eastern Finland for public examination in Auditorium, Mediteknia building, Kuopio, on Friday, December 20th 2013, at 12 noon

Author's address: Department of Clinical Nutrition
Institute of Public Health and Clinical Nutrition
University of Eastern Finland
KUOPIO
FINLAND

Supervisors: Adjunct Professor Leena Pulkkinen, Ph.D.
Department of Clinical Nutrition
Institute of Public Health and Clinical Nutrition
University of Eastern Finland
KUOPIO
FINLAND

Adjunct Professor Marjukka Kolehmainen, Ph.D.
Department of Clinical Nutrition
Institute of Public Health and Clinical Nutrition
University of Eastern Finland
KUOPIO
FINLAND

Professor Matti Uusitupa, M.D., Ph.D.
Department of Clinical Nutrition
Institute of Public Health and Clinical Nutrition
University of Eastern Finland
KUOPIO
FINLAND

Professor Jussi Pihlajamäki, M.D., Ph.D.
Department of Clinical Nutrition
Institute of Public Health and Clinical Nutrition
University of Eastern Finland
KUOPIO
FINLAND

Reviewers: Adjunct Professor Olavi Ukkola, M.D., Ph.D.
Department of Internal Medicine
University of Oulu
OULU
FINLAND

Professor Atso Raasmaja, M.D., Ph.D.
Faculty of Pharmacy
University of Helsinki
HELSINKI
FINLAND

Opponent: Adjunct Professor Kirsi Virtanen, M.D., Ph.D.
Turku PET Centre
Turku University Hospital
TURKU
FINLAND

ABSTRACT:

Obesity is associated with low-grade inflammation and extracellular matrix (ECM) remodeling, both of which also contribute to adipose tissue dysfunction. Obesity is a major risk factor for the development of type 2 diabetes and cardiovascular diseases. Previous studies by our group have demonstrated that weight reduction changes the gene expression profile of white adipose tissue (wAT) in overweight subjects with features of metabolic syndrome. Among the down-regulated genes in response to weight loss were microfibrillar-associated protein 5 (*MFAP5*) and a calcineurin-like phosphoesterase domain containing 1 (*CPPED1*).

Previous studies on MFAP5 demonstrate that it is involved in elastinogenesis, cell survival and angiogenesis, but the function of CPPED1 is unknown. The overall aim of this thesis work was to investigate whether changes in gene expressions of *MFAP5* and *CPPED1* were associated with changes in wAT biology in obesity and to elucidate the possible roles of *MFAP5* and *CPPED1* in controlling adipocyte function, inflammation and glucose metabolism at cellular level in cultured adipocytes.

MFAP5 expression was correlated with factors related to ECM remodeling and inflammation in wAT samples. Modified expression of *MFAP5* by down-regulation in cultured adipocytes decreased the expression of ECM components and inflammatory genes. Furthermore, inflammatory factors increased the expression of *MFAP5* in preadipocytes. *CPPED1* expression was positively correlated with the expression of inflammatory genes in wAT and peripheral blood mononuclear cells (PBMCs), and the expression of *CPPED1* tended to increase in response to tumour necrosis factor alpha (TNFα) treatment in cultured adipocytes. Decreased expression of *CPPED1* enhanced insulin-stimulated glucose uptake, possibly through a phosphatidyl inositol 3-kinase (PI3K) -dependent pathway, and adiponectin signaling pathway could either directly or indirectly play a role in this regulation.

In conclusion, the present results demonstrate that MFAP5 and CPPED1 have a role in wAT biology and could contribute to complications in dysfunctional wAT, which could be in part reversed by weight reduction. Furthermore, the results suggest that *MFAP5* is involved in ECM function and wAT inflammation. Reduction in *CPPED1* expression enhances insulin-stimulated glucose uptake, possibly via adiponectin-mediated mechanisms.

TIIVISTELMÄ:

Lihavuus on yhteydessä matala-asteiseen tulehdustilaan ja sidekudoksen muokkaukseen. Molemmat myös vaikuttavat rasvakudoksen toimintahäiriöön. Lihavuus on myös merkittävä riskitekijä tyypin 2 diabeteksen ja sydän- ja verisuonitautien kehittymiselle. Tutkimusryhmämme aikaisemmat tutkimukset ovat osoittaneet, että laihduttaminen voi muuttaa valkoisessa rasvakudoksessa ilmentyvien geenien profiilia ylipainoisilla henkilöillä, joilla on metabolisen oireyhtymän piirteitä. *MFAP5* (microfibrillar-associated protein 5) ja *CPPED1* (calcineurin-like phosphoesterase domain containing 1) olivat niiden geenien joukossa, joiden ilmentyminen väheni laihdutuksen myötä.

Aikaisemmat tutkimukset MFAP5:stä osoittavat sen olevan yhteydessä elastinogeneesiin, verisuonten uudismuodostukseen ja solujen eloonjäämiseen, mutta CPPED1:n toiminto on kuitenkin tuntematon. Tämän väitöskirjatyön kokonaistavoite oli tutkia ovatko *MFAP5*- ja *CPPED1*-geenien ilmentymisen muutokset yhteydessä muutoksiin lihavuuden valkoisen rasvakudoksen biologiaan, ja selventää *MFAP5*:n ja *CPPED1*:n mahdollisia tehtäviä rasvasolun toiminnassa, tulehdustilassa ja glukoosiaineenvaihdunnassa solutasolla rasvasoluviljelmässä.

Rasvakudosnäytteissä *MFAP5* ilmentyminen korreloi sidekudoksen muokkaukseen ja tulehdustilaan liittyvien tekijöiden kanssa. *MFAP5*:n ilmentymisen vähentäminen rasvasoluviljelmässä vähensi sidekudoskomponenttien ja tulehdukseen liittyvien geenien ilmentymistä. Lisäksi tulehdustekijät lisäsivät *MFAP5*:n ilmentymistä rasvasolun esiastesoluissa, preadiposyyteissä. Valkoisessa rasvakudoksessa ja perifeerisen veren mononukleaarisoluissa *CPPED1* ilmentyminen korreloi positiivisesti tulehdustilaan liittyvien geenien ilmentymisen kanssa ja tuumorinekroositekijä alfa (TNFα) -altistuksella oli taipumus lisätä sen ilmentymistä rasvasoluviljelmässä. Vähentynyt *CPPED1*:n ilmentyminen lisäsi insuliinilla stimuloitua glukoosin sisäänottoa soluun mahdollisesti fosfatidyyli-inositoli 3 kinaasi (PI3K) -riippuvaisen signaalitien kautta ja adiponektiinin signalointireitillä saattaa olla rooli tässä säätelyssä, joko suorasti tai epäsuorasti.

Yhteenvetona tulokset näyttävät, että MFAP5:llä ja CPPED1:llä on rooli valkoisen rasvakudoksen biologiassa ja ne saattavat vaikuttaa valkoisen rasvakudoksen toimintahäiriöihin, jotka osittain voidaan korjata laihduttamisella. Tulokset osoittavat myös, että *MFAP5* on yhteydessä sidekudoksen toimintaan ja valkoisen rasvakudoksen tulehdustilaan, kun taas *CPPED1* ilmentymisen vähentyminen tehostaa insuliinilla stimuloitua glukoosin sisäänottoa soluun, mahdollisesti adiponektiinivälitteisten mekanismien kautta.

Acknowledgements

Kuopio, November 2013

Maija Vaittinen

"Rohkeutta on, että uskaltaa, vaikka pelottaa"

List of the original publications

This dissertation is based on the following original publications:

I   Vaittinen M, Kolehmainen M, Schwab U, Uusitupa M and Pulkkinen L. Microfibrillar-associated protein 5 is linked with markers of obesity-related extracellular matrix remodeling and inflammation. *Nutrition and Diabetes 1, e15; doi:10.1038/nutd.2011.10, 2011.*

II   Vaittinen M, Kolehmainen M, Rydén M, Eskelinen M, Wabitsch M, Pihlajamäki J, Uusitupa M and Pulkkinen L. Expression of microfibrillar-associated protein 5 (*MFAP5*) is modified by inflammatory factors in preadipocytes *in vitro*. *Submitted.*

III Vaittinen M, Kaminska D, Käkelä P, Eskelinen M, Kolehmainen M, Pihlajamäki J, Uusitupa M and Pulkkinen L. Down-regulation of calcineurin-like phosphoesterase domain containing 1 (*CPPED1*) expression improves glucose metabolism *in vitro* in adipocytes. *Diabetes 62(11): 3747-3750, 2013.*

In addition, some previously unpublished, complementary data are presented.

The publications were adapted with the permission of the copyright owners.

Contents

Abbreviations

ADAM12	A disintegrin and metalloproteinase domain 12		GPDH	Glycerol-3-phosphate dehydrogenase
ADIPOR1	Adiponectin receptor 1		HIF1α	Hypoxia-inducible factor, alpha
ADIPOQ	Adiponectin		HMW	High molecular weight
Akt/PKB	Protein kinase B		HSL	Hormone-sensitive lipase
AMPK	AMP-activated protein kinase		IFG	Impaired fasting glucose
ANOVA	Analysis of variance		IGT	Impaired glucose tolerance
AT	Adipose tissue		IKBα	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
AU	Arbitrary unit			
BMI	Body mass index			
CCND2	Cyclin D2			
cDNA	Complementary deoxyribonucleic acid		IKBβ	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta
C/EBPβ	CCAAT/enhancer binding proteins, beta		IKKβ	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
COL1A	Type I collagen, alpha			
CPM	Counts per minute			
CPPED1	Calcineurin-like phosphoesterase domain containing 1		IL1β	Interleukin 1 beta
			IL1Ra	Interleukin 1 receptor antagonist
CPT1B	Carnitine palmitoyltransferase 1B		IL-6	Interleukin 6
			IL-10	Interleukin 10
ECM	Extracellular matrix		IR	Insulin resistance
ELISA	Enzyme-linked immunosorbent assay		IRS	Insulin receptor substrate
			JNK	c-Jun N-terminal kinase
FAK	Focal adhesion kinase		KRH	Krebs Ringer HEPES
FBS	Fetal bovine serum		LEP	Leptin
FFA	Free fatty acid		MCP-1	Monocyte chemoattractant protein 1
FSIGT	Frequently sampled intravenous glucose tolerance test		MFAP5	Microfibrillar-associated protein 5
			MMPs	Metalloproteinases
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase		mRNA	Messenger ribonucleic acid
			NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
GLM	General linear model			
GLUT1	Glucose transporter 1		NOTCH1	Notch Homolog 1
GLUT4	Glucose transporter 4		OGTT	Oral glucose tolerance test

PAI-1	Plasminogen activator inhibitor 1
PBS	Phosphate buffered saline
PBMCs	Peripheral blood mononuclear cells
PFA	Paraformaldehyde
PI3K	Phosphatidylinositol 3-kinase
PPARγ	Peroxisome proliferator-activated receptor gamma
PPIA	Cyclophilin A
PVDF	Polyvinylidene fluoride
RNA	Ribonucleic acid
RNAi	RNA interference
rpm	Revolutions per minute
RT	Room temperature
RT-qPCR	Reverse-transcriptase quantitative polymerase chain reaction
18S	Ribosomal RNA 18S
sc	Subcutaneous
scAT	Subcutaneous adipose tissue
SD	Standard deviation
SEM	Standard error of mean
SGBS	Simpson-Golabi-Behmel-Syndrome
siRNA	small interfering RNA
SVF	Stromal-vascular fraction
TBP	TATA box binding protein
TGFβ	Transforming growth factor beta
TIMPs	Tissue inhibitor of metalloproteinases
TNFα	Tumour necrosis factor alpha
WHO	World Health Organization
WR	Weight reduction

*the genes are indicated with *italic* font and proteins with normal font

1 Introduction

During the last decades, obesity has been considered a major public health problem (1) and is associated with an increased incidence and prevalence of obesity-related co-morbidities, such as insulin resistance (IR), type 2 diabetes, hypertension, cardiovascular diseases and several types of cancer (2-4). The burden related to obesity and its co-morbidities has increased the basic and applied research on the role of white adipose tissue (wAT) in regulating physiological and pathological processes (5).

WAT is a highly active metabolic and endocrine organ. It is one of the key tissues having an effect on human energy metabolism and inflammation, thus, having also a major role in the development of metabolic abnormalities in obesity (6). Enlargement of wAT is associated with adipocyte hypertrophy followed by increased low-grade inflammation, abnormal production of adipokines, immune cell infiltration into wAT and the extracellular matrix (ECM) remodeling (7-11), finally leading to dysfunctional wAT (12).

The ECM maintains the integrity of wAT and is composed of collagens, glycoproteins and proteoglycans (13-15). In obesity, the synthesis of ECM components is increased, potentially leading to fibrosis which links obesity to diabetes and cardiovascular diseases in association with inflammation (11,16).

Lifestyle modification aiming at weight reduction has been shown to prevent many obesity associated co-morbidities and to reduce the risk of type 2 diabetes via improving insulin and glucose metabolism and attenuating the low-grade chronic inflammatory state (17-19). Furthermore, ECM remodeling could be reversed by weight loss. Changes in gene expression of ECM during weight gain and losss suggest a role of ECM in wAT biology in obesity (9).

The strategy of our research group has been to identify new putative genes related to obesity and type 2 diabetes. Alterations in body weight have multitude of changes in wAT gene expression. In a previous study of our group, microfibrillar-associated protein 5 (*MFAP5*) and a calcineurin-like phosphoesterase domain containing 1 (*CPPED1*) were down-regulated during weight reduction in overweight subjects with impaired fasting glucose or impaired glucose tolerance, supporting them as putative genes involved in wAT biology, obesity and its related conditions (20).

The purpose of this thesis work was to investigate the possible roles of *MFAP5* and *CPPED1* in controlling adipocyte function, inflammation and glucose metabolism at the cellular level using human peripheral tissues and adipocyte cell culture models. Specifically, 1) the relationship between the expression of target genes and changes in adiposity, glucose metabolism and inflammation, 2) the functions of target genes by gene silencing technology on adipocyte function and glucose metabolism in adipocyte cell cultures and 3) the effect of inflammatory cytokines on the expression of target genes in adipocyte cell cultures were studied.

2 Review of literature

2.1 OBESITY

During positive energy balance wAT stores excess of lipids as triglycerides which leads to wAT enlargement and development of obesity (21), which increases the risk for type 2 diabetes and cardiovascular diseases (22). Traditionally, body mass index (BMI) is used as a common indicator to measure body fat and to diagnose underweight and overweight (23). Overweight is defined as BMI (calculated dividing weight by height in meters squared) ≥ 25 kg/m^2 and obesity as BMI ≥ 30 kg/m^2 (24,25).

However, the distribution of fat differs in obesity (24), and BMI does not account for this variation. That is, BMI does not differentiate the weight between muscle and fat tissues or location of wAT (24). Therefore, other measures, such as waist circumference and waist-to-hip ratio, which are more closely associated with the risk of cardiovascular diseases than BMI, more accurately describe fat distribution (26,27). Specifically, abdominal or central obesity has been shown to be associated with adipocyte hypertrophy and development of IR and its complications like type 2 diabetes, hypertension and cardiovascular diseases (28-30). In contrast, peripheral obesity is associated with less metabolic risk (31). There are also regional differences in fat distribution between males and females. Abdominal (upper-body) obesity is more frequent in males, whereas peripheral obesity in gluteal and femoral regions (lower-body) is more common in females (32). There is a great interest towards identifying the main factors contributing to the development of obesity which results from the interaction of lifestyle (unhealthy diet and low physical activity) and genetic factors (21,30,33,34).

The increasing prevalence of obesity is widely documented (24). According to World Health Organization (35) in 2008, 34% of men and 35% of women were overweight, and 10% of men and 14% of women were classified as obese (35). These numbers are in line with the situation in Finland. In Finland, the prevalence of obesity has increased during the last decades (36). Based on the FinRisk 2012 study, 66% of men and 46% of women were classified as overweight (BMI ≥ 25 kg/m^2) and 20% of Finnish people were obese (37).

2.1.1 Obesity-related metabolic changes in white adipose tissue

Enlargement of wAT is associated with increased fat cell volume and recruitment of new fat cells termed as hypertrophy and hyperplasia, and is followed by increased angiogenesis, immune cell infiltration into wAT, and ECM overproduction, also termed wAT remodeling (9,11,38). The increase in wAT mass can have several effects, including hypoxia, endoplasmic reticulum stress, adipocyte cell death, enhanced adipokine secretion and dysregulation in fatty acid fluxes (11,39). These processes are a result of dysregulation of the regulatory system in wAT (21,40,41).

Moreover, obesity is considered as a chronic low-grade inflammatory state (42) with abnormal production of adipokines such as adiponectin, leptin, tumour necrosis factor alpha (TNFα) and interleukin (IL)-6 from wAT (7,8), which results in disturbed insulin

sensitivity and impaired glucose metabolism (12). The source of these adipokines is not completely clear. However, adipocytes and immune cells such as macrophages, which infiltrate into wAT (43), contribute to the secretion of these adipokines from obese wAT (4,30,44). Low-grade inflammation is nowadays regarded as a mediating factor between obesity and its co-morbidities (45). In contrast, weight loss has been shown to be an effective way to improve insulin sensitivity and glucose metabolism (46,47) and reverse the state of inflammation (18).

2.2 WHITE ADIPOSE TISSUE AS AN ENDOCRINE ORGAN

The discovery of leptin (48), and later adiponectin (49,50), changed our view of wAT as only serving for energy storage. Nowadays, wAT is also considered to be an endocrine organ that produces a variety of hormones and bioactive proteins, termed adipokines or adipocytokines (Table 1), which act in an autocrine, paracrine, or endocrine manner (8,51). These bioactive molecules regulate biological processes like energy homeostasis, adipocyte proliferation and differentiation, blood pressure, lipid and glucose metabolism, angiogenesis and inflammation, thus affecting the metabolism of many organs (13,51,52). The hormones secreted by major adipocytes, such as adiponectin and leptin, are considered true adipokines (53) whereas other bioactive proteins, like TNFα and IL1β, which are secreted by adipocytes and other cell types in the wAT, are usually called adipocytokines (54). Table 1 presents key adipokines and adipocytokines associated with glucose metabolism and obesity-related inflammation in wAT.

Table 1. Key adipokines and adipocytokines secreted from wAT, modified from (13,54)

Secreted factors	Change in obesity	Effects on
Leptin	↑	food intake, glucose metabolism
Adiponectin	↓	insulin sensitivity, fatty acid oxidation, inflammation
Omentin	↓	insulin sensitivity
Tumour necrosis factor α	↑	insulin resistance, adipogenesis, apoptosis
Interleukin 1β	↑	insulin resistance, inflammation
Interleukin 6	↑	insulin resistance, inflammation
Monocyte chemoattractant protein 1	↑	macrophage infiltration
Resistin	↑	weight regulation, insulin resistance, inflammation
Visfatin	↑	weight regulation, insulin secretion, insulin resistance
Plasminogen activator inhibitor 1	↑	fibrinolysis

WAT also produces adipocytokines like TNFα, IL1β and IL-6, and other factors like monocyte chemoattractant protein (MCP) -1, resistin and plasminogen activator inhibitor (PAI-1), which have also been shown to originate from other cell types in the stromal-vascular fraction (SVF), such as immune cells (8,30,44,54). WAT also secretes many other hormones, e.g. omentin and visfatin, but their roles in glucose metabolism in humans need to be further elucidated (26). The function of these adipokines and adipocytokines is

important for cross-talk with other organs (40). In obesity the secretion and expression of adipokines and adipocytokines is dysregulated and contributes to the development of metabolic abnormalities and the risk of cardiovascular diseases (51,52).

2.2.1 Factors involved in white adipose tissue biology

Leptin levels are increased in obesity (Figure 1) and correlate directly with wAT mass (5,53). The main function of leptin is to control appetite and energy homeostasis (8). In addition, leptin is also involved in the inflammatory response, regulation of glucose homeostasis and insulin sensitivity, hematopoiesis, angiogenesis, bone development and carcinogenesis (5,8,13,55).
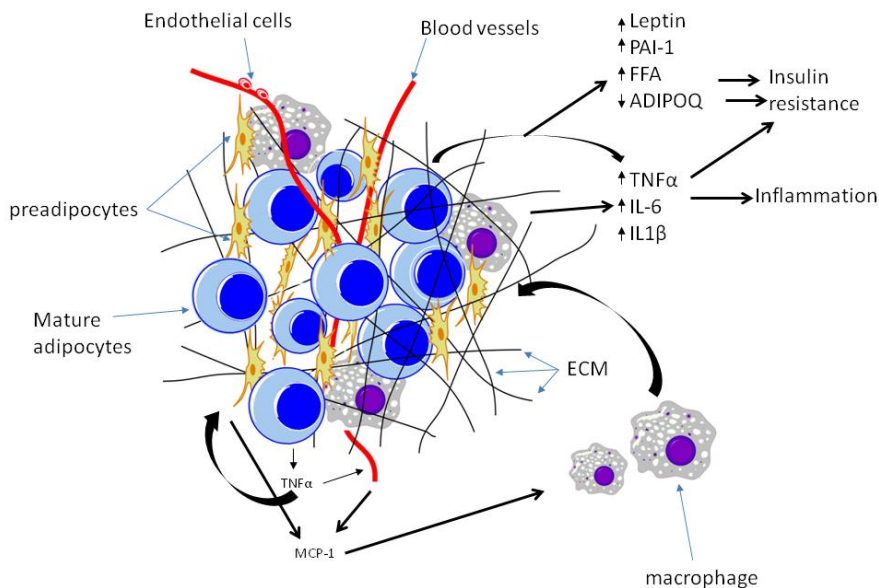
Figure 1. Factors involved in dysregulation of wAT in obesity.

The level of adiponectin is reduced in obesity, IR and type 2 diabetes, and is inversely correlated to adiposity. Adiponectin levels are increased by weight reduction and improved insulin sensitivity (3,56). Adiponectin is involved in the regulation of insulin sensitivity, inhibition of gluconeogenesis and enhancement of fatty acid oxidation in liver, skeletal muscle and in wAT (57,58). In addition, adiponectin has been demonstrated to have anti-inflammatory properties via down-regulation of the inflammatory factors *TNFα* and *IL-6* through inhibition of NF-κB activation and induction of the anti-inflammatory factors *IL-10* and interleukin 1 receptor antagonist *IL1Ra* (5). There are several isoforms of adiponectin circulating in the blood, including full-length or high-molecular weight (HMW), medium-molecular weight and low-molecular weight adiponectin isoforms. Of these, particularly the HMW isoform, which is proposed to be the biologically active form,

has been shown to be negatively associated with IR, metabolic syndrome and cardiovascular disease (13,59).

The production of TNFα, IL-6, IL1β and the anti-inflammatory IL1Ra is increased in obesity and is correlated with BMI and IR (5,60-62). Mature adipocytes are capable of producing inflammatory cytokines, but SVF cells and macrophages are the main source of TNFα, IL-6 and IL1β in obese wAT (30,38,45). TNFα has been shown to inhibit adipocyte differentiation by down-regulating the expression of transcription factors involved in adipogenesis, stimulates lipolysis and contributes adipocyte apoptosis (63-65).

Increased infiltration of macrophages into the wAT is observed in obesity, and many factors may be involved. Macrophage accumulation is associated with adipocyte hypertrophy and increased expression of inflammatory factors in wAT, suggesting a role of macrophages in wAT remodeling (38,40,66). The production of TNFα is increased in enlarged adipocytes, which triggers MCP-1 secretion from preadipocytes and endothelial cells and subsequently attracts macrophages into the wAT (Figure 1) (65,67,68). TNFα also contributes to inflammatory status in AT by increasing the production of inflammatory factors (40,56). Furthermore, hypoxia stimulates macrophage infiltration through hypoxia-inducible factor (HIF)1α activation (69). Weight reduction decreases macrophage infiltration into wAT (45).

In addition to adipocytes, PAI-1 is produced by other cell types of the SVF. PAI-1 is an inhibitor of fibrinolysis and thus enhances the formation of thrombosis, leading to increased cardiovascular disease risk (26). Furthermore, it has a role in adipocyte differentiation, insulin signaling (70), tumor growth, metastasis and angiogenesis via interaction with components of the ECM (26,68). Some studies have shown that the expression of resistin is increased in obesity and is associated with the development of IR. However, the results have demonstrated some discrepancy, and the role of resistin needs to be further clarified (13,58,71).

2.3 INSULIN ACTION AND INSULIN RESISTANCE

In wAT, insulin regulates adipocyte biology by inducing the differentiation of preadipocytes to mature adipocytes, stimulating glucose uptake and triglyceride synthesis (lipogenesis), and inhibiting the release of fatty acids via reduced lipolysis by inhibiting hormone-sensitive lipase (HSL) activity in mature adipocytes (72). In the liver, insulin reduces the release of glucose by inhibiting glycogenolysis and increasing glucose storage (10). In addition to traditional insulin target tissues (liver, muscle, AT), insulin activates intracellular signaling pathways in many other tissues, like the vascular endothelium, nervous system, kidney and brain (73,74).

In wAT and skeletal muscle, insulin action is initiated by binding of insulin to its receptor and phosphorylation of insulin receptor substrates (IRSs), leading to activation of phosphatidylinositol 3-kinase (PI3K) and downstream signaling molecules, including protein kinase B (Akt/PKB) and atypical PKCs, with glucose transporter 4 (GLUT4) translocation to the plasma membrane and glucose uptake, as illustrated in figure 2 (10,72,75). Insulin signaling can be effected by e.g. inflammatory factors and free fatty acids leading to IR through several mechanisms and pathways as discussed in the following section.
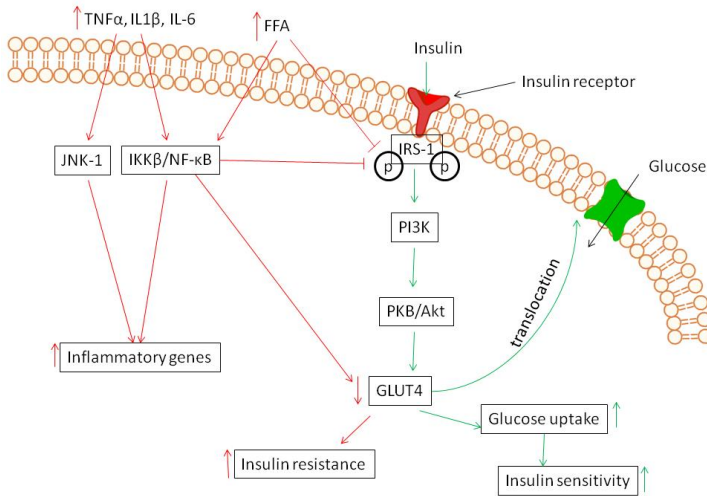
Figure 2. Insulin action and factors involved in impaired insulin signaling and development of IR in skeletal muscle and wAT.

Obesity-related IR has shown to precede type 2 diabetes (66). Complex mechanisms, as shown in figure 2, include long-term energy surplus, increased fatty acid release and low-grade inflammation (10). The term IR is defined as a decreased tissue response of circulating insulin to stimulate glucose utilization in skeletal muscle, liver and wAT (10,34,74). The metabolic effects of impaired insulin sensitivity in these tissues are decreased insulin-stimulated glucose uptake and metabolism in adipocytes and skeletal muscle, and decreased insulin-stimulated inhibition of both glucose production in liver and lipolysis in wAT, leading to whole body IR (10,34,74). However, the mechanisms are not completely understood (66).

Several factors are capable of inducing IR (Figure 2). One of the primary causes, and consequences, of IR is increased release of free fatty acids (FFA) from wAT due to increased lipolysis. This induces ectopic lipid accumulation in liver, muscle, pancreas and epicardium and blood vessel walls (34,38,51,54). FFA-induced IR may be mediated through reduced phosphorylation of IRS-1 and GLUT4 translocation, activation of NF-κB pathway and inflammatory cytokine production (31,68). Lipodystrophy, the lack of wAT, induces IR via increased circulating triglycerides and fatty acids in humans. Therefore, the presence of functional wAT is needed for normal storing of lipids, adipokine secretion, insulin sensitivity and glucose metabolism (2,76).

WAT is involved in maintaining glucose homeostasis and insulin sensitivity by taking up glucose through GLUT4 transporter in adipocytes in response to insulin (51). Down-regulation of *GLUT4* expression and decreased GLUT4 translocation to plasma membrane has been shown to contribute to the development of IR in adipocytes. However, in skeletal muscle the expression of *GLUT4* is not changed, and the impaired glucose uptake is due to decreased translocation of GLUT4 to the plasma membrane (72,77).

Based on experimental and human studies, it could be proposed that also activation of many inflammatory pathways are involved, which was first shown by Hotamisligil et al in

1993 (30,42,66). As mentioned earlier, several inflammatory factors, like TNFα, IL1β and IL-6 are increased in obesity and are also associated with the development of IR (66) mainly through activation of inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) - pathway, but also through other pathways, like c-Jun N-terminal kinase (JNK)1/2-pathway (4,13,56,78). TNFα induces IR in wAT and skeletal muscle by phosphorylation of the insulin receptor, IRS-1 and down-regulation of adiponectin and *GLUT4* gene expression and translocation (5,34,54,56,71). IL-6 induces IR through tyrosine phosphatase activation or interaction between suppressor of cytokine signaling (SOCS) proteins and the insulin receptor (56). There is evidence showing that IL1β could stimulate IR in a similar way as TNFα (45,60). Furthermore, adipocyte hypertrophy leads to macrophage infiltration into wAT, where they release inflammatory cytokines which can induce IR in neighboring adipocytes via paracrine effects (10).

Weight reduction, caloric restriction and the use of neutralizing antibodies against inflammatory factors have been shown to reduce inflammation and IR and increase anti-inflammatrory factors in wAT (18,45,54,61,62,66).

2.4 CONSTITUENTS OF ADIPOSE TISSUE

There are two types of AT: white and brown (44,79). In addition to energy storage, wAT is highly active metabolic and endocrine organ involved in modulating appetite, insulin sensitivity, bone metabolism, inflammation and immunity (5,44). WAT is a heterogeneous tissue composed of lipid-filled mature adipocytes and a stromal-vascular fraction (SVF) that in turn is comprised of preadipocytes, fibroblasts, endothelial cells, immune cells and several other cell types (13,30,51,74).

In human adults, most AT is white. There is also evidence that adults have functional brown AT, which is involved in facultative nonshivering thermogenesis through the action of UCP-1 (80-82). Brown adipocytes have multilocular lipid droplets and are associated with fatty acid oxidation (83). It has been demonstrated that brown adipocytes are present in white AT, though it is unclear whether they originate from trans-differentiating white adipocytes or *de novo* differentiating preadipocytes (84,85).

2.4.1 White adipose tissue remodeling

Preadipocytes can differentiate into lipid-filled mature adipocytes in a phenomenon termed as adipogenesis (79). Adipogenesis is an ongoing process throughout the life-span enabling enlargement of wAT during development of obesity (79). Recently, Spalding et al proposed that the number of adipose cells is constant in adulthood in obese and lean subjects indicating that there is a continuous turnover of adipose cells (86). In fact, 10% of adipose cells are renewed annually in adulthood (86). Adipogenesis is highly regulated system by transcription factors and other regulators (21). As shown in figure 3, the preadipocyte differentiation into mature adipocyte is induced with a well characterized differentiation cocktail and is associated with sequential activation of transcription factors, notably CCAAT/enhancer binding proteins (*C/EBP*)-β, peroxisome proliferator-activated receptor (*PPAR*)-γ, and *C/EBP-α* and adipocyte-specific genes (40,41,87).
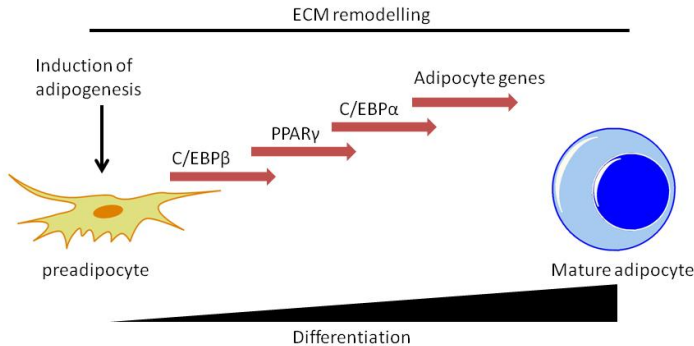
Figure 3. Preadipocyte differentiation and factors involved in the process

During wAT enlargement and adipogenesis the change from a fibroblast-like preadipocyte into mature adipocytes is associated with remodeling of the ECM (Figure 3) (14,41) which takes place simultaneously with activation and repression of the transcription factors (15). However, the molecular mechanisms have not been fully defined (16). In addition, angiogenesis is strongly associated with adipogenesis and angiogenesis enhances during wAT enlargement and development of obesity in mice (4).

2.4.2 Extracellular matrix in white adipose tissue

ECM is enclosing mature adipocytes and maintains the integrity and structure of wAT (13,14,51) and is composed of collagens, adhesive glycoproteins and proteoglycans (15). ECM regulates physiological and pathological processes in wAT, such as development and tissue repair and inflammation (15). Signals from ECM to intracellular compartments are mediated through plasma membrane integrins which in turn are involved in cell proliferation, differentiation, migration, apoptosis, gene induction and fatty acid oxidation in mitochondria (9,52). Components of the ECM are capable of modifying immune functions, e.g. immune cell migration into and within inflamed tissues (88). The expression of ECM proteins is increased in obesity (11), and many cell types of wAT are capable of producing ECM proteins (52). However, there is strong evidence that the ECM molecules are predominantly expressed in SVF of wAT (52).

WAT fibrosis, defined as an excessive synthesis of various ECM molecules, including collagens (collagens I and III) and glycoproteins (laminin, fibronectin) (52,89), may link obesity to diabetes or cardiovascular diseases in association with inflammation (16). Recent studies have demonstrated that collagen VI is highly expressed in obesity, limits wAT expansion and is associated with obesity-related comorbidities. There is also

evidence that a loss of collagen VI in wAT leads to improvements of metabolic profile (14,90,91). There is strong evidence that preadipocytes may contribute to the development of fibrosis (9). It has been shown that preadipocytes are able to increase the production of many ECM molecules in an inflammatory environment, and this is associated with increased proliferation and migration of preadipocytes (15). It cannot be ruled out, however, that also mature adipocytes could produce ECM proteins (52).

2.4.3 Extracellular matrix remodeling and factors involved

The amount of ECM molecules during development of obesity or after weight reduction is balanced by the rate of their synthesis and degradation (14). This is regulated by several factors (Table 2), like matrix metalloproteinases (MMPs) and their tissue inhibitors (14). In addition, growth factors like transforming growth factor beta (TGFβ), and cytokines (TNFα, IL1β, IL-6) modulate ECM synthesis (88,92). *TGFβ*, which is up-regulated in human and animal models of obesity (9), is a major regulator of ECM synthesis through induction of gene expression of matrix and tissue inhibitors of metalloproteinases (TIMPs) (92). Moreover, *TGFβ* is suggested to be involved in enlargement of wAT and inhibition of adipogenesis (93). Recently, apart from its role in adipogenesis, *PPARγ* has been shown to be an antifibrotic factor and to down-regulate the synthesis of type I collagen, possibly through reduced *TGFβ* activity (14,94).

Table 2. Factors involved in ECM remodeling

Factors	Effects on ECM
MMPs	degradation of ECM components
TIMPs	inhibition of ECM degradation
TGFβ	synthesis of ECM components
cytokines	modification of ECM through MMPs/TIMPs
PPARγ	down-regulation of ECM components
insulin	modification of ECM through MMPs/TIMPs
adiponectin	modification of ECM through MMPs/TIMPs
leptin	modification of ECM through MMPs/TIMPs
angiotensin	modification of ECM through MMPs/TIMPs
estrogen	modification of ECM through MMPs/TIMPs

There are also other factors that are capable of modifying ECM synthesis. Insulin regulates the production of ECM components through transcription of genes for protein-modifying and –processing enzymes (95). Furthermore, adiponectin, leptin, angiotensin and estrogen are involved in the modification of ECM protein expression through regulation of MMP/TIMP activity (96-98). These hormones and their receptors are expressed in wAT. Thus, it could be postulated that they are capable of regulating ECM remodeling also in wAT (98).

Little is known about the effect of nutrition or single nutrients, except obesity and weight changes, on ECM remodeling in wAT. Although cellular interactions in wAT has been studied extensively (14), the molecular mechanisms involved in ECM remodeling are

poorly understood in humans (9), despite the fact that ECM is an important constituent of wAT physiology (14).

2.5 EFFECT OF WEIGHT REDUCTION ON WHITE ADIPOSE TISSUE BIOLOGY/FUNCTION

Lifestyle modification, such as weight reduction, healthy diet and increased physical activity, has been shown to prevent many obesity associated co-morbidities and to reduce the risk of type 2 diabetes in long-term and to improve insulin and glucose metabolism (17,19). Even a modest weight reduction improves metabolic status and decreases the risk factors associated with obesity (30), suggesting an essential role of wAT in obesity-related complications (99). Weight loss is associated with a reduced production and expression of inflammatory factors, and increased adiponectin secretion from wAT (18,30,45). Moreover, weight loss is known to decrease macrophage infiltration and the expression of inflammation-related genes in wAT (18,45,100).

A series of studies have aimed to identify new putative genes related to obesity and type 2 diabetes. A genome-wide transcriptomics analysis performed on subcutaneous (sc) wAT samples from persons with metabolic syndrome participating in the Gene Expression in Obesity and Insulin Resistance (GENOBIN) study (20) demonstrated changes in the expression of 105 genes in response to weight reduction, and at the same time glucose metabolism and insulin sensitivity improved. The major gene clusters that responded to weight reduction included ECM and cell death (20). Among the down-regulated genes after weight loss were ECM-related gene microfibrillar-associated glycoprotein 5 (*MFAP5*) and a calcineurin-like phosphoesterase domain containing 1 (*CPPED1*). These genes are therefore interesting putative genes involved in obesity-related conditions (20).

2.5.1 Microfibrillar-associated protein 5 (MFAP5)
MFAP5, also known as MAGP-2 (microfibrillar-associated glycoprotein 2), is located in the microfibrils of elastin networks in a number of tissues, and it has been suggested to be involved in cell signaling during microfibril assembly, elastinogenesis (101-103) and cell survival (104,105) through activation of $\alpha_v\beta_3$ integrin and focal adhesion kinase (FAK) (105,106). *MFAP5* has pro-angiogenic activity and it interacts with *NOTCH1* either directly or through NOTCH1 ligands (107,108). It seems that *MFAP5* mediates cell specific functions, because it has been shown to activate (108) or inhibit (107) NOTCH1 signaling depending on the cell type involved. ECM is functionally important to wAT biology (14) and is involved in wAT inflammation. As a constituent of ECM, MFAP5 is a putative molecule involved in wAT biology; in this regard it has not been studied before.

2.5.2 Calcineurin-like phosphoesterase domain containing 1 (CPPED1)
CPPED1 has been shown to be expressed in melanoma and peripheral blood mononuclear cells (PBMCs) (109,110). However, the function of CPPED1 in wAT, in which we observed down-regulation in gene expression after weight loss (20), is completely unknown. By using EFICAz (Enzyme Function Inference by a Combined Approach), *CPPED1* has been predicted as an endopolyphosphatase, which degrades long polyphosphate chains into shorter chains (111). Since macrophages infiltrate into wAT in obesity and cause inflammatory responses, we hypothesized that CPPED1 could be a novel molecule involved in wAT inflammatory processes.

3 Aims of the Study

The increased low-grade inflammation in obesity with abnormal production of adipokines and adipocytokines alters gene expression in wAT. Consequently, altered levels of inflammatory factors and gene expression profile in wAT modulate insulin sensitivity by targeting molecules regulating the insulin signaling pathway, eventually leading to IR, a process that could be reversed by weight reduction. The expression of *MFAP5* and *CPPED1* was down-regulated in response to weight reduction, supporting them as putative genes involved in wAT biology. The general objective of this thesis work was to study the roles of *MFAP5* and *CPPED1* in wAT biology, and particularly whether changes in their gene expression in response to weight reduction are associated with the magnitude of weight reduction.

Specifically, the aim was to elucidate the possible roles of *MFAP5* and *CPPED1* in controling adipocyte function, inflammation and glucose metabolism at the cellular level using human peripheral tissues and adipocyte cell culture models. The aims in detail were:

1. To identify the relationship between the expression of target genes and changes in adiposity, glucose metabolism and inflammation
2. To elucidate the functions of target genes by RNA interference technology on adipocyte metabolism and insulin sensitivity
3. To investigate the effect of inflammatory cytokines on the expression of target genes

4 Materials and Methods

4.1 HUMAN STUDIES

4.1.1 Study populations and study designs

The Gene Expression in Obesity and Insulin Resistance (GENOBIN) Study—Originally, 75 overweight/obese (BMI 28-40 kg/m^2) subjects aged 40 to 70 years were recruited to the GENOBIN study as described earlier (20,112). The subjects had impaired fasting glucose (fasting plasma glucose concentration 5.6 – 6.9 mmol/L) or impaired glucose tolerance (2-h plasma glucose concentration 7.8 – 11.0 mmol/L) and at least two other features of the metabolic syndrome according to the Adult Treatment Panel III criteria (113) as modified by the American Heart Association (22). Subjects were randomly assigned to one of the following groups: a weight reduction group (WR, n=28), an aerobic exercise training group (n=15), a resistance exercise training group (n=14) or a control group (n=18) (20,112). Subjects were matched for age, gender, BMI and the status of glucose metabolism. In the current thesis work, only the data from the weight reduction and control groups were included and analyzed (*study I and* unpublished, complementary data).

The weight reduction group had an intensive weight reduction period lasting for 12 weeks during which study subjects followed detailed instructions given by a clinical nutritionist. These instructions were based on a 4-day dietary record and an interview. Between weeks 12 and 33 the aim was to maintain the achieved reduction in weight. The control group was asked not to change their lifestyle habits.

The Kuopio obesity surgery (KOBS) study—The design of the study has been reported earlier (114). All subjects undergoing the Roux-en-Y gastric bypass surgery at Kuopio University Hospital were recruited to this study. Altogether 95 morbidly obese (BMI 45.0 ± 5.9 kg/m^2) subjects aged 36 to 54 years participated in the study. Open subcutaneous wAT biopsies and blood samples were drawn at baseline and after 12 months of follow-up to collect wAT and PBMC samples, respectively. Weight loss in this study during the follow-up period was -24±9%. Gene expression data from the subcutaneous wAT and PBMC samples at baseline and at follow-up were analyzed in this study.

4.1.2 Approval of the Ethics Committee

The clinical studies (*study I and* unpublished, complementary data) and experiments concerning SVF isolation and immunohistochemistry (*study II* and unpublished, complementary data) were performed in accordance with the standards of the Helsinki Declaration and the Ethics Committee of the District Hospital Region of Northern Savo approved the study plan. All participants gave a written informed consent.

4.1.3 Methods

4.1.3.1 Anthropometric and biochemical measurements (*study I* and unpublished, complementary data)

Anthropometric and biochemical measurements were taken at baseline and end of the intervention. Weight and height were measured in light indoor clothes, and BMI was calculated as weight (kg) divided by height (m) squared. Waist circumference was measured halfway between the lowest rib and iliac crest.

In the GENOBIN study, plasma glucose concentration was measured with the hexokinase method (Thermo Clinical Labsystems, Vantaa, Finland) and serum insulin was determined with a chemiluminescence sandwich method (ACS 180 Plus Automated Chemiluminescence System; Bayer Diagnostics, Tarrytown, NY). Hs-CRP was measured by Immage Immunochemistry System (Immulite 2000; DPC, 134 Los Angeles, CA). TNFα, IL1β and IL1Ra concentrations were measured by solid phase ELISA (Quantikine, R&D Systems, Minneapolis, MN). Commercial RIA kit (Linco Research Inc., St. Louis, MO) was used for the analysis of serum leptin concentration and ELISA kit (R&D Systems Inc., MN) for serum adiponectin measurement. Glucose metabolism was measured using an oral 2-hour glucose tolerance test (OGTT) and frequently sampled intravenous glucose tolerance test (FSIGT) according to the Minimal Model method (115) at baseline and at the end of the intervention (20). Insulin sensitivity index (S_I), glucose effectiveness (S_G) and acute phase insulin response to glucose (AIR) were calculated by the MINMOD Millenium software (116).

In the KOBS study, plasma glucose was analyzed with an enzymatic hexokinase photometric assay (Konelab Systems Reagents, Thermo Fischer Scientific, Vantaa, Finland) and serum insulin concentrations with an immunoassay (ADVIA Centaur Insulin IRI, no 02230141, Siemens Medical Solutions Diagnostics, Tarrytown, NY).

4.1.3.2 Adipose tissue biopsies (*studies I and III*)

WAT samples of the GENOBIN study were taken by a needle biopsy after a 12-hour overnight fasting from superficial abdominal subcutaneous wAT at the midpoint between umbilicus and iliac crest at baseline and after the intervention under local anesthesia (lidocaine 10 mg/ml without adrenaline) to collect 0.5 - 5 g of wAT. WAT samples were washed twice with phosphate-buffered saline (PBS) and treated with RNAlater according to the instructions provided by the manufacturer (Ambion, Austin, TX, USA). After removal of RNAlater the samples were stored in -80°C until used for RNA extraction (20).

WAT samples of the KOBS study were taken as an open biopsy from subcutaneous and visceral wAT during the Roux-en-Y gastric bypass surgery operation (at baseline) and 12 months after the surgery (follow-up) under local anesthesia (lidocaine 10 mg/ml with adrenaline) (114).

4.1.3.3 Isolation of PBMC cells (unpublished, complementary data)

PBMCs of the GENOBIN study were isolated from anticoagulated peripheral blood by density centrifugation for 10 min at 900 g and for another 10 min at 300 g using a reagent (Lymphoprep; Axis-Shield, Oslo, Norway) (100), and PBMCs of the KOBS study were

collected from whole blood samples with BD vacutainer CPT™ Tubes according to the manufactures protocol (Becton Dickinson, USA) (114).

4.1.3.4 Isolation of stromal-vascular fraction cells and mature adipocytes (*study II* and unpublished, complementary data)

Subcutaneous abdominal wAT samples were taken by needle biopsy from eight male donors (age 59.2 ± 7.7 years, BMI 25.7 ± 3.26 kg/m^2, fasting plasma glucose and insulin (5.8 ± 0.68 mmol/L, 10.0 ± 6.15 mU/L), respectively) under local anesthesia (lidocaine 10 mg/mL). AT samples were washed twice with phosphate-buffered saline (PBS) and digested with type II collagenase. The cell suspension was centrifuged (400 g, 4 min, +25^{0}C) to obtain SVF cells (cell pellet) and mature adipocytes.

4.2 CELL STUDIES

4.2.1 Methods

4.2.1.1 Differentiation of SGBS cells (*studies II and III*)

Human preadipocyte cell strain Simpson - Golabi - Behmel syndrome (SGBS) is characterized by a high capacity for adipogenic differentiation, and are derived from human SVF of subcutaneous wAT (117). The preadipocytes were cultured at 37^{0}C in a humidified atmosphere of 5 % CO$_2$ in air. The cells were maintained in basal medium 1, OF [DMEM/F12 Nut mix (Lonza, Verviers, Belgium) medium, 33 µmol/L biotin (Sigma-Aldrich), 17 µmol/L pantothenate (Sigma-Aldrich), 1% antibiotics (penicillin/streptomycin, Lonza)], supplemented with 10% FBS (Lonza) until reaching confluence.

To induce adipocyte differentiation, cells were washed several times with PBS (Lonza) and cultured in basal medium 2, 3FC [DMEM/F12, 33 µmol/L biotin, 17 µmol/L pantothenate, 1% antibiotics, 10 µg/mL transferrin (Sigma-Aldrich), 20 nmol/L insulin (Sigma-Aldrich), 0.1 µmol/L hydrocortisone (Sigma-Aldrich), 0.2 nmol/L triiodothyronine], supplemented with 25 nmol/L dexamethasone (Sigma-Aldrich), 0.5 µmol/L 1-methyl-3-isobutylxanthine (IBMX, Sigma-Aldrich) and 2 µmol/L BRL 49653 (rosiglitazone, PPARγ agonist, Cayman Chemical, USA) for 4 days. After 4 days, the medium was replaced with 3FC medium supplemented with 25 nmol/L dexamethasone and 0.5 µmol/L IBMX for 3 days. After 3 days, the medium was replenished twice a week with 3FC medium.

4.2.1.2 Oil Red O –staining (*study II*)

SGBS preadipocytes were grown and differentiated as described above. At indicated time points (shown in Figure 7F) the cells were washed with PBS and fixed in 4% paraformaldehyde (PFA) for 5 min and after discarding, fresh PFA was added for 1.5h at room temperature (RT). After fixation, the cells were washed twice with milliQH$_2$O and once with 60% isopropanol for 5 min at RT. Next, Oil Red O solution (filtered 0.3 g/100 mL isopropanol in milliQH$_2$O 3:2 (v/v)) was added and incubated for 25min at RT. Finally, the cells were washed three times with milliQH$_2$O, and the cells were visualized using microscopy (Nikon Eclipse TE300, Japan).

4.2.1.3 Cytokine treatments (*study II and unpublished, complementary data*)

SGBS preadipocytes were cultured in 12-well plates until they reached confluence and were washed once with PBS. The medium was changed to pre-incubation medium 1 [DMEM/F12 Nut mix (1:1) supplemented with 17 μmol/L pantothenate and 33 μmol/L biotin] for 24h. The following day the cells were cultured in the presence of TNF-α (Sigma-Aldrich), IL1β (PeproTech Inc., NJ, USA), TGFβ1 (ProSpec-Tany TechnoGene Ltd., Rehovot, Israel) or IL-6 (PeproTech) with various concentrations (0.1–10.0 ng/mL) and several time points (0, 3, 6, 12, 24, 48, 72h). Control cells were treated with appropriate vehicle and wells incubated in pre-incubation medium 1 without cytokines were used as controls.

For cytokine-treated mature adipocytes, preadipocytes were cultured in 12-well plates and induced into mature adipocytes as described in 4.2.1.1. On day 14 of differentiation the medium was changed to pre-incubation medium 2 (DMEM/F12 Nut mix (1:1) supplemented with 10 μg/mL transferrin, 33 μmol/L biotin and 17 μmol/L pantothenate) for 24h. The cytokine treatment was performed for fully differentiated cells on day 15 of post-differentiation. The cells were cultured in the presence of cytokines as mentioned above. Control cells were treated with appropriate vehicle and wells incubated in pre-incubation medium 2 without cytokines were used as controls.

In *study II*, the data from the cytokine treatment of TNF-α, IL1β, TGFβ1 and IL-6 for 48h in preadipocytes is included, showing the most pronounced effects on the mRNA expression of *MFAP5*. In addition, the data from the cytokine treatment of TNF-α for 24h both in preadipocytes and adipocytes is included in this thesis work, showing the most pronounced effects on the mRNA expression of *CPPED1*.

4.2.1.4 Knock-down experiments (*studies II and III*)

RNA interference (RNAi) was used for knocking down the expression of the target gene in mature adipocytes. ON-TARGETplus SMARTpool SiRNA was used and purchased from Dharmacon (Thermo Scientific Dharmacon®, Lafayette, USA). The four target siRNA sequences included in the pool of the target genes are shown in table 3. The negative control for the siRNA (scrambled) used in the experiments was Allstars negative control SiRNA (Qiagen, Valencia, CA, USA). The siRNA was transfected into the cell by using HiPerFect transfection reagent (Qiagen) according to the instructions.

The SGBS cells were cultured in 12-well plates and induced into mature adipocytes as described in 4.2.1.1. On day 14 of differentiation, the medium was replaced to DMEM/F12 Nut mix (1:1) supplemented with 33 μmol/L biotin, 17 μmol/L pantothenate, 10 μg/mL transferrin, and 20 nmol/L insulin. The cells were transfected with 20 nmol/L of *MFAP5* and 50 nmol/L of *CPPED1* siRNA and incubated for indicated time points. Knock-down of target gene expression was confirmed by quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) and western blot.

Table 3. The siRNA sequences of the target genes used in this thesis work

Gene	siRNA sequence
MFAP5	GGUCAAUAGUCAACGAGGA
	CGAUGUGACUCAAGCGACU
	GCCUAAUAGUUAAUACGAA
	GCAGUUGUCUAGUCGGGAA
CPPED1	AGAAAAUUGUUCACCGAUA
	UAAAUGCACUAAUGCGAAA
	CGGAGGACCUGAAGCGAGU
	CCUUUAAAAUGGAGCGAAU

4.2.1.5 Insulin-stimulated glucose uptake (*studies II and III*)

The SGBS preadipocytes were cultured in 12-well plates and induced into mature adipocytes as described in 4.2.1.1. On day 17 of differentiation, the cells were washed twice with PBS and pre-incubated with Krebs Ringer HEPES (KRH)-buffer [20 mmol/L HEPES (pH 7.4), 118 mmol/L NaCl, 4.8 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$,]. After pre-incubation, the cells were incubated in the presence of 100 nM Wortmannin, a PI3K-specific inhibitor (when indicated, *study III*), and followed by incubation with 1 µmol/L insulin. Next, 0.5 µCi/mL of labeled 2-Deoxy-D-[^{3}H] glucose (Amersham TRK672, GE Healthcare, UK) and 0.2 mmol/L of D-glucose were added. The reaction was terminated by placing the cells onto the ice and washing 3 times with ice-cold PBS.

The cells were solubilized with 0.2 N NaOH/well, and the cell lysate was transferred to a 2.0 mL eppendorf tube, and scintillation liquid was added for radioactivity counting. Glucose uptake was normalized to protein content as measured from the remaining cell lysate by using the Bio-Rad protein assay (Bio-Rad D$_C$ Protein Assay, Bio-Rad Laboratories, Hercules, CA, USA). Protein concentrations were measured according to manufactures instructions (D$_C$ Protein Assay, BIORAD) by using Wallac 1420.

4.2.1.6 Immunohistochemistry (*study II and unpublished, complementary data*)

Immunohistochemistry was performed to test for localization of the target protein expression in human abdominal subcutaneous wAT samples obtained from subjects (age 54 ± 17.4 years, BMI 35 ± 9.3 kg/m2) undergoing an elective surgery at the Kuopio University Hospital using NovoLink™ Min Polymer Detection system (Novocastra laboratories Ltd, UK). Paraffin-embedded human wAT sections were de-paraffinized in xylene and rehydrated in decreasing concentrations of ethanol, via sequential steps with interposed washing steps. Endogenous peroxidase activity was neutralized using Peroxidase Block and this was followed by incubation with Protein Block. WAT sections were stained with 1:10 or 1:500 diluted polyclonal antibody against MFAP5 (Sigma-Aldrich, St. Louis, MO, USA) or 1:10 diluted polyclonal antibody against CPPED1 in a humidity chamber for 1h at room temperature. The wAT sections were incubated with Post Primary block and further with a polymer that recognized mouse and rabbit immunoglobulins. Peroxidase activity was developed with substrate/chromogen, 3,3' -

diaminobenzidine. The sections were counterstained with Hematoxylin and coverslipped. A negative control slide without a primary antibody was included for assessing non-specific staining. Results were interpreted using a light microscope (Zeiss AxioImager M2 (Zeiss, Oberkochen, Germany)) and a camera (Zeiss AxioCam MRc).

4.2.1.7 Immunofluorescence (unpublished, complementary data)

Preadipocytes were grown on Permanox slides (Thermo Scientific) at 80% confluency which after the cells were induced to differentiation as mentioned in 4.2.1.1. Preadipocytes and differentiated adipocytes (day 11 post-differentiation) were washed twice in PBS and fixed with 4% paraformaldehyde (PFA). Next, the cells were washed twice in PBS and plasma membranes were permeabilized in PBS containing 0.01% Saponin (Sigma-Aldrich). The cells were incubated with Image-iT FX Signal enhancer to reduce background and washed twice with PBS. After blocking (0.2 mol/L Glycine + 5% FBS in PBS) the cells were incubated with 1:10 dilution of a rabbit anti-CPPED1 antibody (Sigma-Aldrich) at 4^0C overnight. The next day, after three washes with PBS, the cells were incubated with 1:1500 dilution of an Alexa Fluor 568 goat anti-rabbit secondary antibody (Molecular Probes) and 1:2000 dilution of a Hoechst 33258 nuclear stain (Molecular Probes). After washing with wash buffer and PBS, the cells were mounted with PermaFluor™ Aqueous Mounting Medium (Thermo Scientific) and visualized using an Olympus confocal microscope (OLYMPUS DP50, Japan).

4.2.1.8 Western blot (*studies II and III*)

In *study II*, adipocytes for total protein extraction were rinsed twice with PBS and then lysed in T-PER buffer (PIERCE, Rockford, USA) freshly supplemented with 1 mM Na_3VO_4, 1 mM NaF, and proteinase inhibitor cocktail (Roche Diagnostics, Germany), followed by centrifugation (13000 rpm, 15 min, $+4^0C$) and removal of the soluble fraction. The remaining cell pellet was lysed in UREA buffer [PBS (pH 7.4), 5.0 M Urea (Sigma-Aldrich), 2.0 M Thiourea (Sigma Aldrich), 50 mM DTT (Sigma-Aldrich), 0.1% SDS], followed by centrifugation (13000 rpm, 15 min, $+4^0C$) and removal of supernatant which consists of insoluble fraction (membranes etc.). The protein concentration was determined using Pierce 660nm Protein Assay (Pierce).

In *study III*, adipocytes were rinsed twice with PBS and then lysed in RIPA Lysis and Extraction Buffer (PIERCE, Rockford, USA) freshly supplemented with 1 mM EDTA, 1 mM PMSF, 1 mM Na_3VO_4, 1 mM NaF, and proteinase inhibitor cocktail (Roche Diagnostics, Germany), followed by centrifugation (13000 rpm, 15 min, $+4^0C$) and removal of soluble fraction. The protein concentration was determined using the Bio-Rad protein assay (Bio-Rad D_C Protein Assay, BioRad).

Equal amounts of protein (10 – 20 μg) was separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to the polyvinylidene fluoride (PVDF) membrane (GE Healthcare, Buckinghamshire, UK). A Rabbit anti-human MFAP5, ADAM12 and TGFβ1 (Abcam, Cambridge, UK) or a mouse anti-human IL-6 or a Rabbit anti-human CPPED1 and ADIPOQ (Sigma-Aldrich) were used as primary antibodies. The primary antibodies were detected with goat anti-rabbit or –mouse peroxidase conjugated secondary antibody (Pierce). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Abcam) was used for normalization. Signals were detected using Amersham ECL Advance Western Blotting Detection Kit (GE Healthcare) and ImageQuant Capture-RT ECL for Windows version 1.0.1 (GE Healthcare). In *study II*,

densitometric analysis was performed by Scion Image for Windows version Alpha 4.0.3.2 (Scion Corporation, USA) and in *study III* by ImageJ version 1.45s (ImageJ, National Institutes of Health, Maryland, USA).

4.2.1.9 High molecular weight (HMW) adiponectin enzyme-linked immunosorbent assay (ELISA) (*study III*)

In *study III*, mature adipocytes were treated with 50 nM *CPPED1* siRNA for 48h as mentioned in 4.2.1.4, and the effect of knock-down on HMW adiponectin secretion into the conditioned medium was measured with ELISA (Millipore, St. Charles, Missouri, USA) with a sensitivity of 0.5 ng/mL. After treatment the conditioned medium was collected, centrifuged at 1000 rpm for 10 min at room temperature (RT) and stored -80⁰C until measured. The samples were pre-treated with digestion solution that selectively degraded LMW and MMW adiponectin isoforms by proteolytic actions to enable measurement of HMW adiponectin levels. The standards, digestion controls and samples were added into the microtiter plate and incubated at RT for 2h with shaking. Next, unbound materials were washed with wash buffer and afterwards biotinylated polyclonal anti-adiponectin antibody was added and incubated at RT for 1h with shaking. Again, unbound materials were washed with wash buffer. Next, streptavidin-horseradish peroxidase conjugate was added for 30 min at RT with shaking and afterwards washed of excess of free enzyme conjugates with wash buffer. Next, TMB substrate was added for 5 to 20 min with shaking, and the enzyme activity was measured spectrophotometrically at 450-590 nm. The increase in absorbance is directly proportional to the amount of captured human HMW adiponectin.

4.2.1.10 Quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) (*studies I-III*)

Total RNA from wAT samples of the GENOBIN study was extracted using the TRIzol method (Invitrogen, Carlsbad, CA, USA) followed by further purification with RNeasy Mini Kit columns (Qiagen, Valencia, CA, USA). For the wAT and PBMC samples of the KOBS study, PBMCs of the GENOBIN study, SVF cells, mature adipocytes and cultured SGBS cells, the RNeasy Mini Kit was used for the total RNA extraction (Qiagen). The High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used for cDNA synthesis of AT and PBMC samples and iScript cDNA synthesis kit (Bio-Rad) for SVF cells, mature adipocytes and SGBS cells according to instructions provided by the manufacturer.

   RT-qPCR with TaqMan® chemistry (Applied Biosystems ®) by using ABI Prism 7500 analyzer (Applied Biosystems) was used for the determination of mRNA expression levels, and the TaqMan gene expression assays that were used in this thesis work are presented in table 4. The analysis for the relative quantity of a specific gene before and after the intervention in wAT and PBMCs of the GENOBIN study was performed as follows. Quantities of gene expression on each plate were corrected by the calibrator of the corresponding plate and the relative amount of expression was normalized with the corresponding values of endogenous control cyclophilin A1 (*PPIA*) expression for wAT and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) for PBMC samples of the GENOBIN study. Final gene expression levels are expressed as arbitrary units (AU). Expression of target genes were normalized to *PPIA* expression for wAT samples of the KOBS study, SVF cells, mature adipocytes and SGBS cells, and *GAPDH* for PBMC samples of the KOBS study. Expression of the target genes in wAT and PBMC samples of the KOBS

study, SVF cells, mature adipocytes and in cultured SGBS cells were normalized to the endogenous control using the formula $2^{\wedge -ddCt}$ (118).

Table 4. Genes investigated in this thesis work

Study	Tissue/ cells	Gene symbol	Gene description	Assay ID	Function
I,II	AT, SGBS	*ADAM12*	a disintegrin and metalloproteinase domain 12	Hs01106111_m1	involved in cell-cell, cell-matrix interactions, and early steps of adipogenesis
I	AT	*ADAM22*	a disintegrin and metalloproteinase domain 22	Hs00244640_m1	involved in cell-cell and cell-matrix interactions
III	AT, SGBS	*ADIPOR1*	adiponectin receptor 1	Hs00360422_m1	a receptor for adiponectin through which adiponectin regulates fatty acid catabolism and glucose uptake by adiponectin
III	AT	*ADIPOR2*	adiponectin receptor 2	Hs00226105_m1	mediates increased AMPK, PPARα ligand activity, fatty acid oxidation and glucose uptake by adiponectin
I-III	AT, SGBS	*ADIPOQ*	adiponectin	Hs00605917_m1	involved in insulin sensitivity and glucose uptake, fatty acid oxidation and inflammation
I	AT	*CCND2*	cyclin D2	CCND2 – E4	involved in regulating G1/S transition of cell cycle
III	AT, PBMC, SGBS	*CPPED1*	calcineurin-like phosphoesterase domain 1	Hs00217887_m1	unknown function, predicted to be endopolyphosphatase
III	AT, SGBS	*CPT1B*	carnitine palmitoyltransferase 1B	Hs00189258_m1	catalyzes long-chain fatty acid beta-oxidation in muscle mitochondria
III	PBMC, SGBS	*GAPDH*	glyceraldehyde-3-phosphate dehydrogenase	Hs99999905_m1	reference gene, involved in carbohydrate metabolism
II,III	SGBS	*GLUT1*	glucose transporter 1	Hs00892681_m1	responsible for constitutive or basal glucose uptake
II,III	SGBS	*GLUT4*	glucose transporter 4	Hs00168966_m1	Insulin-regulated facilitative glucose transporter
III	AT, PBMC	*IKBKB (IKKβ)*	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	Hs00233284_m1	plays an essential role in the NF-κB signaling pathway
I-III	AT, SGBS	*IL1β*	interleukin 1 beta	Hs00174097_m1	involved in the inflammatory response
I,III	AT	*IL1Ra*	interleukin 1 receptor antagonist	Hs00277299_m1	Inhibits the activity of interleukin-1
I-III	AT, SGBS	*IL-6*	interleukin 6	Hs00174131_m1	functions in inflammation and the maturation of B cells

I-III	AT, SGBS	*LEP*	leptin	Hs00174877_m1	a major role in the regulation of body weight, involved in the regulation of immune and inflammatory responses, hematopoiesis, angiogenesis
I,II	AT, SGBS	*MFAP5*	microfibrillar-associated protein 5	Hs00969606_m1	Component of the elastin-associated microfibrils
III	AT, PBMC	*NFKBIA (IKBα)*	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	Hs00153283_m1	Inhibits the activity of dimeric NF-κB
III	AT, PBMC	*NFKBIB (IKBβ)*	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta	Hs00182115_m1	Inhibits NF-κB by complexing with and trapping it in the cytoplasm
I,II	AT, SGBS	*NOTCH1*	notch1	Hs01062012_m1	Affects the implementation of differentiation, proliferation and apoptotic programs
I	AT	*NOTCH2*	notch2	Hs01050729_m1	Affects the implementation of differentiation, proliferation and apoptotic programs
I-III	AT, SGBS	*PPARγ2*	peroxisome proliferator-activated receptor gamma 2	PPARgamma2_REVP	a regulator of adipocyte differentiation and glucose homeostasis
I-III	AT, SGBS	*PPIA*	peptidylprolyl isomerase A (cyclophilin A)	Hs99999904_m1	accelerate the folding of proteins
I,II	AT, SGBS	*TGFβ1*	transforming growth factor, beta 1	Hs00932734_m1	regulator of ECM production and is involved in proliferation, differentiation, and other functions in many cell types
I,III	AT	*TNFα*	tumor necrosis factor alpha	Hs00174128_m1	involved in cell proliferation, differentiation, apoptosis, lipid metabolism, and impairs insulin signaling

4.3 STATISTICAL ANALYSES

4.3.1 Human studies

The GENOBIN study—The data were analyzed using the SPSS software for Windows version 14.0 (SPSS Inc., Chicago, IL, USA). Data are given as mean ± SD, unless otherwise indicated. The normality of distributions of the variables was tested with the Kolmogorov-Smirnov test with Lilliefors' significance correction. Logarithmic transformation was used to achieve normal distribution whenever needed (indicated in the tables and figures). General linear models (GLM) for univariate analysis were used to test the difference in fold change values of mRNA expression between the groups. A paired samples *t*-test was

used for comparing the baseline and endpoint measurements within the study group. Correlation analyses were done using Pearson's correlation coefficient. Partial correlation analysis with adjustment for weight at baseline and gender was used when appropriate. The WR and control groups were combined in the correlation analysis at baseline and when studying the correlations of change- values because the participants represent a similar population in the beginning of the study. Thus, the participants were homogenous regarding the selection criteria. After the intervention the two treatment groups were analyzed separately because of potential treatment effect. A value of $p<0.05$ was considered statistically significant.

The KOBS study—The expression data were analyzed using the SPSS software for Windows version 14.0 (SPSS Inc., Chicago, IL, USA). Data are given as mean ± SD, unless otherwise indicated. Paired samples *t*-test was used for comparing the baseline and endpoint gene expressions.

4.3.2 Cell studies

Results of the SVF cells, mature adipocytes and SGBS cell culture studies were analyzed using the GraphPad Prism5 software for Windows version 5.03 (GraphPad Software, San Diego California USA), and the results are expressed as mean ± SEM. Statistical significance was determined with the independent samples *t*-test, one-way analysis of variance with Bonferroni's correction for multiple comparisons, or one-sample *t*-test (indicated in figures). A value of $p<0.05$ was considered statistically significant.

5 Results

5.1 HUMAN STUDIES (*study I* and unpublished, complementary data)

Baseline values for body weight, BMI, fasting plasma glucose and serum insulin concentration in the GENOBIN and KOBS studies are presented in table 5. Participants in the KOBS study were younger, morbidly obese and their serum insulin levels were much higher than in the GENOBIN study.

Table 5. Baseline characteristics of the GENOBIN and KOBS studies

	GENOBIN (n=46)	KOBS (n=95)
Age, years	59 ± 7	45 ± 8
Body mass index, kg/m^2	32.9 ± 3.2	45.0 ± 6.3
Fasting plasma glucose, mmol/L	6.2 ± 0.4	6.7 ± 1.7
Fasting serum insulin, mU/L	11.5 ± 5.4	22.4 ± 22.0

5.1.1 Gene expression of *MFAP5* and *CPPED1* in adipose tissue of the GENOBIN study (*studies I and III*) and in adipose tissue and peripheral blood mononuclear cells of the KOBS study (unpublished, complementary data)

Previously published reductions in *MFAP5* and *CPPED1* expression in wAT after weight reduction using Affymetrix microarrays (20) were confirmed with RT-qPCR (Figure 4). As shown in figure 4A, *MFAP5* expression in wAT decreased by 13% (p=0.017) in response to weight loss, while no change was observed in the control group (data not shown). *MFAP5* was not expressed in PBMCs of the GENOBIN study (data not shown).

   As shown in figure 4B, RT-qPCR results confirmed the previously published reduction in the expression of *CPPED1* in wAT after weight loss in the GENOBIN study using microarray (*study III*) (20). We demonstrated a similar decrease in PBMCs of the GENOBIN and KOBS studies that was seen in wAT samples of the GENOBIN study after weight loss (Figure 4C). The down-regulation of *CPPED1* expression in wAT along with weight loss could not be verified in wAT samples from the KOBS study (Figure 4B). The expression of *CPPED1* did not change in the control group in wAT or PBMC samples of the GENOBIN study (data not shown).
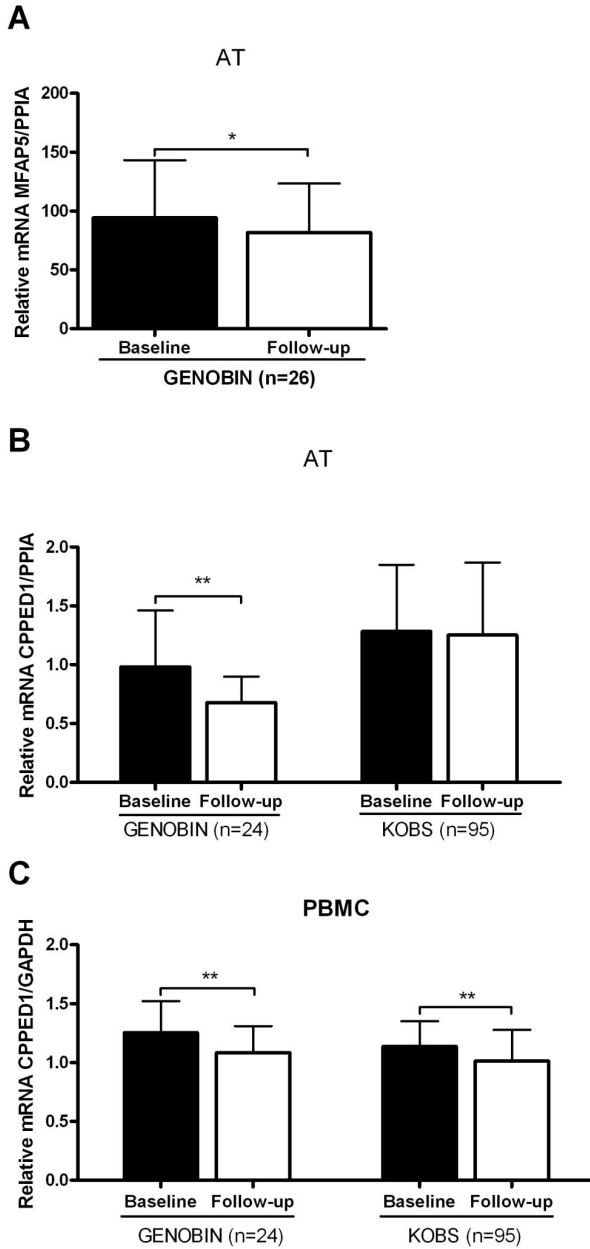
Figure 4. (A) A relative mRNA expression of *MFAP5* at baseline and after weight loss (follow-up) in AT of the GENOBIN study. (B) A relative mRNA expression of *CPPED1* at baseline and after weight loss (follow-up) in AT and (C) in PBMCs of the GENOBIN and KOBS studies. The values are expressed as relative gene expression levels normalized to endogenous control *PPIA* for the AT samples and *GAPDH* for the PBMC samples. $p<0.05$ was considered statistically significant. The comparisons were made within groups: black bars; expression at baseline, white bars; expression after the follow-up.

5.1.2 Correlation analyses

5.1.2.1 Correlation of *MFAP5* with anthropometric and biochemical measures and with selected genes (*study I*)

Correlation analyses at baseline in the GENOBIN study showed that the expression of *MFAP5* mRNA correlated positively with body mass index (Table 6). Moreover, the change in *MFAP5* mRNA expression correlated with the change of body fat mass (r=0.392, p=0.009). The expression of *MFAP5* correlated with fasting serum insulin concentration at baseline even after adjustment for body weight (Table 6). There was no correlation between *MFAP5* mRNA expression and fasting plasma glucose levels.

Furthermore, the expression of *MFAP5* correlated negatively with concentration of fasting serum adiponectin and positively with serum concentration and wAT expression of leptin (Table 6). The change of *MFAP5* expression level correlated significantly with the change of adiponectin expression in wAT (r=0.433, p=0.004). Moreover, a positive correlation was also found between *MFAP5* expression and fasting plasma IL1Ra concentrations, and a negative correlation with fasting plasma IL1β concentrations (adjusted for body weight). *MFAP5* expression did not correlate with the gene expression level of *IL1Ra* and *IL1β* in subcutaneous wAT (Table 6).

Interestingly, *MFAP5* expression correlated positively with the mRNA expression of a master regulator of adipogenesis, *PPARγ*, cyclin D2 (*CCND2)* and disintegrin and metalloproteinase domain 12 (*ADAM12)* at baseline when adjusted for body weight (Table 6). Furthermore, the change of *MFAP5* expression level correlated with the change of *ADAM12* expression in wAT (r=0.343, p=0.026) when adjusted for the change of body weight.

Table 6. Baseline partial correlations of *MFAP5* mRNA expression in subcutaneous wAT with anthropometric, biochemical measures, and with selected genes expressed in subcutaneous wAT in the combined weight reduction and control groups.

	Groups combined (n=46)	
	0 wk	
	r^a	p
Anthropometric and biochemical measures		
Body mass index (kg m^{-2})^b	**0.369$^{####}$**	**0.014**
Body weight (kg)^b	0.043$^{###}$	0.785
Body fat mass (kg)^b	0.277$^{####}$	0.069
Waist circumference (cm)	0.282$^{###}$	0.071
S_I ((mU l^{-1})$^{-1}$ x min^{-1})	-0.260$^{##}$	0.101
fS-adiponectin (µg/ml)	**-0.378$^{###}$**	**0.014**
fS-leptin (ng/ml)	**0.361$^{###}$**	**0.019**
fS-insulin (pmol/l)	**0.397$^{##}$**	**0.010**
fP-glucose (mmol/l)	0.132$^{##}$	0.41
fS-TNFα (pg/ml)	-0.162$^{###}$	0.305
fP-IL1β (pg/ml)	**-0.401$^{###}$**	**0.009**
fP-IL1Ra (pg/ml)	**0.347$^{###}$**	**0.024**
hsCRP (mg/l)	-0.021$^{###}$	0.895
Gene expression in adipose tissue (AU)		
LEP	**0.338$^{###}$**	**0.028**
ADIPOQ	0.033$^{###}$	0.838
PPARγ	**0.511$^{###}$**	**0.001**
TNFα	0.132$^{###}$	0.404
IL1β	0.071$^{##}$	0.659
IL1Ra	0.106$^{###}$	0.504
IL-6	0.007$^{###}$	0.966
TGFβ1	0.25$^{#}$	0.12
NOTCH1	-0.277$^{#}$	0.083
NOTCH2	0.191$^{#}$	0.239
CCND2	**0.523$^{###}$**	**<0.001**
ADAM22	0.248$^{###}$	0.114
ADAM12	**0.584$^{###}$**	**<0.001**

Values were logarithmized for analyses when appropriate. ^{a} adjusted for body weight (kg); ^{b} correlation analyses done using Pearson's method, no adjustments; $^{#}$ n = 41; $^{##}$ n = 42; $^{###}$ n = 43; $^{####}$ n = 44; S_I = insulin sensitivity index; fS = fasting serum; fP = fasting plasma; TNFα = tumour necrosis factor alpha; IL1β = interleukin 1 beta; IL1Ra = interleukin 1 receptor antagonist; hsCRP = high sensitivity C reactive protein; AU = arbitrary unit; LEP = leptin; ADIPOQ = adiponectin; PPARγ = Peroxisome proliferator-activated receptor gamma; IL-6 = interleukin 6; TGFβ1 = transforming growth factor beta 1; NOTCH1 = notch1 preproprotein; NOTCH2 = notch2 preproprotein; CCND2 = cyclin D2; ADAM22 = A disintegrin and metalloproteinase domain 22; ADAM12 = A disintegrin and metalloproteinase domain 12; bolded font indicates a statistically significant correlation (p<0.05)

5.1.2.2 Correlation of *CPPED1* with selected genes (unpublished, complementary data)

Due to down-regulated expression of *CPPED1* in PBMCs of both human studies and in wAT in the GENOBIN study, we examined whether *CPPED1* expression correlated with

the expression of inflammatory genes in wAT and PBMCs from the GENOBIN study. The expression of *CPPED1* mRNA in wAT correlated strongly with the mRNA expression levels of inflammatory genes, including *IL1Ra*, *TNFα*, *IL1β*, and inhibitors of the NF-κB pathway, i.e. *IKBα*, *IKBβ* and *IKKβ*, when adjusted for baseline body weight. In addition, there was a positive correlation between the mRNA expression of *CPPED1* and *IKBα* in PBMCs (Table 7). Furthermore, the change of *CPPED1* mRNA expression correlated significantly with the changes in expression of *TNFα* and *IL1β* (r=0.664, p<0.001; r=0.778, p<0.001) in wAT and with the expression change of *IKBβ* (r=0.428, p=0.016) in PBMCs.

Moreover, *CPPED1* mRNA expression was positively correlated with the mRNA expression levels of adiponectin receptor 1 (*ADIPOR1*) at baseline (Table 7), and the change in *CPPED1* mRNA expression in wAT correlated strongly with the change of *ADIPOR1* expression in wAT (r=0.779, p<0.001). There were no significant correlations between *CPPED1* expression and anthropometric or biochemical measures (data not shown).

Table 7. Partial correlations of *CPPED1* mRNA expression in subcutaneous wAT and PBMCs of the GENOBIN study with selected genes expressed in subcutaneous wAT and PBMCs at baseline of the intervention in the combined weight reduction and control groups.

	Groups combined (n=46), 0 months		Groups combined (n=34), 0 months	
	wAT		PBMC	
	r[a]	p	r[a]	p
Gene expression (AU)				
PPARγ	**-0.358[###]**	**0.020**	-	
LEP	-0.093[###]	0.559	-	
ADIPOQ	-0.228[###]	0.146	-	
ADIPOR1	**0.775[###]**	**<0.001**	-0.090[+++++]	0.626
ADIPOR2	-0.020[###]	0.901	-0.033	0.857
IL1Ra	**0.364[###]**	**0.018**	-0.061[++]	0.759
TNFα	**0.695[###]**	**<0.001**	-0.178[++]	0.364
IL1β	**0.699[##]**	**<0.001**	-0.047[++]	0.813
IL-6	0.117[###]	0.462	-0.069[++]	0.726
IKBα	**0.331[#]**	**0.037**	**0.385[+++++]**	**0.030**
IKBβ	**0.431[#]**	**0.005**	0.299[+++++]	0.096
IKKβ	**0.428[#]**	**0.006**	-0.293[++]	0.130
CPT1B	**0.348[###]**	**0.024**	-	

Values are logarithmized, when appropriate. wAT = white adipose tissue; PBMC = peripheral blood mononuclear cell; [a] adjusted for body weight (kg); AU = arbitrary unit; [#] n = 41; [##] n = 42; [###] n = 43; [++] n = 29; [+++++] n = 33; PPARγ = Peroxisome proliferator-activated receptor gamma; LEP = leptin; ADIPOQ = adiponectin; ADIPOR = adiponectin receptor; IL1Ra = interleukin 1 receptor antagonist; TNFα = tumor necrosis factor alpha; IL1β = interleukin 1 beta; IL-6 = interleukin 6; IKBα = nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; IKBβ = nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, beta; IKKβ = inhibitor of κB kinase-β; CPT1β = Carnitine palmitoyltransferase 1 beta; - not tested; bolded font indicates a statistically significant correlation (p<0.05)

5.2 EXPERIMENTS IN WHITE ADIPOSE TISSUE AND ADIPOCYTE CELL CULTURE

5.2.1 Protein expression in human wAT (*study II* and unpublished, complementary data) and in preadipocytes and adipocytes (unpublished, complementary data)

To examine the localization of protein expression in formalin-fixed paraffin-embedded sections of human subcutaneous wAT and in cultured preadipocytes and adipocytes, the wAT sections (Figure 5) and cells (Figure 6) were stained with an antibody against target proteins MFAP5 (*study II*) or CPPED1 (unpublished, complementary data).

 As shown in figure 5(B-C), MFAP5 protein was expressed in the interstitial space, connective tissue (Figure 5B) and blood vessel walls (Figure 5C) of human wAT, indicating that MFAP5 is expressed in the ECM of human wAT. Type I collagen (COL1A), which was used as a positive control for ECM staining, showed a similar localization as MFAP5 and was expressed in the connective tissue (Figure 5A). Immunohistochemistry of human subcutaneous wAT sections also showed staining of CPPED1 protein in the cytoplasm of adipocytes (Figure 5D).
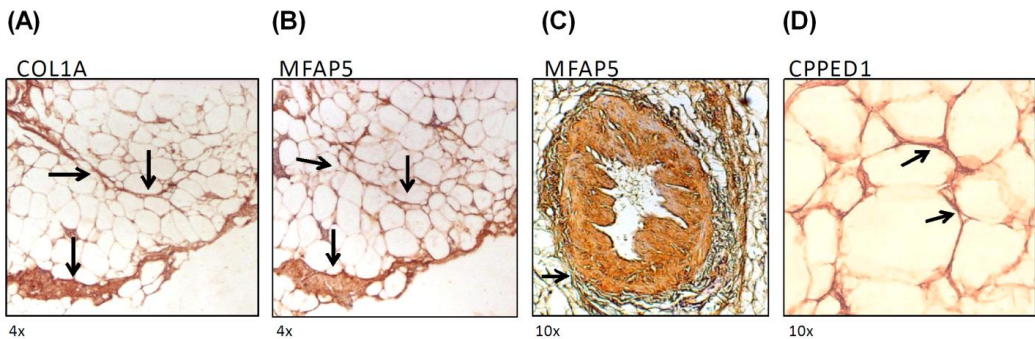
Figure 5. Protein expression of (A) COL1A (1:10 dilution),-(B-C) MFAP5 (1:10 and 1:500 dilutions) and (D) CPPED1 (1:10 dilution) in human subcutaneous wAT sections by immunohistochemistry (brownish color: positive staining of target protein, indicated with arrows; magnification is indicated in figures). Representative figures from 3 independent stainings.

 Immunofluorescence of preadipocytes and differentiating adipocytes showed a cytoplasmic localization of the CPPED1 protein expression (Figure 6A-B), respectively.
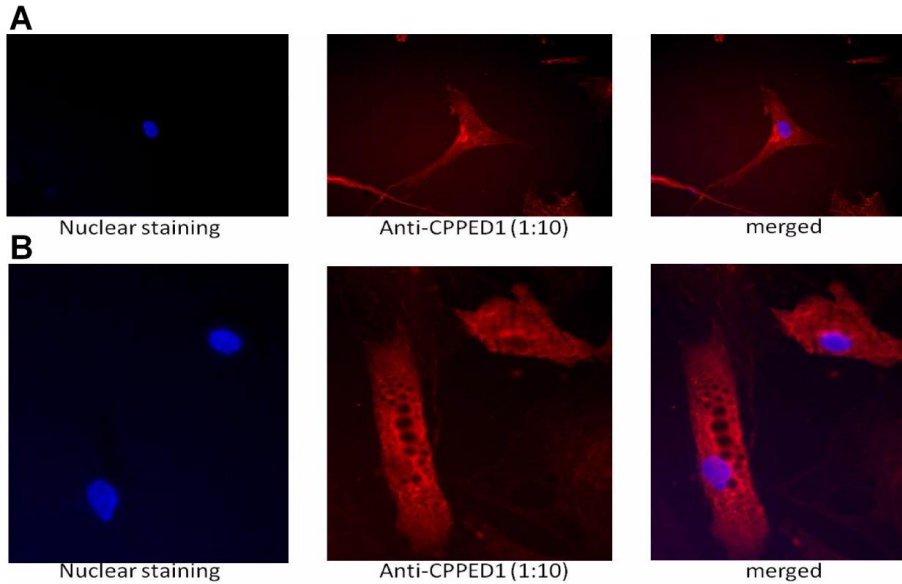
Figure 6. An immunofluorescence staining of CPPED1 protein in cultured (A) preadipocytes and (B) differentiating adipocytes. Blue (1:2000 nuclear stain, Hoechst 33258, red (1:10 anti-CPPED1).

5.2.2 Gene expression in SVF/mature adipocytes, and during preadipocyte differentiation (*study II* and unpublished, complementary data)

Gene expressions of *MFAP5* and *CPPED1* were higher in SVF cells than in mature adipocytes isolated from human subcutaneous wAT samples (37%, p<0.01; 81%, p<0.01), respectively (Figure 7A-B). Target gene expression was determined during differentiation of SGBS cells. The expression of *MFAP5* was maximal in preadipocytes, and decreased during differentiation of preadipocytes (Figure 7C), while the expression of *CPPED1* mRNA did not change during preadipocyte differentiation (Figure 7D). The expression of *PPARγ2*, which was used as a marker gene for preadipocyte differentation, increased constantly during the adipocyte differentiation process, as expected (Figure 7E). In addition, the differentiation was also confirmed with increased staining of lipid droplets during differentiation by Oil Red O staining (Figure 7F) which was performed to detect mature adipocytes.
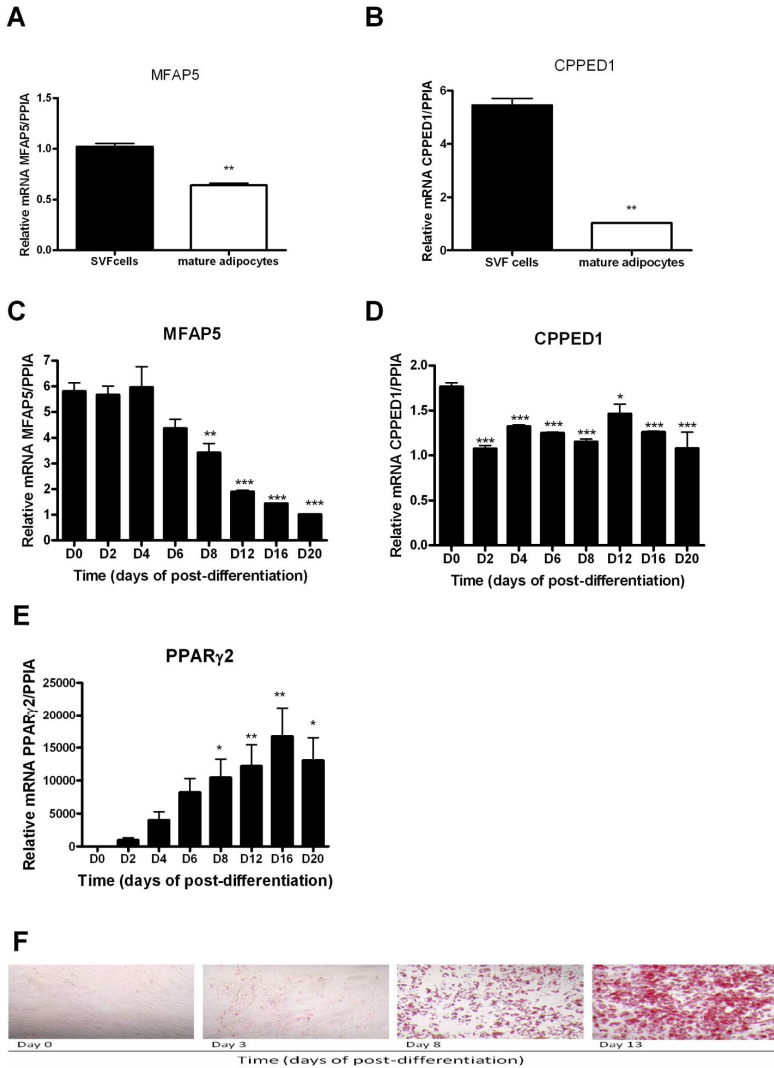
Figure 7. The expression of (A) *MFAP5* and (B) *CPPED1* in stromal-vascular fraction (SVF) cells (black bar) and in mature adipocytes (white bar). The graph shows the means ±SEM from two independent experiments, and the values are expressed as relative gene expression levels normalized to endogenous control *PPIA*. ** $p<0.01$ (mature adipocytes vs. SVF cells). The expression of (C) *MFAP5*, (D) *CPPED1* and (E) *PPARγ2* in cultured SGBS cells during preadipocyte differentiation. The SGBS cells were induced to differentiate and were harvested in different time points during adipocyte differentiation. Statistical significance was tested using one-way ANOVA with Bonferroni's multiple comparison test. The graph shows the means ±SEM from four independent experiments, and the values are expressed as relative gene expression levels normalized to endogenous control *PPIA*. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (indicated time point versus Day (D)0).(F) Oil-Red-O staining during adipocyte differentiation (10x magnification)

5.2.3 Knock-down experiments (*studies II and III*)

Based on the correlation results, we wanted to better understand the role of the target genes, *MFAP5* and *CPPED1*, in adipocyte metabolism. Therefore, the effect of target gene knock-down by siRNA in SGBS adipocytes on the expression of genes involved in adipogenesis, glucose metabolism, inflammation and the ECM was studied.

5.2.3.1 MFAP5 in adipocyte function (*study II*)

The knock-down of *MFAP5* expression by siRNA induced a significant down-regulation of MFAP5 protein expression (Figure 8A). At the mRNA level, *MFAP5* knock-down decreased the expression of several genes related to ECM function and inflammation (Figure 8B). However, no significant effects were observed on the expression levels of genes involved in adipogenesis, like *PPARγ*, leptin and adiponectin or in glucose metabolism, like *GLUT1* and *GLUT4* (Figure 8B). Furthermore, *MFAP5* knock-down did not affect insulin-stimulated glucose uptake in SGBS adipocytes (data not shown). In *study I*, we found correlations between the expressions of *MFAP5* and *ADAM12* and factors related to inflammation in human AT samples. Therefore, we wanted to examine whether MFAP5 could modulate the expression of these genes in SGBS adipocytes. The *MFAP5* knock-down decreased protein expression of ADAM12 (Figure 8C). *MFAP5* knock-down decreased protein expression of TGFβ1 and IL-6 (Figure 8D-E) which are known to be involved in ECM remodeling.
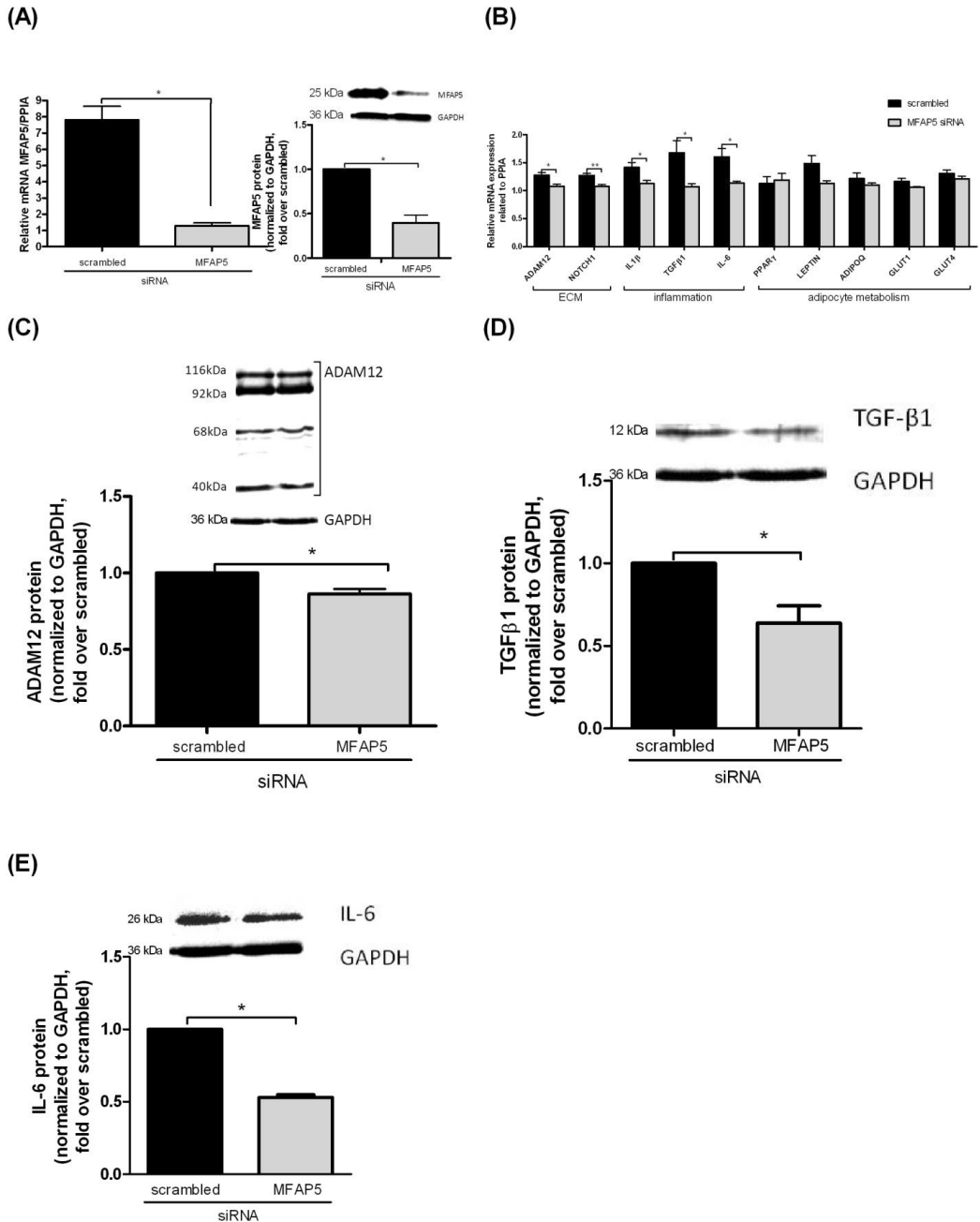
Figure 8. The effect of *MFAP5* knock-down (A) on the mRNA and protein expression of MFAP5, (B) on the mRNA expression of selected genes involved in adipocyte metabolism, ECM and inflammation, on the protein expressions of (C) ADAM12, (D) TGFβ1 and (E) IL-6. The graph shows the means ±SEM from three independent experiments, and the values are expressed as relative gene expression levels normalized to reference gene *PPIA* for mRNA experiments and GAPDH for protein experiments. * p<0.05, ** p<0.01 (MFAP5 siRNA/scrambled)

5.2.3.2 CPPED1 in adipocyte function (*study III*)

Knock-down of *CPPED1* expression decreased the protein expression of CPPED1 time-dependently (Figure 9A). Moreover, the *CPPED1* knock-down for 48h increased mRNA expression of *ADIPOQ, ADIPOR1* and *GLUT4*, and decreased mRNA expression of *GLUT1* and leptin (Figure 9B).

   CPPED1 knock-down modified the expression of genes related to glucose metabolism suggesting an impact of *CPPED1* in adipocyte metabolism. Therefore, we examined insulin-stimulated glucose uptake in cells treated with or without *CPPED1* siRNA. As expected, insulin-stimulated glucose uptake increased in control cells (Figure 9C). Interestingly, *CPPED1* knock-down increased insulin-stimulated glucose uptake ($p<0.05$) compared to insulin-treated control cells. In *CPPED1* siRNA transfected cells, Wortmannin treatment reduced insulin-stimulated glucose uptake ($p<0.01$) compared to the insulin-treated cells, suggesting that the *CPPED1* siRNA induced effect on insulin-stimulated glucose uptake could be mediated through a PI3K-dependent pathway (Figure 9C).

   The protein expression of ADIPOQ increased time-dependently (Figure 9D), leading to a significant increase in ADIPOQ protein at 96h after *CPPED1* siRNA treatment ($p<0.05$). Furthermore, the reduction of *CPPED1* expression for 48h tended to increase HMW adiponectin secretion ($p=0.057$) into the conditioned medium (Figure 9E). Finally, there was a tendency ($p=0.0686$, $p=0.1663$, $p=0.1051$, respectively) for time-dependent up-regulation of GLUT4 protein in response to *CPPED1* knock-down (Figure 9F).
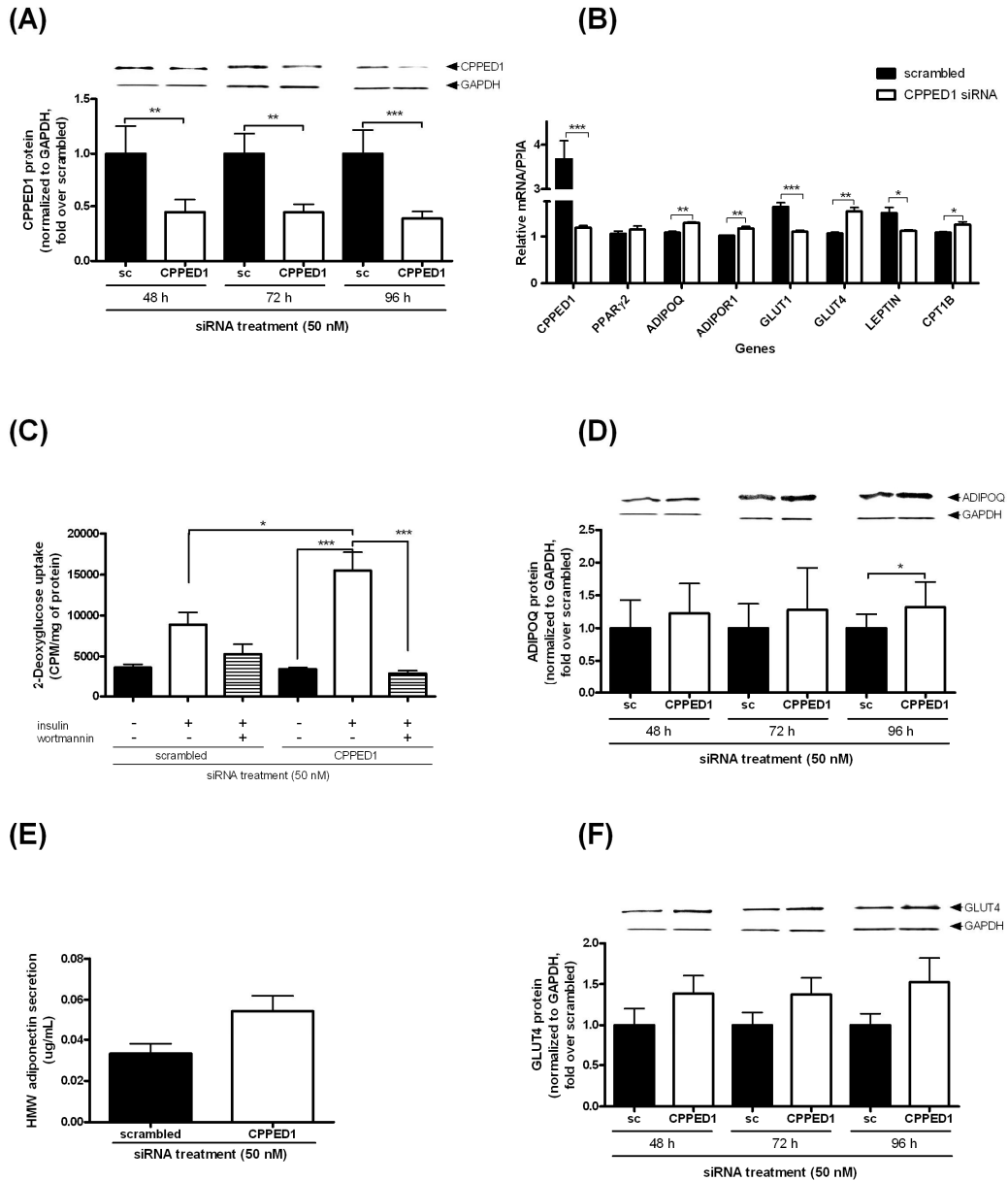
Figure 9. (A) Time-course effect of *CPPED1* knock-down on the protein expression of CPPED1. (B) The effect of *CPPED1* knock-down on the mRNA expressions of selected genes. (C) The effect of *CPPED1* knock-down for 48h on insulin-stimulated glucose uptake (the values are expressed as counts per minute (CPM) normalized to the protein amount (mg) of corresponding well). Statistical significance was tested using one-way ANOVA with Bonferroni's multiple comparison test. (D) Time-course effect of *CPPED1* knock-down on the protein expression of ADIPOQ. (E) The effect of *CPPED1* knock-down for 48 h on the secretion of HMW adiponectin into the conditioned medium. (F) Time-course effect of *CPPED1* knock-down on the

protein expression of GLUT4. The values for the mRNA and protein expression results of *CPPED1* knock-down are normalized to *PPIA* and GAPDH, respectively; and statistical significance was tested using independent samples *t*-test. The graph shows the means ±SEM and/or representative figures within time of siRNA exposure from at least three independent experiments.* p<0.05, ** p<0.01, *** p<0.001; sc: scrambled

5.2.4 Cytokine treatments (*study II* and unpublished, complementary data)

In *study II*, we investigated the effect of inflammatory factors on the expression of *MFAP5* in SGBS preadipocytes. Incubation of preadipocytes for 48h showed that the lowest concentration (0.1 ng/mL) of TNF-α and IL-6 (Figure 10A,D, respectively) increased the mRNA expression of *MFAP5* (p<0.01, p<0.05, respectively). The highest concentration (10 ng/mL) of TGFβ1 up-regulated *MFAP5* mRNA expression (p<0.05) (Figure 10C). In contrast, increasing concentrations of IL1β decreased the mRNA expression of *MFAP5* (Figure 10B).
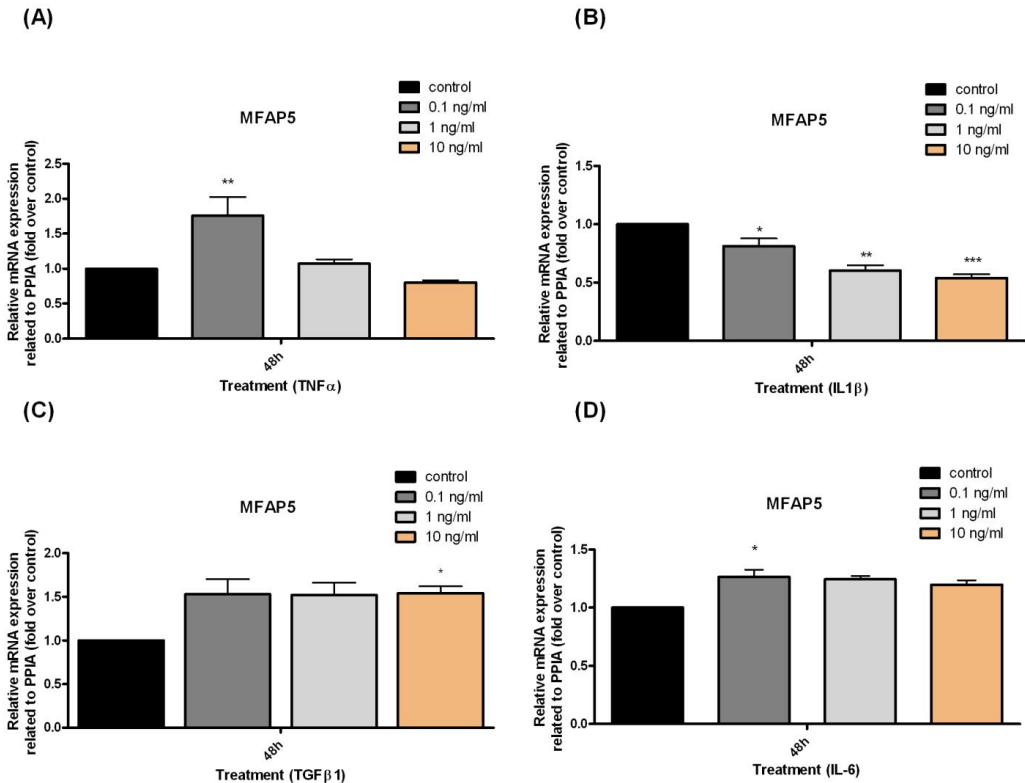
Figure 10. The effect of (A) TNF-α, (B) IL1β, (C) TGFβ1 or (D) IL-6 treatment at different concentrations (0.1–10.0 ng/mL) for 48 h on the mRNA expression of *MFAP5* in preadipocytes. The graph shows the means ±SEM from three independent experiments, and the values are

expressed as relative gene expression levels normalized to reference gene *PPIA*. * p<0.05, **
p<0.01, *** p<0.001 compared with control

Our results showed that treatments with TNFα and IL1β (10 ng/mL) for 24 h induced IR in
differentiated adipocytes as measured by glucose uptake, with a significant reduction in
insulin-stimulated glucose uptake (Figure 11A). As shown in figure 11B, treatment of
preadipocytes with 1.0 and 10.0 ng/mL of TNF-α for 24h up-regulated the *CPPED1* mRNA
expression. Treatment of mature adipocytes with TNF-α tended to increase the *CPPED1*
mRNA expression, but the results were not significant (Figure 11C).

(A)

(B) preadipocyte
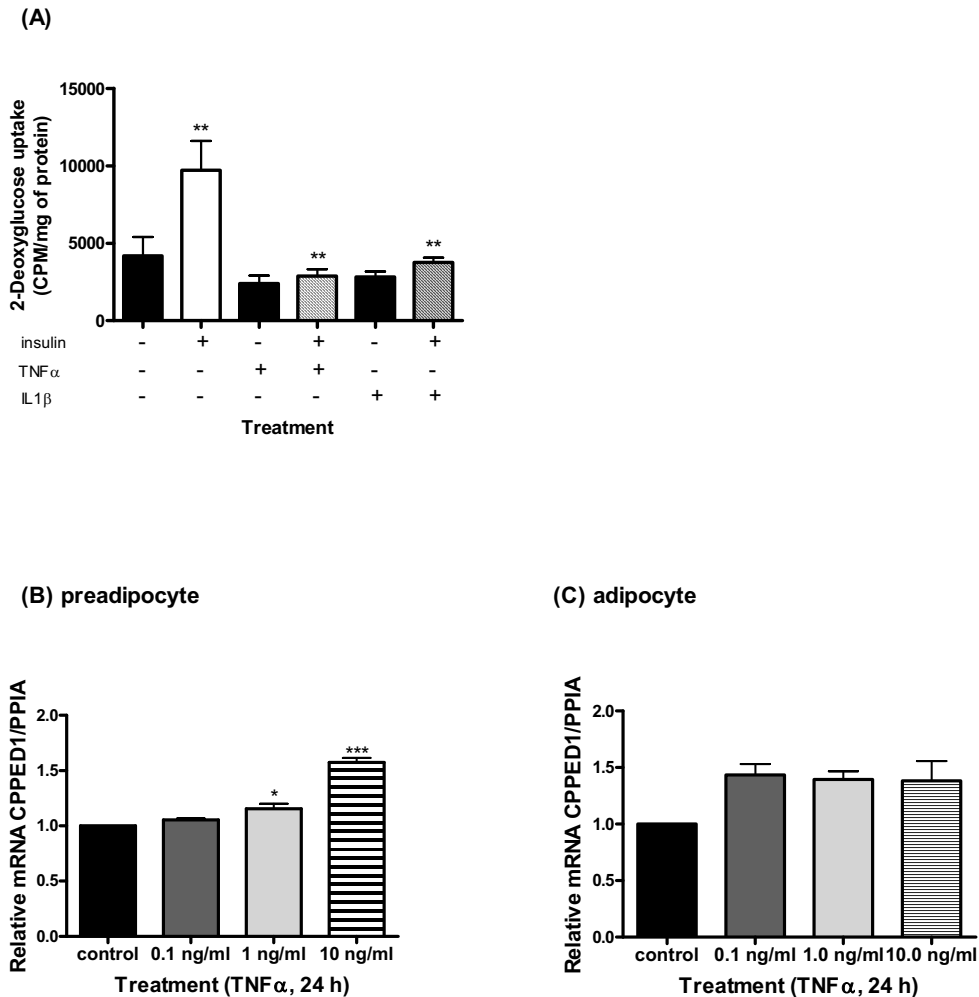
(C) adipocyte

Figure 11. The effect of cytokine treatment on (A) glucose uptake (the values are expressed as
counts per minute (CPM) normalized to the protein amount (mg) of corresponding well), and
expression of *CPPED1* (B) in preadipocytes and (C) in mature adipocytes. (The graph shows

the means ±SEM from three independent experiments, and the values are expressed as relative gene expression levels normalized to the reference gene *PPIA*. * p<0.05, ** p<0.01, *** p<0.001 compared with control)

6 Discussion

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Subjects

In this study, subjects were carefully selected and well characterized and thus formed a homogenous study population (119). The study subjects in GENOBIN were overweight or obese with impaired glucose tolerance or impaired fasting glucose and additionally had two other features of the metabolic syndrome and thus were at high risk of developing type 2 diabetes and cardiovascular diseases (120). The subjects in the KOBS also had abnormal glucose metabolism, but they were morbidly obese.

6.1.2 Adipose tissue biopsy techniques

Microarray has shown to be an efficient tool to investigate simultaneously thousands of genes in a single experiment thus enabling to investigate new players of wAT in human obesity (121). The development of obesity surgery programs has increased the accessibility of wAT samples for gene expression experiments. However, the biopsy technique using a surgical incision was more laborious than the needle biopsy method, which is easier to obtain and repeat in the same subject, better enabling measurement of the individual response to an intervention (122). There is evidence showing that although the needle biopsy method is a rapid and easy technique to obtain wAT samples, it may result in underrepresentation of SVF of wAT because the components of ECM that reside in SVF are poorly aspirated by this technique (122). Therefore, it would be preferable to use wAT samples obtained with the surgical biopsy method when investigating the biology of SVF in wAT. Good practice would be to replicate the findings of MFAP5 in other studies, like KOBS, in which surgical biopsy technique was used.

A drawback is that we used two different wAT biopsy sampling methods to obtain the wAT samples. It has been demonstrated that gene expression profile could differ between wAT samples obtained with needle or open biopsy methods (122) and thus the results need to be interpreted with caution. Moreover, a limitation in *studies I* and *II* was that we measured mRNA levels and lack the protein samples due to the limited sample size in needle-biopsy technique used for wAT samples in the GENOBIN study.

In *studies I-III*, the gene expression analyses were investigated in subcutaneous wAT samples and cells isolated from subcutaneous wAT. There is easy accessibility of human subcutaneous wAT of the abdomen, for which reason it is routinely used in both *in vivo* and *in vitro* studies (51). Both white adipose depots, visceral and subcutaneous, have been shown to be associated with obesity-related complications like IR, type 2 diabetes and cardiovascular disease (18,123). However, subcutaneous and visceral fat have been demonstrated to be metabolically different, resulting in differences in their gene expression patterns (124). In addition, visceral wAT has a unique inflammatory pattern compared to subcutaneous wAT (123). Visceral fat is usually obtained during surgical operations from morbidly obese subjects. Therefore, in *study I* these samples were not

considered, however, we have gene expression data in visceral wAT related to *study III* (unpublished, complementary data).

6.1.3 Statistical issues

A limitation in the GENOBIN study was the small sample size. Statistical power is limited when carrying out statistical analyses in small groups. However, despite having small groups we were able to find significant correlations. Different methods were used for wAT biopsies and for normalization of RT-qPCR data in the study on CPPED1 in human samples (*study III* and unpublished, complementary data), and this may complicate the comparison of the results between the two studies, GENOBIN and KOBS. We used the standard curve method for wAT samples of the GENOBIN study and $2^{-\Delta Ct}$ method for wAT samples of the KOBS study. It could be argued whether using two different methods is appropriate, but these two methods have been demonstrated to give equivalent results (125).

Pearson's correlation coefficient is widely used in clinical studies to measure a relationship between two variables (126). In *studies I* and unpublished, complementary data related to *study III*, it was used for quantifying relationships between the expressions of genes of interest and anthropometric and clinical and selected gene expression data. However, it should be noted that correlation does not necessarily imply for causation (127). Therefore, functional studies were performed (*study II and III*) to investigate the mechanisms behind the correlations.

A drawback in *study II* is the lack of statistics due to inadequate sample size in the experiments concerning isolated SVF and mature adipocytes of wAT. The reason for this is that there were not enough wAT samples for RNA extraction, and we needed to pool together samples so that there was enough material for further experiments. In the future, to resolve this problem the number of biological replicates should be increased to ensure the adequacy of material.

6.1.4 Gene expression

RT-qPCR is a sensitive and powerful method to quantify gene expression and to validate the data obtained from microarray analysis (118,128). The data in RT-qPCR is relative, and normalization is crucial for correcting variations in total RNA amount, reverse-transcription and amplification efficiency. Several methods can be used to normalize data using a reference gene (99,129). *GAPDH* and *18S* are most commonly used reference genes (99). However, the expression of reference genes have shown to differ greatly, as seen e.g. in *GAPDH* response to dietary intervention (99) and in the *in vitro* experiments of SGBS cells during differentiation in this thesis work (data not shown). Moreover, *18S* has been shown to have high expression levels (99), also in our samples, and thus was not suitable as a reference gene. Because the level of expression were much lower in the target genes, and normalization to the expression of *18S* would have blunted the results of the target genes. We tested also other possible candidate reference genes like beta actin and TATA box binding protein (*TBP*), but *PPIA* was chosen as a reference gene because its expression was the least affected by our *in vitro* experiments. In human samples of the GENOBIN study the optimal reference genes were tested using a TaqMan kit of endogenous controls from Applied Biosystems.

The $2^{-\Delta Ct}$ method is commonly used for the normalization of RT-qPCR data (99,129), and it was used for normalization of our *in vitro* experiments. Another method that is generally used is relative standard curve which was also used for normalization of RT-qPCR data of

our human samples in the GENOBIN study with *PPIA* as a reference gene in wAT samples and *GAPDH* in PBMCs. The reason why we changed to using the $2^{-\Delta Ct}$ method is that there is no need to make standard dilutions, and the possible errors made during dilutions are eliminated. In addition, this method requires fewer reagents and less space in the plate compared to standard curve method (125).

6.1.5 Protein expression

In *studies II* and *III,* western blot, immunohistochemistry and –fluorescence was used for quantification of protein levels. However, it is generally acknowledged that the mRNA and protein expression levels do not necessarily correlate with each other (130); and therefore, immunohistochemistry and –fluorescence, western blot or other protein methods do not necessarily support the mRNA data obtained from cellular experiments. The mRNA expression level does not tell whether the mRNA is translated to a functional protein or not, and the mRNA could be regulated only at the transcriptional level (129). Immunohistochemistry was used for localization of MFAP5 and CPPED1 in wAT sections (*study II* and unpublished, complementary data).

In intact wAT, adipocytes are round and filled with a large lipid droplet and a thin rim of cytoplasm between the lipid droplet and plasmamembrane. This causes special challenges for morphological analyses like immunohistochemistry because the sections from wAT are scarce, very difficult to prepare and give a limited view of the cell/tissue. Due to this it could be possible that proteins are unevenly distributed and the cytoplasm is hard to see (131). To overcome this problem we also used immunofluorescence to detect the localization of CPPED1 protein expression (unpublished, complementary data). Immunofluorescence maintains the cell structure and enables localization of the protein at the time of fixation (132).

6.1.6 SGBS cell strain

Experimental models of obesity are needed for the research of biochemical, physiological and pathological conditions of human obesity (34). An *in vitro* model of obesity is needed and at the moment, there is no human adipocyte cell line available for the obesity research and thus, murine cell lines have been widely used. However, there is strong evidence showing that all pathological conditions of human obesity do not occur in animal models (34), and there are several differences between human and murine models of adipocyte biology e.g. altered cell growth and adipokine expression.

Although a murine cell line is a validated model, it would be important to have a human adipocyte cell line, which would be more clinically relevant for human diseases. Simpson – Golabi – Behmel syndrome (SGBS) cells are derived from SVF of subcutaneous wAT of an infant with X-linked congenital overgrowth syndrome (117). The SGBS cells have been shown to be morphologically, biochemically and functionally similar to primary human adipocytes, showing e.g. a similar glycerol-3-phosphate dehydrogenase (GPDH) activity and the expression of genes related to adipocyte differentiation, suggesting that these cells could be used for investigating adipocyte function and as a model of adipogenesis (133-135). Furthermore, SGBS cells can be used for investigating adipocyte-specific metabolic functions like insulin-stimulated glucose uptake, lipogenesis and lipolysis (136). Due to the fact that the amount of preadipocytes obtained from subcutaneous wAT biopsies is restricted (134) and that SGBS cells are a human cell strain derived from subcutaneous

wAT, they are an important model to investigate human obesity and adipocyte function (135). For these reasons, SGBS cells were chosen for this thesis work (*studies II and III* and unpublished, complementary data).

6.1.7 siRNA experiments

RNA interference (RNAi) technique using double-stranded small interfering RNA (siRNA) is a powerful, quick and easy technique to decrease the expression of gene of interest and to investigate the role of a particular gene in cellular functions (137). However, adipocytes have been shown to be a difficult cell type for gene knock-down due to their lipid content (137). Therefore, the optimization of siRNA experiments is important for achieving sufficient and specific silencing in healthy adipocytes (138). We used a commercial pool of four siRNA oligonucleotides in *studies II* and *III* to minimize the off-target effects and to yield >70% reduction in target gene, and a nonspecific siRNA oligonucleotide as a negative control.

6.2 GENERAL DISCUSSION

6.2.1 Studies on MFAP5

The effect of weight reduction on gene expression of MFAP5

ECM-associated genes and cytoskeleton components have been shown to be up-regulated by a high-fat diet and correlated with body weight in mice (41,139). However, not only weight gain, but also weight loss induces ECM remodeling and modifies the expression of ECM molecules (140). The role of the ECM during weight reduction or in negative energy balance is unknown. However, it could be suggested that the synthesis of ECM molecules is reduced through transcriptional regulation (98).

Kolehmainen et al. (20) have previously shown using Affymetrix microarrays that the expression of *MFAP5* was down-regulated in wAT after weight reduction in individuals with features of the metabolic syndrome. In *study I*, we confirmed the microarray data and showed that the expression of *MFAP5* mRNA in wAT was decreased after weight loss, thus supporting it as a putative molecule involved in wAT biology.

Expression patterns of MFAP5

ECM proteins are predominantly expressed in SVF of wAT (52). In line with this, our results showed that MFAP5 was expressed in ECM of subcutaneous wAT sections and the expression of *MFAP5* was higher in SVF than in mature adipocytes, but there was not a formal statistical analysis to support this finding due to the small sample size (*study II*). In addition, *MFAP5* expression was down-regulated during adipocyte differentiation (*study II*) similarly to another component of the ECM, fibronectin which together with other ECM molecules are strongly associated with adipogenesis in order to accommodate wAT growth (9,15,41,141). Taken together, our results show that preadipocytes and SVF cells are important source of the MFAP5 expression, and that MFAP5 may be a relevant marker particularly for the biology of SVF cells.

MFAP5, adipokines and factors involved in ECM remodeling in wAT
In *study I,* the associations between the mRNA levels of *MFAP5* and bio-clinical and selected gene expression data were investigated. We showed that *MFAP5* expression was correlated with BMI, the change of body fat mass, and fasting concentrations of adiponectin, leptin, IL1β and IL1Ra.

In humans, the level of leptin is directly and adiponectin inversely associated with obesity and IR (142). In addition, IL1β and IL1Ra are correlated with BMI and development of IR. Moreover, adiponectin and leptin are involved in obesity-related angiogenesis and ECM remodeling in other tissues (142,143). Recent studies have demonstrated that ECM remodeling is important for enlargement of wAT and that positive correlations exist between the components of ECM in subcutaneous wAT and BMI (144). Type VI collagen is highly expressed in obese wAT and associated with increased BMI and inflammation (90). There is also evidence that a loss of collagen VI in wAT leads to improvements of metabolic profile (14).

We found correlations between the mRNA expressions of *MFAP5* and *PPARγ2* and *ADAM12* (*study I*), which are both involved in the development of wAT and adipocyte differentiation (87,145,146). Therefore, we suggest that MFAP5 is involved in ECM remodeling in wAT and possibly wAT inflammation. Thus, MFAP5 may have a role in wAT function through modulation of adipokines or factors related to adipogenesis during development of obesity and reduction of body fat mass in response to weight reduction.

The effect of MFAP5 knock-down
Due to the fact that correlations cannot prove causality, functional studies are necessary to elucidate the possible mechanisms. Therefore, the role of MFAP5 in adipocyte function was investigated in an SGBS adipocyte cell culture model.

In *study II*, we found that MFAP5 is involved in ECM function and wAT inflammation. In that study, we demonstrated that reduction in *MFAP5* expression decreased the expression of ECM-related genes, *ADAM12* and *NOTCH1*, and genes involved in wAT inflammation, *IL1β*, *TGFβ1* and *IL-6.* Previous studies have shown that MFAP5 interacts with NOTCH1 in a cell-dependent manner (107,108). Furthermore, *ADAM12* is shown to be up-regulated by *NOTCH1* activation (147). NOTCH1 is a transmembrane receptor which determines cell fate, participates in cell-to-cell signaling (148), and notch signaling is involved in activation of inflammatory processes (149). There is evidence that NOTCH1 contributes to tissue remodeling in macrophages (149), but it is not known whether a similar phenomenon could happen also in wAT.

ADAM12 is a cell surface protein involved in cell signaling, cell-ECM interactions and ECM protein degradation (150,151). In addition, both NOTCH1 and ADAM12 have been suggested to be involved in the development of wAT, differentiation and adipogenesis (145,146,148). Furthermore, it has been suggested that ADAM12 is contributes to fibrotic processes in liver (150), and that MFAP5 is increased in a fibrotic skin disorder associated with increased deposition of collagen type I (106). Therefore, we suggest that MFAP5 is associated with remodeling of ECM in wAT, and possibly development of fibrosis, through the NOTCH1 signaling pathway, involving also ADAM12 (152).

Interestingly, we found that *MFAP5* siRNA treatment decreased the expression levels of factors related to inflammation (*study II*), suggesting a possible negative feedback loop in the expression of inflammatory genes. TGFβ1 is regulated by ECM, e.g. microfibrillar ECM proteins (153). Furthermore, there is evidence that MFAP5 could mediate its effects through $α_vβ_3$ integrin (105,106) which regulates NF-κB activation (154). Therefore, reduction in the *MFAP5* expression might decrease the inflammatory status in wAT. Even

though the effects were statistically significant, it should be noted that they were modest, and thus may not be reflected at the physiological level.

The effect of cytokine treatments on the expression of MFAP5

At the cellular level, IR in adipocytes is a characteristic feature of type 2 diabetes. IR is induced by TNFα, IL1β and IL-6 in experimental model systems *in vitro*, in e.g. adipocytes (30,45,60,155). Therefore, in our experiments TNFα, IL1β, IL-6 and TGFβ1 were chosen to illustrate the chronic low-grade inflammatory state present in obese wAT. Our results showed that treatments with TNFα and IL1β abolished the effect of insulin to take up glucose in adipocytes, a hallmark of insulin resistance (unpublished, complementary data).

Interestingly, preadipocytes treated with inflammatory factors TGFβ1, IL-6 or the lowest concentration of TNFα increased the *MFAP5* mRNA expression (*study II*). It has previously been shown that preadipocytes are able to increase the synthesis of ECM molecules in an inflammatory environment (52). In addition, inflammatory factors induce IR through activation of NF-κB pathway (78,156), which is also needed for deposition and remodeling of ECM proteins and proliferation of preadipocytes in response to inflammation (15).

TGFβ1 is a strong pro-fibrotic factor, a modulator of ECM synthesis and a candidate for adipocyte proliferation (93). Prolonged TGFβ1 stimulation has been shown to promote excessive ECM deposition and may contribute to the development of fibrosis in wAT (9,14,157). Furthermore, TGFβ has been demonstrated to increase MFAP5 production in murine fibroblasts (106).

It has also been shown that low levels of TNF-α stimulates proliferation of SVF cells of wAT and fibroblast cell lines (158). This suggests that MFAP5 may be involved in the deposition of ECM in response to inflammatory factors, and this could be mediated through NF-κB. Even though we did not measure proliferation as such, it could be possible that MFAP5 may be related to proliferation of SVF or preadipocytes in response to inflammatory factors.

On the other hand, treatment of preadipocytes with IL1β decreased the expression of *MFAP5*. The expression of ECM proteins have been shown to be modified by IL1β (88) possibly involving matrix metalloproteinases (MMPs) that degrade ECM proteins (159,160). Therefore, the discordance in *MFAP5* expression in response to different cytokines could be due to effects of MMPs or the activation of different signaling pathways in response to cytokines. At present, the role of MMPs or the involvement of NF-κB in the regulation of *MFAP5* expression remains to be defined.

6.2.2 Studies on CPPED1

The effect of weight reduction on the gene expression of CPPED1

We continued to study the role of CPPED1 in wAT (*study III* and unpublished, complementary data) and PBMCs (unpublished, complementary data). We showed that in addition to wAT, the expression of *CPPED1* was also decreased in PBMCs after weight loss in the GENOBIN and KOBS studies. This is in line with a previous finding demonstrating that *CPPED1* is expressed in PBMCs (110). Moreover, we found that *CPPED1* expression was higher in subcutaneous wAT than in wAT (+31.2%, p<0.0001) at baseline in the KOBS (unpublished, complementary data). There is evidence showing that gene expression pattern could differ between these two wAT depots (124,161), suggesting that CPPED1 may be more relevant for the biology of subcutaneous wAT than visceral wAT.

The reason why *CPPED1* expression was not significantly reduced after weight loss in the KOBS study could be in part explained by differences in the study designs, e.g. the mechanism of weight reduction, the amount of fat mass lost and the duration of follow-up. The individuals in the KOBS study were morbidly obese (BMI 45 kg/m^2), underwent bariatric surgery and lost 24% of their original weight. In the GENOBIN study participants were less obese (BMI 32.9 kg/m^2), and their weight reduction was modest (~5%). Moreover, the differences in study design may have different effects on possible compensatory mechanisms. A further reason could be the use of two different wAT biopsy methods, which affects the gene expression patterns in wAT (122).

CPPED1 and factors involved in AT inflammation
Our results showed that *CPPED1* expression correlated with the expression of genes related to inflammation, such as *TNFα*, *IL1β*, *IL1Ra*, and inhibitors of NF-κB pathway (*IKBα*, *IKBβ*, *IKKβ*) in wAT, indicating that *CPPED1* could be involved in wAT inflammation. In addition, our results suggest that *CPPED1* may contribute to the development of IR in response to TNFα and IL1β treatments at the cellular level (unpublished, complementary data).

In obesity, the production and expression of many inflammatory cytokines, like *TNFα* and *IL1β*, is increased (7,8,60). The effects of these cytokines are mainly mediated through activation of IKK/NF-κB pathway (78,156) along with increasing adiposity, and recent studies have highlighted the role of IKKβ and NF-κB and its target genes in the development of IR and type 2 diabetes (78).

The expression of *IL1Ra* is increased in obesity and has been suggested to be associated with the development of IR (60). Conversely, reduction in the *IL1Ra* expression in response to weight loss has been suggested to reflect improvements in insulin and glucose metabolism (162,163).

Moreover, immune cells and macrophage infiltration into wAT may have a central role in the development of IR in adipocytes (60), since it has been shown that factors secreted by macrophages are capable of impairing insulin sensitivity, e.g. through increased lipolysis (44). Thus, monocytes, which are a cell type in PBMCs and differentiate into macrophages in wAT, could be used as a model to study inflammation and its association with IR (45).

Based on correlation results in humans and *in vitro* experiments with inflammatory factors, *CPPED1* may be associated with the development of IR in wAT. Further investigations are necessary, however, to determine which mechanisms might be involved in the regulation of the *CPPED1* expression in adipocytes in response to inflammation.

Expression pattern of CPPED1
CPPED1 protein was expressed in wAT sections, and there was a higher expression of *CPPED1* in SVF than in mature adipocytes (unpublished, complementary data). Moreover, the expression pattern of *CPPED1* was not changed during adipocyte differentiation (*study III*). We also showed cytoplasmic expression of CPPED1 protein in preadipocytes and adipocytes (unpublished, complementary data).

WAT is a heterogeneous pool of different types of cells like adipocytes and SVF cells, including preadipocytes, endothelial cells and several immune cells (164). It has been shown that adipocytes and immune cells are in close proximity in wAT (12,156). Therefore, it could be suggested that both cellular fractions of wAT, adipocytes and SVF cells, are contributing to *CPPED1* expression in wAT. The present results showed that at least preadipocytes and adipocytes are involved in the expression of CPPED1. However, specific cell types could not be distinguished, and further experiments are needed.

The effect of CPPED1 knock-down

The possible effect of *CPPED1* knock-down in insulin sensitivity and glucose metabolism was examined by measuring insulin-stimulated glucose uptake in mature adipocytes. The down-regulation of *CPPED1* mRNA as such enhanced insulin-stimulated glucose uptake, which was inhibited by treatment with wortmannin, a PI3K-specific inhibitor. Therefore, the *CPPED1* knock-down induced improvement in glucose uptake may be mediated through a PI3K/Akt-dependent pathway.

The knock-down of *CPPED1* expression in mature adipocytes increased the gene and protein expression of ADIPOQ. There was also a trend for enhanced secretion of HMW adiponectin into the conditioned medium indicating that *CPPED1* may regulate adiponectin signaling in wAT. Furthermore, gene expression levels of *ADIPOR1* and *GLUT4* were also increased, and there was a tendency for increased GLUT4 protein expression due to *CPPED1* knock-down. The expression of *CPT1B* mRNA was also increased in response to *CPPED1* knock-down. In human data from the present study (unpublished, complementary data), there was a clear correlation between expression of *CPPED1* and *ADIPOR1* and *CPT1B*.

The effects of adiponectin are mediated through adiponectin receptors 1 and 2 (13,59). Adiponectin has been shown to be directly involved in enhancing insulin sensitivity through phosphorylation of the insulin receptor and GLUT4 translocation. Adiponectin also indirectly improves insulin sensitivity via 5'-AMP-activated protein kinase (AMPK) activation, which leads to decreased malonyl-coenzyme A production and increased *CPT1* activity, fatty acid oxidation and reduction in FFA levels in the liver and skeletal muscle (3,57,68,165). Furthermore, the expression of adiponectin receptors is down-regulated in obesity (13). However, there is some controversy because previously it has been shown that the expression of *ADIPOR1* may be increased in skeletal muscle in obese subjects (3). This could be because higher expression level of adiponectin receptors may compensate for the decreased levels of circulating adiponectin seen in obesity and IR (3).

The main tissue for insulin-stimulated glucose uptake is skeletal muscle (166). In addition, there is evidence showing that reduction in *GLUT4* expression and glucose uptake can induce IR in AT (166). The role of the GLUT4 transporter in insulin-stimulated glucose uptake has also been shown to be important for glucose homeostasis in AT (72). As shown in mice, *GLUT4* over-expression has increased insulin sensitivity. Moreover, *GLUT4* knock-out from fat tissue has induced IR, as seen in muscle-specific *GLUT4* knock-out (72). However, insulin-stimulated glucose uptake induces the GLUT4 translocation to the plasma membrane rather than up-regulating *GLUT4* intrinsic activity in adipocytes (75,167). On the other hand, the expression of *GLUT4* is reduced in adipocytes, but not in muscle. This suggests that the regulation of *GLUT4* expression in adipocytes could be important in the development of obesity, although the role of decreased *GLUT4* expression in the development of IR is not completely understood (166,168). Overall, insulin-stimulated glucose uptake in wAT has an important role for glucose homeostasis, and the obesity-induced reduction in *GLUT4* expression is involved in the development of IR and subsequently type 2 diabetes (166).

Furthermore, the present results showed that the *CPPED1* knock-down decreased the mRNA expression of leptin (*study III*). There is evidence that leptin is involved in enhancing insulin sensitivity by inducing fatty acid oxidation and decreasing ectopic fat accumulation in non-adipose tissues through AMPK activation (71). This suggests that also leptin may be involved in *CPPED1*-induced effects on insulin action via indirect actions on glucose uptake.

Taken together, these results suggest improved glucose metabolism in response to *CPPED1* reduction, which may be a result from the concomitant increase in adiponectin levels and activation of its signaling pathway either directly or indirectly (Figure 12).

6.3 FUTURE IMPLICATIONS

Current publications about MFAP5 demonstrate its role in elastinogenesis, cell survival and angiogenesis. The results presented in this thesis provide novel information about the roles of MFAP5 and CPPED1 in wAT and adipocyte function, which, to our knowledge, has never been studied before.

The production of various cytokines and adipokines is altered in obesity, and the function of these adipokines and adipocytokines is important for cross-talk with other organs. There is a complex interaction between cytokines and ECM, which is essential in maintaining tissue homeostasis and regulating ECM deposition (4,158,169). We could not show a clear dose-response effect of the cytokines examined suggesting that treatment may not have a direct effect on the expression of target genes. If the observed changes on the *MFAP5* and *CPPED1* expression also occur *in vivo*, different cytokines, separately or in combination, may influence the their properties in wAT. Further studies are necessary to unravel whether *MFAP5* is involved in ECM remodeling, and whether *MFAP5* expression is regulated by MMPs. In addition, the role of *MFAP5* in proliferation of preadipocytes in response to inflammation needs to be examined.

Since macrophages and adipocytes are in close proximity in wAT, and *CPPED1* was found to be expressed in wAT, PBMCs and adipocytes, a macrophage-adipocyte co-culture system could provide novel information about the intercellular cross-talk and shed light on the relationship between CPPED1 and inflammation. Furthermore, it would be interesting to investigate the role of *CPPED1* in macrophages.

More mechanistic studies are needed to investigate the role of *CPPED1* in the phosphorylation status of insulin signaling molecules, such as IRS and other downstream targets in order to find specific effects at the molecular level. Moreover, the interaction between CPPED1 and adiponectin needs to be investigated in more detail. It would be also interesting to examine whether over-expression of *CPPED1* could induce IR at the cellular level.

6.4 CONCLUSIONS

The possible processes involved in the regulation of *MFAP5* and *CPPED1* expression in wAT and adipocytes are illustrated in figure 12. Obesity leads to increased low-grade inflammation and ECM remodeling, both of which are reduced after weight loss and concomitant improved glucose metabolism and insulin sensitivity in wAT. In obese wAT, inflammation induces ECM overproduction, which may finally lead to the development of fibrosis.

The present results show that *MFAP5* expression in wAT decreases after weight loss and correlates with factors related to ECM remodeling and wAT inflammation. In addition, inflammatory factors up-regulate the expression of MFAP5 in preadipocytes. These findings suggest that obesity-related inflammation either directly or indirectly increases *MFAP5* expression in wAT through effects on ECM. Furthermore, the down-regulated

MFAP5 expression further decreases the expression of ECM components involved in adipogenesis and ECM remodeling, and decreases inflammatory status in adipocytes. In this way, down-regulation of *MFAP5* expression possibly forms a negative feedback loop and thereby may reduce the obesity-induced inflammation and ECM remodeling in wAT. These results suggest that MFAP5 is associated with wAT inflammation and ECM function.

The present results hypothesize that increased wAT inflammation is responsible for the up-regulated *CPPED1* expression, possibly through activation of IKKβ/NF-κB, leading to impaired insulin signaling and development of IR. The improvement in glucose uptake after weight loss could be partly due to the decreased expression of *CPPED1* through a PI3K-dependent pathway, and possibly adiponectin signaling could either directly or indirectly play a role in this regulation. This work demonstrates that MFAP5 and CPPED1 have a role in wAT biology and could contribute to complications in dysfunctional wAT. The studies presented in this thesis work demonstrate that moderate weight reduction down-regulated the expression of *MFAP5* and *CPPED1* in human subcutaneous wAT and PBMC samples. The health benefits of moderate weight reduction may originate from reduced overall adiposity and inflammation and modulated production of adipokines and ECM proteins. Furthermore, down-regulated expression of the target genes *in vitro* showed beneficial effects on adipocyte metabolism, but whether it could be reflected also *in vivo* needs to be investigated.

Taken together, the results provide novel insights about the function of *MFAP5* in ECM remodeling and wAT inflammation and the role of *CPPED1* in glucose metabolism, possibly via adiponectin-mediated mechanisms, which are important processes in the development of obesity- related morbidities, like IR, type 2 diabetes and cardiovascular diseases. Finally, these results support the strategy to find new putative genes that might be related to obesity-related wAT dysfunction. Along with this line, we have identified genes whose expression levels are modified with weight change and which are involved in processes relevant for wAT biology, like glucose metabolism, inflammation and ECM function.
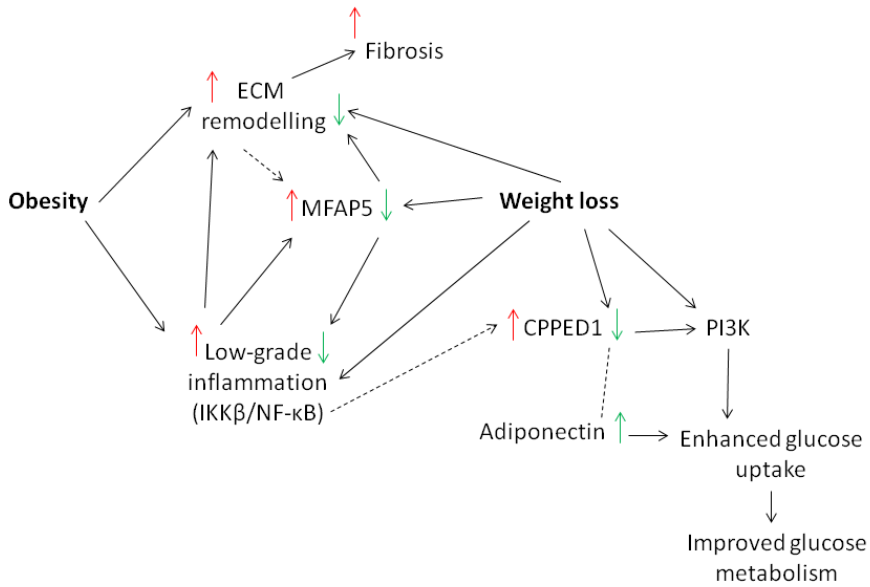
Figure 12. Possible roles of MFAP5 and CPPED1 in wAT biology and adipocyte function. Intact arrows: known effects in wAT samples/cell experiments; Dashed arrows/lines: possible effects;↑ up-regulation; ↓down-regulation (red arrows: unbeneficial, green arrows: beneficial)

7 Summary

The most important results and methods of this thesis work are presented in table 8.

Table 8. The key methods and results of this thesis work

Methodology	Results	
	MFAP5	CPPED1
Human samples		
The effect of weight reduction on gene expression	↓ in wAT of the GENOBIN study (*study I*)	↓ in wAT and PBMCs of the GENOBIN study and in PBMCs of the KOBS study (*study III*, unpublished, complementary data)
Correlation analysis in the GENOBIN study	Correlated with factors related to ECM remodeling and wAT inflammation (*study I*)	Correlated with the expression of inflammatory genes in wAT and PBMCs (unpublished, complementary data)
Immunohistochemistry of human wAT samples	Protein expressed in ECM and blood vessels (*study II*)	Protein expressed in cytoplasm of adipocytes (unpublished, complementary data)
Isolation of SVF from human wAT samples	Gene expression higher in SVF than in mature adipocytes (unpublished, complementary data)	Gene expression higher in SVF compared to mature adipocytes (unpublished, complementary data)
SGBS cell culture		
Adipocyte differentiation	Gene expression was down-regulated during adipocyte differentiation (*study II*)	Gene expression was not changed during adipocyte differentiation (*study III*)
Immunofluorescence	*	Protein expression was localized in cytoplasm of preadipocytes and mature adipocytes (unpublished, complementary data)
Cytokine treatment	TNFα, TGFβ1 and IL-6 treatments increased *MFAP5* gene expression in preadipocytes (*study II*)	TNFα treatment increased *CPPED1* gene expression in preadipocytes and mature adipocytes (unpublished, complementary data)
siRNA treatment	↓ the expression of ECM components and inflammatory genes/proteins (*study II*)	↑ the expression of genes/proteins related glucose metabolism (*study III*)
Glucose uptake	*MFAP5* siRNA treatment did not have an effect on glucose uptake (*study II*)	*CPPED1* siRNA treatment ↑ insulin-stimulated glucose uptake (*study III*)
ELISA	*	*CPPED1* siRNA treatment tended to increase ADIPOQ secretion (*study III*)

MFAP5, microfibrillar-associated protein 5; CPPED1, calcineurin-like phosphoesterase domain 1; wAT, white adipose tissue; PBMCs, peripheral blood mononuclear cells; GENOBIN, gene expression in obesity and insulin resistance; KOBS, Kuopio obesity surgery; ECM, extracellular matrix; SVF, stromal-vascular fraction; TNFα, tumour necrosis factor alpha; TGFβ1, transforming growth factor beta 1; IL-6, interleukin 6; siRNA, small interfering RNA; ELISA, enzyme-linked immunosorbent assay; * was not measured; ↑ up-regulation; ↓down-regulation

References

(1) Chan RS, Woo J. Prevention of overweight and obesity: how effective is the current public health approach. Int J Environ Res Public Health 2010 Mar;7(3):765-783.

(2) Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 2008 May;9(5):367-377.

(3) Bluher M, Bullen JW,Jr, Lee JH, Kralisch S, Fasshauer M, Kloting N, et al. Circulating adiponectin and expression of adiponectin receptors in human skeletal muscle: associations with metabolic parameters and insulin resistance and regulation by physical training. J Clin Endocrinol Metab 2006 Jun;91(6):2310-2316.

(4) Ye J, McGuinness OP. Inflammation during obesity is not all bad: Evidence from animal and human studies. Am J Physiol Endocrinol Metab 2012 Dec 26.

(5) Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005 May;115(5):911-9; quiz 920.

(6) Ahima RS, Flier JS. Adipose tissue as an endocrine organ. Trends Endocrinol Metab 2000 Oct;11(8):327-332.

(7) Hotamisligil GS. Inflammatory pathways and insulin action. Int J Obes Relat Metab Disord 2003 Dec;27 Suppl 3:S53-5.

(8) Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004 Jun;89(6):2548-2556.

(9) Divoux A, Clement K. Architecture and the extracellular matrix: the still unappreciated components of the adipose tissue. Obes Rev 2011 May;12(5):e494-503.

(10) Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. J Clin Invest 2008 Sep;118(9):2992-3002.

(11) Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. J Clin Invest 2011 Jun 1;121(6):2094-2101.

(12) Iyer A, Fairlie DP, Prins JB, Hammock BD, Brown L. Inflammatory lipid mediators in adipocyte function and obesity. Nat Rev Endocrinol 2010 Feb;6(2):71-82.

(13) Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. Mol Med 2008 Nov-Dec;14(11-12):741-751.

(14) Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia M, Abate N, et al. Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. Mol Cell Biol 2009 Mar;29(6):1575-1591.

(15) Keophiphath M, Achard V, Henegar C, Rouault C, Clement K, Lacasa D. Macrophage-secreted factors promote a profibrotic phenotype in human preadipocytes. Mol Endocrinol 2009 Jan;23(1):11-24.

(16) Chun TH. Peri-adipocyte ECM remodeling in obesity and adipose tissue fibrosis. Adipocyte 2012 Apr 1;1(2):89-95.

(17) Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001 May 3;344(18):1343-1350.

(18) Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 2004 Nov;18(14):1657-1669.

(19) Lindstrom J, Peltonen M, Eriksson JG, Ilanne-Parikka P, Aunola S, Keinanen-Kiukaanniemi S, et al. Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). Diabetologia 2013 Feb;56(2):284-293.

(20) Kolehmainen M, Salopuro T, Schwab U, Kekäläinen J, Kallio P, Laaksonen D, et al. Weight reduction modulates expression of genes involved in extracellular matrix and cell death: the GENOBIN study. IJO 2008;32:292-303.

(21) Bernhard F, Landgraf K, Kloting N, Berthold A, Buttner P, Friebe D, et al. Functional relevance of genes implicated by obesity genome-wide association study signals for human adipocyte biology. Diabetologia 2013 Feb;56(2):311-322.

(22) Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive Summary. Crit Pathw Cardiol 2005 Dec;4(4):198-203.

(23) Carroll JF, Chiapa AL, Rodriquez M, Phelps DR, Cardarelli KM, Vishwanatha JK, et al. Visceral fat, waist circumference, and BMI: impact of race/ethnicity. Obesity (Silver Spring) 2008 Mar;16(3):600-607.

(24) World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000;894:i-xii, 1-253.

(25) Dolan CM, Kraemer H, Browner W, Ensrud K, Kelsey JL. Associations between body composition, anthropometry, and mortality in women aged 65 years and older. Am J Public Health 2007 May;97(5):913-918.

(26) Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J 2008 Dec;29(24):2959-2971.

(27) Huxley R, Mendis S, Zheleznyakov E, Reddy S, Chan J. Body mass index, waist circumference and waist:hip ratio as predictors of cardiovascular risk--a review of the literature. Eur J Clin Nutr 2010 Jan;64(1):16-22.

(28) Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000 Dec;21(6):697-738.

(29) Isakson P, Hammarstedt A, Gustafson B, Smith U. Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor-alpha, and inflammation. Diabetes 2009 Jul;58(7):1550-1557.

(30) Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr 2011 Dec;106 Suppl 3:S5-78.

(31) Piya MK, McTernan PG, Kumar S. Adipokine inflammation and insulin resistance: the role of glucose, lipids and endotoxin. J Endocrinol 2013 Jan 2;216(1):T1-T15.

(32) Krotkiewski M, Bjorntorp P, Sjostrom L, Smith U. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. J Clin Invest 1983 Sep;72(3):1150-1162.

(33) Achike FI, To NH, Wang H, Kwan CY. Obesity, metabolic syndrome, adipocytes and vascular function: A holistic viewpoint. Clin Exp Pharmacol Physiol 2011 Jan;38(1):1-10.

(34) Pereira-Lancha LO, Campos-Ferraz PL, Lancha AH J. Obesity: considerations about etiology, metabolism, and the use of experimental models. Diabetes Metab Syndr Obes 2012;5:75-87.

(35) WHO. Available at: http://www.who.int/gho/ncd/risk_factors/obesity_text/en/.

(36) Pajunen P, Vartiainen E, Mannisto S, Jousilahti P, Laatikainen T, Peltonen M. Intra-individual changes in body weight in population-based cohorts during four decades: the Finnish FINRISK study. Eur J Public Health 2012 Feb;22(1):107-112.

(37) Männistö S, Laatikainen T, Vartiainen E. Suomalaisten lihavuus ennen ja nyt. Tutkimuksesta tiiviisti: 2012_4 2012.

(38) Itoh M, Suganami T, Hachiya R, Ogawa Y. Adipose tissue remodeling as homeostatic inflammation. Int J Inflam 2011;2011:720926.

(39) Hotamisligil GS. Inflammation and endoplasmic reticulum stress in obesity and diabetes. Int J Obes (Lond) 2008 Dec;32 Suppl 7:S52-4.

(40) Gustafson B, Gogg S, Hedjazifar S, Jenndahl L, Hammarstedt A, Smith U. Inflammation and impaired adipogenesis in hypertrophic obesity in man. Am J Physiol Endocrinol Metab 2009 Jul 21;297:E999-E1003.

(41) Taleb S, Cancello R, Clement K, Lacasa D. Cathepsin s promotes human preadipocyte differentiation: possible involvement of fibronectin degradation. Endocrinology 2006 Oct;147(10):4950-4959.

(42) Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993 Jan 1;259(5091):87-91.

(43) 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 Dec;112(12):1796-1808.

(44) Sharma AM, Staels B. Review: Peroxisome proliferator-activated receptor gamma and adipose tissue--understanding obesity-related changes in regulation of lipid and glucose metabolism. J Clin Endocrinol Metab 2007 Feb;92(2):386-395.

(45) Permana PA, Menge C, Reaven PD. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. Biochem Biophys Res Commun 2006 Mar 10;341(2):507-514.

(46) Wing RR, Blair EH, Bononi P, Marcus MD, Watanabe R, Bergman RN. Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. Diabetes Care 1994 Jan;17(1):30-36.

(47) Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. Diabetes 1999 Apr;48(4):839-847.

(48) 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 Dec 1;372(6505):425-432.

(49) Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996 May 3;271(18):10697-10703.

(50) 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 Nov 10;270(45):26746-26749.

(51) Wronska A, Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. Acta Physiol (Oxf) 2012 Jun;205(2):194-208.

(52) Henegar C, Tordjman J, Achard V, Lacasa D, Cremer I, Guerre-Millo M, et al. Adipose tissue transcriptomic signature highlights the pathological relevance of extracellular matrix in human obesity. Genome Biol 2008 Jan 21;9(1):R14.

(53) 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 May;145(5):2273-2282.

(54) O'Rourke R. Inflammation in obesity-related diseases. Surgery 2009;145:255-259.

(55) Prieto-Hontoria PL, Perez-Matute P, Fernandez-Galilea M, Bustos M, Martinez JA, Moreno-Aliaga MJ. Role of obesity-associated dysfunctional adipose tissue in cancer: a molecular nutrition approach. Biochim Biophys Acta 2011 Jun;1807(6):664-678.

(56) Leal V, Mafra D. Adipokines in obesity. Clinica Chimica Acta 2013;419:87-94.

(57) Deepa SS, Dong LQ. APPL1: role in adiponectin signaling and beyond. Am J Physiol Endocrinol Metab 2009 Jan;296(1):E22-36.

(58) Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol 2010 Mar 25;316(2):129-139.

(59) Ziemke F, Mantzoros CS. Adiponectin in insulin resistance: lessons from translational research. Am J Clin Nutr 2010 Jan;91(1):258S-261S.

(60) Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulange A, Capeau J, et al. Long-term treatment with interleukin-1beta induces insulin resistance in murine and human adipocytes. Diabetologia 2006 Sep;49(9):2162-2173.

(61) Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer JM. IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? J Clin Endocrinol Metab 2002 Mar;87(3):1184-1188.

(62) Pihlajamaki J, Kuulasmaa T, Kaminska D, Simonen M, Karja V, Gronlund S, et al. Serum interleukin 1 receptor antagonist as an independent marker of non-alcoholic steatohepatitis in humans. J Hepatol 2012 Mar;56(3):663-670.

(63) Zhang HH, Halbleib M, Ahmad F, Manganiello VC, Greenberg AS. Tumor necrosis factor-alpha stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. Diabetes 2002 Oct;51(10):2929-2935.

(64) Torti FM, Torti SV, Larrick JW, Ringold GM. Modulation of adipocyte differentiation by tumor necrosis factor and transforming growth factor beta. J Cell Biol 1989 Mar;108(3):1105-1113.

(65) Schaffler A, Scholmerich J, Buchler C. Mechanisms of disease: adipocytokines and visceral adipose tissue--emerging role in intestinal and mesenteric diseases. Nat Clin Pract Gastroenterol Hepatol 2005 Feb;2(2):103-111.

(66) Wieser V, Moschen AR, Tilg H. Inflammation, cytokines and insulin resistance: a clinical perspective. Arch Immunol Ther Exp (Warsz) 2013 Apr;61(2):119-125.

(67) Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003 Dec;112(12):1821-1830.

(68) van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. Cancer Epidemiol Biomarkers Prev 2009 Oct;18(10):2569-2578.

(69) Wang B, Wood IS, Trayhurn P. Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. Pflugers Arch 2007 Dec;455(3):479-492.

(70) Liang X, Kanjanabuch T, Mao SL, Hao CM, Tang YW, Declerck PJ, et al. Plasminogen activator inhibitor-1 modulates adipocyte differentiation. Am J Physiol Endocrinol Metab 2006 Jan;290(1):E103-E113.

(71) Vettor R, Milan G, Rossato M, Federspil G. Review article: adipocytokines and insulin resistance. Aliment Pharmacol Ther 2005 Nov;22 Suppl 2:3-10.

(72) Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000 Aug;106(4):473-481.

(73) Thirone AC, Huang C, Klip A. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. Trends Endocrinol Metab 2006 Mar;17(2):72-78.

(74) Lee DE, Kehlenbrink S, Lee H, Hawkins M, Yudkin JS. Getting the message across: mechanisms of physiological cross talk by adipose tissue. Am J Physiol Endocrinol Metab 2009 Jun;296(6):E1210-29.

(75) Prasad CN, Anjana T, Banerji A, Gopalakrishnapillai A. Gallic acid induces GLUT4 translocation and glucose uptake activity in 3T3-L1 cells. FEBS Lett 2010 Feb 5;584(3):531-536.

(76) Arioglu E, Rother KI, Reitman ML, Premkumar A, Taylor SI. Lipoatrophy syndromes: when 'too little fat' is a clinical problem. Pediatr Diabetes 2000 Sep;1(3):155-168.

(77) Herman MA, Peroni OD, Villoria J, Schon MR, Abumrad NA, Bluher M, et al. A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature 2012 Apr 19;484(7394):333-338.

(78) Chen F. Is NF-KB a culprit in type 2 diabetes? Biochemical and biophysical research communications 2005;332:1-3.

(79) Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci 2013 Apr 20;9(2):191-200.

(80) Matthias A, Ohlson KB, Fredriksson JM, Jacobsson A, Nedergaard J, Cannon B. Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty scid-induced thermogenesis. J Biol Chem 2000 Aug 18;275(33):25073-25081.

(81) Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009 Apr 9;360(15):1509-1517.

(82) Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, et al. Functional brown adipose tissue in healthy adults. N Engl J Med 2009 Apr 9;360(15):1518-1525.

(83) Vosselman MJ, van Marken Lichtenbelt WD, Schrauwen P. Energy dissipation in brown adipose tissue: From mice to men. Mol Cell Endocrinol 2013 Apr 28.

(84) Harwood HJ. The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. Neuropharmacology 2012;63:57-75.

(85) Himms-Hagen J, Melnyk A, Zingaretti MC, Ceresi E, Barbatelli G, Cinti S. Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. Am J Physiol Cell Physiol 2000 Sep;279(3):C670-81.

(86) Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature 2008 Jun 5;453(7196):783-787.

(87) Rosen E, Spiegelman B. Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol 2000;16:145-171.

(88) Sorokin L. The impact of the extracellular matrix on inflammation. Nat Rev Immunol 2010 Oct;10(10):712-723.

(89) Divoux A, Tordjman J, Lacasa D, Veyrie N, Hugol D, Aissat A, et al. Fibrosis in human adipose tissue: composition, distribution, and link with lipid metabolism and fat mass loss. Diabetes 2010 Nov;59(11):2817-2825.

(90) Pasarica M, Gowronska-Kozak B, Burk D, Remedios I, Hymel D, Gimble J, et al. Adipose tissue collagen VI in obesity. J Clin Endocrinol Metab 2009 Dec;94(12):5155-5162.

(91) Spencer M, Yao-Borengasser A, Unal R, Rasouli N, Gurley CM, Zhu B, et al. Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. Am J Physiol Endocrinol Metab 2010 Dec;299(6):E1016-27.

(92) Chen MB, McAinch AJ, Macaulay SL, Castelli LA, O'brien PE, Dixon JB, et al. Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese type 2 diabetics. J Clin Endocrinol Metab 2005 Jun;90(6):3665-3672.

(93) Hausman DB, DiGirolamo M, Bartness TJ, Hausman GJ, Martin RJ. The biology of white adipocyte proliferation. Obes Rev 2001 Nov;2(4):239-254.

(94) Xu Y, Farmer SR, Smith BD. Peroxisome proliferator-activated receptor gamma interacts with CIITA x RFX5 complex to repress type I collagen gene expression. J Biol Chem 2007 Sep 7;282(36):26046-26056.

(95) Wang P, Keijer J, Bunschoten A, Bouwman F, Renes J, Mariman E. Insulin modulates the secretion of proteins from mature 3T3-L1 adipocytes: a role for transcriptional regulation of processing. Diabetologia 2006 Oct;49(10):2453-2462.

(96) Schram K, Sweeney G. Implications of myocardial matrix remodeling by adipokines in obesity-related heart failure. Trends Cardiovasc Med 2008 Aug;18(6):199-205.

(97) Schram K, De Girolamo S, Madani S, Munoz D, Thong F, Sweeney G. Leptin regulates MMP-2, TIMP-1 and collagen synthesis via p38 MAPK in HL-1 murine cardiomyocytes. Cell Mol Biol Lett 2010 Dec;15(4):551-563.

(98) Mariman EC, Wang P. Adipocyte extracellular matrix composition, dynamics and role in obesity. Cell Mol Life Sci 2010 Apr;67(8):1277-1292.

(99) Viguerie N, Montastier E, Maoret JJ, Roussel B, Combes M, Valle C, et al. Determinants of human adipose tissue gene expression: impact of diet, sex, metabolic status, and cis genetic regulation. PLoS Genet 2012 Sep;8(9):e1002959.

(100) de Mello VD, Kolehmainen M, Schwab U, Mager U, Laaksonen DE, Pulkkinen L, et al. Effect of weight loss on cytokine messenger RNA expression in peripheral blood mononuclear cells of obese subjects with the metabolic syndrome. Metabolism 2008 Feb;57(2):192-199.

(101) Gibson MA, Finnis ML, Kumaratilake JS, Cleary EG. Microfibril-associated glycoprotein-2 (MAGP-2) is specifically associated with fibrillin-containing microfibrils but exhibits more restricted patterns of tissue localization and developmental expression than its structural relative MAGP-1. J Histochem Cytochem 1998 Aug;46(8):871-886.

(102) Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. J Cell Sci 2002 Jul 15;115(Pt 14):2817-2828.

(103) Lemaire R, Bayle J, Mecham R, Lafyatis R. Microfibril-associated MAGP-2 stimulates elastic fiber assembly. J Biol Chem 2007;282:800-808.

(104) Mok SC, Bonome T, Vathipadiekal V, Bell A, Johnson ME, Wong KK, et al. A gene signature predictive for outcome in advanced ovarian cancer identifies a survival factor: microfibril-associated glycoprotein 2. Cancer Cell 2009 Dec 8;16(6):521-532.

(105) Spivey KA, Banyard J. A prognostic gene signature in advanced ovarian cancer reveals a microfibril-associated protein (MAGP2) as a promoter of tumor cell survival and angiogenesis. Cell Adh Migr 2010 Apr-Jun;4(2):169-171.

(106) Lemaire R, Korn JH, Shipley JM, Lafyatis R. Increased expression of type I collagen induced by microfibril-associated glycoprotein 2: novel mechanistic insights into the molecular basis of dermal fibrosis in scleroderma. Arthritis Rheum 2005 Jun;52(6):1812-1823.

(107) Albig AR, Becenti DJ, Roy TG, Schiemann WP. Microfibril-associate glycoprotein-2 (MAGP-2) promotes angiogenic cell sprouting by blocking notch signaling in endothelial cells. Microvasc Res 2008 May;76(1):7-14.

(108) Miyamoto A, Lau R, Hein PW, Shipley JM, Weinmaster G. Microfibrillar proteins MAGP-1 and MAGP-2 induce Notch1 extracellular domain dissociation and receptor activation. J Biol Chem 2006 Apr 14;281(15):10089-10097.

(109) UCSC. Available at: http://genome.ucsc.edu.

(110) Smith AP, Weeraratna AT, Spears JR, Meltzer PS, Becker D. SAGE identification and fluorescence imaging analysis of genes and transcripts in melanomas and precursor lesions. Cancer Biol Ther 2004 Jan;3(1):104-109.

(111) Arakaki AK, Tian W, Skolnick J. High precision multi-genome scale reannotation of enzyme function by EFICAz. BMC Genomics 2006 Dec 13;7:315.

(112) Mager U, Kolehmainen M, de Mello VD, Schwab U, Laaksonen DE, Rauramaa R, et al. Expression of ghrelin gene in peripheral blood mononuclear cells and plasma ghrelin concentrations in patients with metabolic syndrome. Eur J Endocrinol 2008 Apr;158(4):499-510.

(113) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001 May 16;285(19):2486-2497.

(114) Kaminska D, Kuulasmaa T, Venesmaa S, Kakela P, Vaittinen M, Pulkkinen L, et al. Adipose Tissue TCF7L2 Splicing Is Regulated by Weight Loss and Associates With Glucose and Fatty Acid Metabolism. Diabetes 2012 Nov;61(11):2807-2813.

(115) Sarkkinen E, Schwab U, Niskanen L, Hannuksela M, Savolainen M, Kervinen K, et al. The effects of monounsaturated-fat enriched diet and polyunsaturated-fat enriched diet on lipid and glucose metabolism in subjects with impaired glucose tolerance. Eur J Clin Nutr 1996 Sep;50(9):592-598.

(116) Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol Ther 2003;5(6):1003-1015.

(117) Wabitsch M, Brenner RE, Melzner I, Braun M, Moller P, Heinze E, et al. Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. Int J Obes Relat Metab Disord 2001 Jan;25(1):8-15.

(118) Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001 Dec;25(4):402-408.

(119) Kere J. Human population genetics: lessons from Finland. Annu Rev Genomics Hum Genet 2001;2:103-128.

(120) Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009 Oct 20;120(16):1640-1645.

(121) Gomez-Ambrosi J, Catalan V, Diez-Caballero A, Martinez-Cruz LA, Gil MJ, Garcia-Foncillas J, et al. Gene expression profile of omental adipose tissue in human obesity. FASEB J 2004 Jan;18(1):215-217.

(122) Mutch DM, Tordjman J, Pelloux V, Hanczar B, Henegar C, Poitou C, et al. Needle and surgical biopsy techniques differentially affect adipose tissue gene expression profiles. Am J Clin Nutr 2009 Jan;89(1):51-57.

(123) Alvehus M, Buren J, Sjostrom M, Goedecke J, Olsson T. The human visceral fat depot has a unique inflammatory profile. Obesity (Silver Spring) 2010 May;18(5):879-883.

(124) Baranova A, Collantes R, Gowder SJ, Elariny H, Schlauch K, Younoszai A, et al. Obesity-related differential gene expression in the visceral adipose tissue. Obes Surg 2005 Jun-Jul;15(6):758-765.

(125) Guan H, Yang K. RNA isolation and real-time quantitative RT-PCR. Methods Mol Biol 2008;456:259-270.

(126) Francis DP, Coats AJ, Gibson DG. How high can a correlation coefficient be? Effects of limited reproducibility of common cardiological measures. Int J Cardiol 1999 May 15;69(2):185-189.

(127) Aldrich J. Correlations Genuine and Spurious in Pearson and Yule. Statistical Science 1995;10:364-376.

(128) Goni R, García P, Foissac S.
The qPCR data statistical analysis Integromics SL 2009.

(129) Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009 Apr;55(4):611-622.

(130) Cagney G, Park S, Chung C, Tong B, O'Dushlaine C, Shields DC, et al. Human tissue profiling with multidimensional protein identification technology. J Proteome Res 2005 Sep-Oct;4(5):1757-1767.

(131) Malide D. Application of immunocytochemistry and immunofluorescence techniques to adipose tissue and cell cultures. Methods Mol Biol 2008;456:285-297.

(132) DiDonato D, Brasaemle D.
Fixation Methods for the Study of Lipid Droplets by Immunofluorescence Microscopy The Journal of Histochemistry & Cytochemistry 2003;51:773-780.

(133) Fischer-Posovszky P, Newell FS, Wabitsch M, Tornqvist HE. Human SGBS cells - a unique tool for studies of human fat cell biology. Obes Facts 2008;1(4):184-189.

(134) Rosenow A, Arrey TN, Bouwman FG, Noben JP, Wabitsch M, Mariman EC, et al. Identification of novel human adipocyte secreted proteins by using SGBS cells. J Proteome Res 2010 Oct 1;9(10):5389-5401.

(135) Allott EH, Oliver E, Lysaght J, Gray SG, Reynolds JV, Roche HM, et al. The SGBS cell strain as a model for the in vitro study of obesity and cancer. Clin Transl Oncol 2012 Oct;14(10):774-782.

(136) Keuper M, Dzyakanchuk A, Amrein KE, Wabitsch M, Fischer-Posovszky P. THP-1 Macrophages and SGBS Adipocytes - A New Human in vitro Model System of Inflamed Adipose Tissue. Front Endocrinol (Lausanne) 2011;2:89.

(137) Shimba S. Application of RNA interference techniques to adipose cell cultures. Methods Mol Biol 2008;456:249-257.

(138) Echeverri CJ, Perrimon N. High-throughput RNAi screening in cultured cells: a user's guide. Nat Rev Genet 2006 May;7(5):373-384.

(139) Poussin C, Hall D, Minehira K, Galzin AM, Tarussio D, Thorens B. Different transcriptional control of metabolism and extracellular matrix in visceral and subcutaneous fat of obese and rimonabant treated mice. PLoS One 2008;3(10):e3385.

(140) Higami Y, Barger JL, Page GP, Allison DB, Smith SR, Prolla TA, et al. Energy restriction lowers the expression of genes linked to inflammation, the cytoskeleton, the extracellular matrix, and angiogenesis in mouse adipose tissue. J Nutr 2006 Feb;136(2):343-352.

(141) Kubo Y, Kaidzu S, Nakajima I, Takenouchi K, Nakamura F. Organization of extracellular matrix components during differentiation of adipocytes in long-term culture. In Vitro Cell Dev Biol Anim 2000 Jan;36(1):38-44.

(142) Nedvidkova J, Smitka K, Kopsky V, Hainer V. Adiponectin, an adipocyte-derived protein. Physiol Res 2005;54(2):133-140.

(143) Christiaens V, Lijnen HR. Angiogenesis and development of adipose tissue. Mol Cell Endocrinol 2010 Apr 29;318(1-2):2-9.

(144) Tam CS, Tordjman J, Divoux A, Baur LA, Clement K. Adipose tissue remodeling in children: the link between collagen deposition and age-related adipocyte growth. J Clin Endocrinol Metab 2012 Apr;97(4):1320-1327.

(145) Kawaguchi N, Sundberg C, Kveiborg M, Moghadaszadeh B, Asmar M, Dietrich N, et al. ADAM12 induces actin cytoskeleton and extracellular matrix reorganization during early adipocyte differentiation by regulating beta1 integrin function. J Cell Sci 2003 Oct 1;116(Pt 19):3893-3904.

(146) Kurisaki T, Masuda A, Sudo K, Sakagami J, Higashiyama S, Matsuda Y, et al. Phenotypic analysis of Meltrin alpha (ADAM12)-deficient mice: involvement of Meltrin alpha in adipogenesis and myogenesis. Mol Cell Biol 2003 Jan;23(1):55-61.

(147) Li H, Solomon E, Duhachek Muggy S, Sun D, Zolkiewska A. Metalloprotease-disintegrin ADAM12 expression is regulated by Notch signaling via microRNA-29. J Biol Chem 2011 Jun 17;286(24):21500-21510.

(148) Garces C, Ruiz-Hidalgo MJ, Font de Mora J, Park C, Miele L, Goldstein J, et al. Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis. J Biol Chem 1997 Nov 21;272(47):29729-29734.

(149) Bansal K, Kapoor N, Narayana Y, Puzo G, Gilleron M, Balaji KN. PIM2 Induced COX-2 and MMP-9 expression in macrophages requires PI3K and Notch1 signaling. PLoS One 2009;4(3):e4911.

(150) Bourd-Boittin K, Le Pabic H, Bonnier D, L'Helgoualc'h A, Theret N. RACK1, a new ADAM12 interacting protein. Contribution to liver fibrogenesis. J Biol Chem 2008 Sep 19;283(38):26000-26009.

(151) Dehmel T, Janke A, Hartung HP, Goebel HH, Wiendl H, Kieseier BC. The cell-specific expression of metalloproteinase-disintegrins (ADAMs) in inflammatory myopathies. Neurobiol Dis 2007 Mar;25(3):665-674.

(152) Kveiborg M, Albrechtsen R, Couchman JR, Wewer UM. Cellular roles of ADAM12 in health and disease. Int J Biochem Cell Biol 2008;40(9):1685-1702.

(153) Sivakumar P, Das AM. Fibrosis, chronic inflammation and new pathways for drug discovery. Inflamm Res 2008 Sep;57(9):410-418.

(154) Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli CM. NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. J Cell Biol 1998 May 18;141(4):1083-1093.

(155) Zhu S, Sun F, Li W, Cao Y, Wang C, Wang Y, et al. Apelin stimulates glucose uptake through the PI3K/Akt pathway and improves insulin resistance in 3T3-L1 adipocytes. Molecular and cellular biochemistry 2011;353:305-313.

(156) Gil A, Aguilera C, Gil-Campos M, Canete R. Altered signalling and gene expression associated with the immune system and the inflammatory response in obesity. British journal of nutrition 2007;98(suppl1):S121-S126.

(157) Kisseleva T, Brenner DA. Mechanisms of fibrogenesis. Exp Biol Med (Maywood) 2008 Feb;233(2):109-122.

(158) Kras KM, Hausman DB, Martin RJ. Tumor necrosis factor-alpha stimulates cell proliferation in adipose tissue-derived stromal-vascular cell culture: promotion of adipose tissue expansion by paracrine growth factors. Obes Res 2000 Mar;8(2):186-193.

(159) O'Hara A, Lim FL, Mazzatti DJ, Trayhurn P. Microarray analysis identifies matrix metalloproteinases (MMPs) as key genes whose expression is up-regulated in human adipocytes by macrophage-conditioned medium. Pflugers Arch 2009 Oct;458(6):1103-1114.

(160) Cho JW, Kang MC, Lee KS. TGF-beta1-treated ADSCs-CM promotes expression of type I collagen and MMP-1, migration of human skin fibroblasts, and wound healing in vitro and in vivo. Int J Mol Med 2010 Dec;26(6):901-906.

(161) Linder K, Arner P, Flores-Morales A, Tollet-Egnell P, Norstedt G. Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. J Lipid Res 2004 Jan;45(1):148-154.

(162) de Mello V, Kolehmainen M, Pulkkinen L, Schwab U, Mager U, Laaksonen D, et al. Downregulation of genes involved in NFKB activation in peripheral blood mononuclear cells after weight loss is associated with the improvement of insulin sensitivity in individuals with the metabolic syndrome: the GENOBIN study. Diabetologia 2008;51:2060-2067.

(163) Ruotsalainen E, Salmenniemi U, Vauhkonen I, Pihlajamaki J, Punnonen K, Kainulainen S, et al. Changes in inflammatory cytokines are related to impaired glucose tolerance in offspring of type 2 diabetic subjects. Diabetes Care 2006 Dec;29(12):2714-2720.

(164) Mack I, BelAiba RS, Djordjevic T, Gorlach A, Hauner H, Bader BL. Functional analyses reveal the greater potency of preadipocytes compared with adipocytes as endothelial cell activator under normoxia, hypoxia, and TNFalpha exposure. Am J Physiol Endocrinol Metab 2009 Sep;297(3):E735-48.

(165) Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002 Nov;8(11):1288-1295.

(166) Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 2001 Feb 8;409(6821):729-733.

(167) Cushman SW, Wardzala LJ. Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. Apparent translocation of intracellular transport systems to the plasma membrane. J Biol Chem 1980 May 25;255(10):4758-4762.

(168) Shepherd PR, Gnudi L, Tozzo E, Yang H, Leach F, Kahn BB. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. J Biol Chem 1993 Oct 25;268(30):22243-22246.

(169) Verrecchia F, Mauviel A. TGF-beta and TNF-alpha: antagonist cytokines controlling type I collagen gene expression. Cell Signal 2004;16:873-880.

Maija Vaittinen
The Role of Weight Reduction Sensitive Genes on Adipose Tissue Biology and Adipocyte Function

Special Emphasis on the Expression of

MFAP5 and CPPED1

This study focused on the roles of microfibrillar-associated protein 5 *(MFAP5)* and calcineurin-like phosphoesterase domain 1 *(CPPED1)* in white adipose tissue (wAT) biology and adipocyte function. The results demonstrate that *MFAP5* and *CPPED1* could contribute to complications in dysfunctional wAT. Furthermore, the results suggest that *MFAP5* is involved in extracellular matrix function and wAT inflammation. In addition, the results provide novel insight about the role of *CPPED1* in glucose metabolism, possibly via adiponectin-mediated mechanisms.

UNIVERSITY OF
EASTERN FINLAND