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

I **Mulder H, Sörhede-Winzell M, Contreras JA, Fex M, Ström K, Ploug T, Galbo H, Arner P, Lundberg C, Sundler F, Ahrén B, Holm C.**
Hormone-sensitive lipase null mice exhibit signs of impaired insulin sensitivity whereas insulin secretion is intact. J Biol Chem. 2003 Sep 19;278(38):36380-8.

II **Hansson O, Ström K, Güner N, Wierup N, Sundler F, Höglund P, Holm C.**
Inflammatory response in white adipose tissue in the non-obese hormone-sensitive lipase null mouse model. J Proteome Res. 2006 Jul;5(7):1701-10.

III **Ström K, Hansson O, Lucas S, Nevsten P, Fernandez C, Klint C, Movérare-Skrtic S, Sundler F, Ohlsson C and Cecilia Holm.**
Attainment of Brown Adipocyte Features in White Adipocytes of Hormone-Sensitive Lipase Null Mice. PLoS ONE. 2008 Mar 12;3(3):e1793.

IV **Ström K, Gundersen TE, Hansson O, Lucas S, Fernandez C, Klint C, Blomhoff R and Cecilia Holm.**
Retinoid metabolism is perturbed in adipose tissue of mice lacking Hormone-sensitive lipase. Manuscript.

ABBREVIATIONS

ADD-1/SREBP-1	Adipocyte determination and differentiation factor 1 /sterol regulatory element-binding protein 1
Adh	Alcohol dehydrogenase
ADRP	Adipocyte differentiation related protein
ATGL	Adipocyte triglyceride lipase
BAT	Brown adipose tissue
C/EBP	CCAAT/enhancer-binding protein
DG	Diglyceride (Diacylglycerol)
GAL-3	Galectin-3
HFD	High-fat diet
HSL	Hormone-sensitive lipase
FABP/aP2	Fatty acid binding protein
FFA	Free fatty acid
IKKβ	Inhibitory kappa B kinaseβ
IL	Interleukin
JNK	c-Jun N-terminal kinase
LPL	Lipoprotein lipase
MCP-1	Monocyte chemoattractant protein 1
MG	Monoglyceride (Monoacylglycerol)
NEFA	Non-esterified fatty acid
NFκB	Nuclear factor kappa B
PGC1	PPARγ coactivator 1
PKA	Protein kinase A (cAMP-dependent protein kinase)
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
RBP	Retinol binding protein
pRB	Retinoblastoma protein
RIP140	Receptor interacting protein 140
RA	Retinoic acid
RALD	Retinaldehyde
Raldh	Retinaldehyde dehydrogenase

RAR	Retinoic acid receptor
RE	Retinyl ester
ROH	Retinol (Vitamin A)
RXR	Retinoid X receptor
SRC	Steroid receptor coactivator
SVF	Stromal-vascular fraction
T2D	Type 2-Diabetes (Mellitus)
TIF	Transcriptional intermediary factor
TZD	Thiazolidinedione
TG	Triglyceride (Triacylglycerol)
TNFα	Tumor necrosis factor α
UCP-1	Uncoupling protein 1
WAT	White adipose tissue

INTRODUCTION

OBESITY

Obesity is one of the greatest public health challenges of the 21st century and the increased prevalence of obesity has focused attention on a worldwide problem. Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Body mass index (BMI) is a simple index of weight-for-height that is commonly used in classifying overweight and obesity in adult populations and individuals. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2). BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults. However, it should be considered as a rough guide because it may not correspond to the same degree of fatness in different individuals. The world health organization (WHO) defines "overweight" as a BMI equal to or more than 25, and "obesity" as a BMI equal to or more than 30. Overweight and obesity lead to serious health consequences including cardiovascular disease and type 2 diabetes and the risk increases progressively as BMI increases. There are two ways of measuring abdominal obesity, measurement of waist to hip ratio (WHR) and of waist circumference (1). A WHR greater than 1.0 in men and 0.85 in women is associated with abdominal fat accumulation. The measurement of waist circumference is the simplest way of measuring abdominal fat accumulation and a value greater than 102 cm in men and 88 cm in women is consistent with abdominal obesity and substantial risk for metabolic complications.

Obesity epidemic

The prevalence of obesity has tripled in many countries in the WHO European Region since the 1980s, and the numbers of those affected continue to rise at an alarming rate, particularly among children. Obesity is already responsible for 2-8% of health costs and 10-13% of deaths in different parts of the region. The latest projection from WHO, indicates that globally in 2005 approximately 1.6 billion adults (age 15+) were overweight whereof at least 400 million were obese. By the year 2015 these figures are projected to increase to approximately 2.3 billion adults being overweight and more than 700 million being obese (WHO, URL: http://www.who.int/mediacentre/factsheets/en/). Global increases in overweight and obesity are attributable to a number of factors including a global shift in diet towards increased intake of energy-dense foods that are high in fat and sugars and decreased physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

Energy imbalance

Obesity is fundamentally the result of energy imbalance and can only develop when energy intake exceeds energy expenditure. Whereas energy intake is almost entirely determined by food intake (minus whatever fails to be absorbed), energy expenditure has more components, including basal metabolism, physical activity and adaptive thermogenesis (2). Adaptive thermogenesis is defined as heat production in response to cold exposure or overfeeding and serves the function of protecting the organism from cold exposure and regulating energy balance after changes in diet (3). The result of a sustained positive energy balance is buffered primarily by an increased storage of energy as fat in the adipose tissue. Accordingly to the

model of "lipid spillover" the extra energy is normally channeled into insulin-sensitive subcutaneous adipose tissue, which is the safe storage of excess energy in the body (4). If the storage capacity in the subcutaneous tissue is exceeded, a surplus of energy will be deposited in the visceral adipose tissue, regarded as an unsafe fat deposit, and eventually, accumulation of fat in non-adipose tissues, i.e. ectopic fat accumulation, will occur in muscle and liver, which is strongly associated with disease.

Visceral obesity

The distribution of body fat is a critical determinant of insulin sensitivity and varies markedly in both lean and obese individuals, with a strong genetic influence (5) (6). Lean individuals with a more peripheral fat distribution are more insulin sensitive than those showing a predominantly central distribution i.e. in the abdominal and chest area. Whereas women accumulate fat in peripheral (gluteal and femoral) regions, men have a preferential abdominal accumulation (1). Triglyceride (TG) levels and fasted glucose levels increase more rapidly with increased body fat in men. Also, women with abdominal (android) fat distribution are more insulin resistant than those with peripheral obesity (5). Increased visceral fat has been shown to have a stronger correlation with waist circumference than with BMI and is further closely linked to cardiovascular disease (CVD). Selective reduction of visceral adiposity is accompanied by improvements in metabolism (6). Pathophysiological mechanisms linking visceral adipose tissue to metabolic disease are probably associated with both anatomical site and altered intrinsic properties of visceral adipocytes. Visceral fat expresses more genes encoding secretory proteins than subcutaneous fat (7). The secretion of adiponectin by visceral adipocytes is more strongly and negatively correlated with BMI, compared to subcutaneous adipocytes. Leptin secretion is greater from subcutaneous fat compared to visceral (6), which could lead to a better control of appetite by this tissue. A higher turnover of TG in abdominal fat resulting in increased plasma NEFA levels has been reported. Visceral fat is more sensitive to lipolytic agents and also less sensitive to the anti-lipolytic effect of insulin than subcutaneous fat (6) (8). The increase in lipolytic response to catecholamines is suggested to be an effect of increased amounts of and sensitivity to adrenergic β-receptors on the surface of the adipocyte (8). Differences in these adipocyte characteristics combined with the proximity of the liver to the intra-abdominal fat depot could result in greater flux of NEFA to the liver, with adverse effects on insulin sensitivity in this organ (9) (7).

Genetic background

Besides changes in food intake and physical activity as underlying causes of obesity, there is compelling evidence that inter-individual differences in susceptibility to obesity have strong genetic determinations (1). One example is the Pima Indian community of Arizona, which has a particularly high prevalence of obesity, with strong genetic linkage. It is frequently assumed that hereditary factors would influence metabolic rate or the selective partitioning of excess calories into fat. However, all monogenic defects causing human obesity actually disrupt hypothalamic pathways and have a profound effect on satiety and food intake, e.g. leptin deficiency. This is also the case in several mouse models of obesity e.g. *ob/ob* (mutation in the leptin gene), *db/db* (mutation in the leptin receptor gene) and Mc4r (mutation in the melanocortin-4 receptor gene) (10). Studies on homozygotic twins have suggested a genetic factor explaining the large variance between individuals in adaptation to long-term

overfeeding (11). Furthermore, the non-exercise activity thermogenesis i.e the thermogenesis associated with maintenance of posture and other physical activities of daily life is suggested to be the main adaptation to increased energy intake and what accounts for the largest part of the susceptibility to weight gain in response to overeating, between individuals (12).

Obesity and disease

The metabolic syndrome (syndrome X) is a frequently used name of a set of interrelated common clinical disorders associated with obesity (abdominal), insulin resistance or diabetes, glucose intolerance, hypertension , dyslipidemia, atherosclerosis and increased risk of developing cardiovascular disease (CVD) (4). Although highly debated, it is recognized as a major risk for developing CVD by many international organs, including the WHO, who recently provided a definition of the syndrome (13). Primary defects in energy balance that results in obesity (visceral adiposity in particular), is sufficient to drive all aspects of the syndrome, and increased non-esterified fatty acids (NEFA) and lipid accumulation in non-adipose tissue seen in obese patients are strong mediators of the insulin resistance (14).

A very large part of patients with type 2 diabetes (T2D) are overweight or obese and it is now clearly established that obesity is one of the most important risk factors for the development of disease (7). The underlying mechanism by which the adipose tissue interplays in the development of disease is not entirely established. However, in obese states normal effects of insulin on adipose tissue are perturbed, resulting in increased plasma levels of NEFAs, negatively affecting key events in the regulation of glucose homeostasis. Since hormone-sensitive lipase (HSL) is an important enzyme for adipocyte lipolysis, and is regulated by insulin, it could have a central role in the generation of disease. Obesity is further associated with increased adipose tissue infiltration of macrophages (15), associated with a proinflammatory state that potentiates insulin resistance and atherosclerosis. Fat-derived adipokines, including TNFα and adiponectin, are implicated as pathogenic contributors and protective factors respectively, and a diabetes-susceptibility locus, mapped to the location of the adiponectin gene, is strongly linked to the metabolic syndrome in individuals of European descent (16). Although a majority of subjects with T2D are overweight or obese, only approximately half of the overweight subjects have abnormal glucose level despite the presence of severe insulin resistance (17). This is due to the compensatory increase in release of insulin from the pancreatic β-cells, overcoming the peripheral insulin resistance. It is only when the β-cells fail to meet the increased demands of insulin that hyperglycemia and diabetes precipitates.

Prevention

Lifestyle prevention is an obvious first-choice action when battling obesity and its related disorders. Through a program of diet control and increased physical activity, leading to weight loss, the complications of obesity-related disorders can be reversed. However, lifestyle prevention requires much effort from the obese subject, and many patients eventually fall out of the program. Also, it is documented that energy homeostasis is regulated to defend the highest weight achieved (18). This system, although beneficial when food is scarce, counteracts a reduced food intake by a reduction in energy expenditure, resulting in sustained body weight. Morbidly obese patients, frequently go through surgery in the form of gastric bypass, to reduce lipid uptake from the diet. Currently there are three

drugs licensed for the treatment of obesity, sibutramine (Reductil®) and rimonabant (Acomplia®) acting by reducing food intake, and orlistat (Xenical®) acting by reducing lipid absorption from the intestine.

INSULIN RESISTANCE

Insulin resistance is a condition in which the cells/tissues of the body become resistant to the effects of insulin, that is, the normal response to a given amount of insulin is reduced. As a result, higher levels of insulin are needed in order for insulin to carry out its effects. Major sites for insulin actions are muscle, liver and the adipose tissue. However, an effect of insulin in the central nervous system, acting in the hypothalamus to regulate food intake and body weight has also been reported (19).

Glucose and insulin resistance

Glucose homeostasis

Glucose is an important source of energy in most organisms. Tissues such as the brain need a constant supply of glucose, and a low plasma glucose concentration can cause consciousness and even be fatal (20). However, prolonged elevation of glucose in poorly controlled diabetes can lead to a number of severe conditions and death. It is therefore important to maintain a relatively constant blood glucose level. Maintenance of normal glucose homeostasis is hormonally regulated and results from the precise orchestration of three processes: intestinal glucose absorption, production by the liver and absorption by nearly all tissues in the body. Under normal conditions, a postprandial increase in plasma levels of glucose is balanced by the release of insulin from the pancreatic β-cells. Insulin is a polypeptide hormone that when released regulate glucose disposal through the binding of insulin receptors located on the plasma membrane target cells. To maintain glucose homeostasis there are primarily three major target tissues on which insulin exerts its effects namely, muscle, liver and adipose tissue. Glucose enters the cell by facilitated diffusion through specific glucose transporters (GLUTs), expressed by most cells, spanning through the plasma membrane of the target cell (21). The two major isoforms expressed in myocytes and adipocytes are GLUT1, which is ubiquitously expressed, and GLUT4, which is regulated by insulin. GLUT2 is expressed at high levels in hepatocytes and pancreatic β-cells and mediates the bidirectional transport of glucose in these cells.

Insulin exerts its effects on target cells by binding to the insulin receptor (IR) on the plasma membrane of the cell. The insulin receptor is a tyrosine kinase and binding of insulin to the receptor initiates a series of autophosphorylation events of the intracellular part of the receptor as well as phosphorylation of insulin receptor substrate (IRS), in turn triggering a downstream signaling cascade, including phosphoinositide-3-OH kinase (PI3K). One important effect of insulin signaling occurring in both myocytes and adipocytes is to mobilize the GLUT4 receptor from internal stores to the surface of the cell, increasing glucose entry to the cell (21). This isoform of GLUT is responsible for the majority of insulin-stimulated glucose uptake into these cells. In the absence of insulin, about 90% of GLUT4 is sequestered intracellularly (20). Insulin signaling is absolutely vital for survival, and mice with a deletion of the insulin receptor die shortly after birth from ketoacidosis (22). A reduction in the expression of IR and IRS-1, leads to a severe hyperinsulinemia, developing into diabetes in

40% of the mice. The importance of intact glucose transport and insulin signaling for glucose homeostasis has been illustrated by a series of studies where tissue specific deletions of GLUT4 or IR have been made.

Defects in glucose uptake in muscle

Skeletal muscle represents the major tissue responsible for insulin-stimulated glucose uptake in humans, and glycogen formation has been estimated to account for ~90% of whole-body glucose metabolism and all non-oxidative glucose disposal (23). It is suggested that the reduced insulin-stimulated muscle glycogen synthesis, which underlies insulin resistance in patients with type 2 diabetes, is attributable mostly to reduced insulin-stimulated glucose transport into myocytes (24). In muscle, GLUT4 mediates glucose transport stimulated by both insulin and muscle contraction. Muscle-specific inactivation of GLUT4 in mouse, results in severe insulin resistance and glucose intolerance from early age (25). Secondary glucotoxic effects leading to reduced ability of insulin to stimulate glucose uptake in adipose tissue and to suppress hepatic glucose production are restored by lowering plasma glucose levels (26). Mice with a muscle specific deletion of the IR remain glucose tolerant, but show secondary defects in adipose tissue, resulting in increased plasma TG and NEFA (25).

Defects in glucose uptake in adipose tissue

Mice with an adipose-specific reduction of GLUT4, are glucose intolerant and show insulin resistance secondarily in liver and muscle, possibly due to impairment of PI3K in the insulin signaling cascade, revealing a critical role of adipose tissue for glucose homeostasis (25). The adipose-selective GLUT4 downregulation seen in human obesity and type 2 diabetes may thus contribute to insulin resistance and development of the disease. In support of this is the fact that overexpression of GLUT4 in adipose tissue reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle (27). Adipose tissue overexpression of GLUT4 in normal mice enhances systemic glucose tolerance and insulin sensitivity (28). Mice with a double deletion of the GLUT4 transporter in both muscle and adipose tissue, display hyperglycemia and impaired glucose tolerance, and further, show an increase in usage of lipid fuels by the liver, demonstrating adaptations to impaired glucose transport (29).

Glucose control by the liver

The liver, unlike myocytes and adipocytes, is freely permeable to glucose. In the fasted state the blood glucose level is maintained largely by the liver and is used by the brain independently of insulin (20). Blood glucose levels are maintained by the rapid mobilization of hepatic glycogen stores (glycogenolysis) stimulated by glucagon released from the pancreatic α-cells and, after prolonged starvation, through gluconeogenesis, i.e. formation of glucose from glycerol, lactate and arginine. Insulin inhibits these two processes and stimulates the synthesis of glycogen from glucose, diminishing the endogenous glucose production from the liver. An increase in gluconeogenesis is believed to be the primary cause of increased efflux of glucose seen in type 2 diabetics (7). Mice with a deletion of the insulin receptor specifically in the liver display hepatic dysfunction and severe insulin resistance, compensated for by an increase in pancreatic β-cell mass (25).

A conclusion from different models where the insulin receptor has been deleted tissue specifically, is that for insulin resistance to have a major impact on disease, it has to occur in

multiple tissues, and for diabetes to develop, a defective insulin signaling in pancreatic β-cells must be present, preventing compensatory action from these cells. This is shown in mice where the insulin receptor gene has been deleted in both liver and β-cells, where an inability of the β-cells to compensate for the insulin resistance by producing more insulin, leads to an early development of diabetes (30).

Lipids and insulin resistance

Historically, T2D has been primarily associated with abnormal glucose metabolism, supported by the fact that chronic elevation of glucose causes many of the microvascular complications of diabetes (31). It is now apparent that impaired lipid metabolism plays a central role in the development of T2D, and there is a tight association between T2D and dyslipidemia i.e. elevated levels of lipid particles in plasma (32).

Insulin effects on adipose tissue

Adipose tissue metabolism is precisely regulated by multiple factors including hormonal and nervous influences. During periods of low energy intake, the body uses its fat reserves, releasing NEFAs from adipose tissue stores for other tissues to use as fuel (31). If plasma NEFA levels are elevated for more than a few hours, they will cause insulin resistance. In certain conditions, such as starvation this is a beneficial effect of NEFA, preserving glucose for tissues that cannot use NEFA for energy such as the brain. In the fed state, insulin exerts many effects on the adipose tissue. Besides stimulating glucose uptake, insulin also inhibits lipolysis, partly through inactivation of HSL, and stimulates lipogenesis in the adipocyte, the two latter mechanisms being the most important effects in lowering plasma NEFA levels. Insulin also lowers plasma TG levels by stimulating the activation of lipoprotein lipase (LPL), on the surface of the endothelial cells, which increases the hydrolysis of TGs. The concentration of insulin needed to obtain an antilipolytic effect is far below that needed to stimulate glucose incorporation in adipose tissue (33). In the adipocyte, both glucose and NEFA are incorporated into lipids, a process called lipogenesis. The human adipocyte has a low capacity for *de novo* fatty acid synthesis (34). Insulin also seems to be important for expansion of the adipose tissue suggested by a mouse model where the insulin receptor gene has been deleted specifically in the adipose tissue, leading to lower fat mass (35).

The Randle cycle

The concept that NEFA interfere with glucose utilization was introduced by Randle *et al* (36), showing that a reciprocal interaction exists between carbohydrate and lipid metabolism. When either of the substrate is present at high amounts, the utilization of the other is suppressed. The concept was named the glucose-fatty acid cycle, more known as the Randle cycle. High levels of NEFA in isolated heart muscle suppressed glucose oxidation by increased fatty acid oxidation, suggested to be the mechanism behind decreased glucose uptake in muscle in starvation but also in insulin resistance and diabetes. He later suggested a more precise mechanism by which NEFA can induce muscle insulin resistance (37). Increased acetyl-CoA and NADH levels arising from increased mitochondrial β-oxidation of NEFA, inactivates pyruvate dehydrogenase. This leads to a rise in intracellular citrate level, which inhibits phosphofructokinase and glucose-6-phosphate (G6P) accumulation. G6P inhibits hexokinase activity, resulting in increased intracellular glucose levels and decreased glucose uptake. This hypothesis has however been challenged by several studies (23) (38).

Although beneficial at starvation, during prolonged periods of energy excess, NEFA-induced insulin resistance becomes counterproductive because there is no need for glucose preservation.

NEFA and insulin resistance

Physiologic increases in plasma NEFA levels cause insulin resistance in both diabetic and non-diabetic subjects by inhibiting insulin-stimulated glucose uptake and glycogen synthesis (39). Elevated plasma NEFA levels have been shown to account for up to 50% of insulin resistance in obese patients with T2D (40). In the liver, the NEFAs are esterified to TG and packed into lipoproteins before being recirculated, resulting in elevated plasma TG levels. As the plasma lipid levels increase, ectopic lipid storage occurs, associated with insulin resistance. Acute and chronic elevations in plasma NEFAs produce muscle and hepatic insulin resistance. A close correlation between intramyocellular TG content and whole-body insulin resistance is seen in patients with obesity and T2D (41). Elevated NEFAs are also associated with perturbations in the insulin-signaling cascade, where activation of atypical PKC negatively affect insulin signaling by inducing serine phosphorylation on IRS-1 and 2 ending in reduced activation of PI3K (42). In liver, NEFAs interfere with insulin effects resulting in increased hepatic glucose production (40). NEFAs also produce a low-grade inflammation in skeletal muscle and liver through activation of nuclear factor-kappaB, resulting in release of several proinflammatory cytokines (43). Plasma NEFAs are often seen as a link between central (visceral) obesity and insulin resistance (39). Regulating the amount of lipid liberated into the plasma, the adipose tissue has a central role in insulin resistance. Circulating NEFAs derived from adipocytes have been suggested to be the single most critical factor in the development of insulin resistance of obesity and diabetes (7). However, fasting plasma NEFAs are not always elevated in insulin-resistant obese subjects.

Besides releasing NEFAs and glycerol, the adipose tissue releases a multitude of other factors commonly known as adipokines, including hormones (e.g. leptin, adiponectin and resistin) and proinflammatoy cytokines (e.g. TNFα and IL-6) that modulate metabolism. In obesity, (visceral in particular) the production of many adipokines is altered, implicated in the development of peripheral insulin resistance. One exception is adiponectin, where decreased plasma levels are associated with obesity and insulin resistance (44). Obesity is strongly connected to a low-grade inflammation, where an expanding adipose tissue releases increased amounts of proinflammatory cytokines that participate in the induction and maintenance of the inflammatory state associated with obesity and insulin resistance (45).

Although over-consumption of lipids and excess adiposity is connected to insulin resistance, the adipose tissue is vital for normal functions of the body to occur. This is shown in a transgenic mouse model where the WAT is absent throughout life, displaying insulin resistance and diabetes with enlarged fatty livers (46). In both mice and humans, a parallel can be seen between obesity and lipodystrophy in that both conditions display loss of appetite regulation and insulin resistance, mediated at least in part by lipid storage in non-adipose tissues (47) (46). Transplantation of WAT or injections of low doses of leptin into mice devoid of adipose tissue reverses hyperphagia and fatty liver, and increases insulin sensitivity (48) (49), showing the importance of WAT, not only as a lipid sink, but also as an endocrine organ controlling feeding behavior.

INSULIN SECRETION

Glucose-stimulated insulin secretion

Pancreatic β-cells are markedly plastic in their ability to regulate insulin release, but do so in a very precise manner. The quantity of insulin released by the β-cells varies according to the nature and quantity of the stimulus, and the prevailing glucose concentration. After a meal, increased plasma levels of glucose are sensed by the pancreatic β-cells, by diffusion of glucose through the permeable GLUT 2 transporter. In order for glucose to promote insulin secretion it needs to be metabolized, generating ATP. This increases the intracellular ratio of ATP/ADP, triggering a closure of the ATP-sensitive potassium (K^+_{ATP}) channel, depolarization of the plasma membrane and influx of calcium through voltage-dependent calcium channels, resulting in insulin granule exocytosis. This process is called glucose-stimulated insulin secretion (GSIS) and is increased in animal models of obesity that maintain normal plasma glucose levels (euglycemia) (7). Non-glucose signals potentiate the response of glucose to yield a maximal response of insulin release.

Adaptation to insulin resistance

In healthy individuals, the β-cells adapt to changes in insulin sensitivity by producing more insulin, occurring both by functional changes in the responsiveness of the β-cell to stimuli and by increasing the β-cell mass (50). Glucokinase, the rate-limiting enzyme in glucose metabolism, functions as a glucose sensor for insulin secretion in β-cells and increase in activity when glucose levels rise. Both glucokinase and IRS-2 are shown to be required for β-cell hyperplasia to occur in response to high fat diet-induced insulin resistance (51). Insulin is a potentially important modulator of islet mass, as activation of the insulin receptor in β-cells leads to phosphorylation of IRS-2 and eventually to activation of mitogen-activated protein kinases. Deletion of the insulin receptors of the β-cells lead to ablation in the first phase of GSIS and a secretory defect similar to that seen in T2D (52).

NEFA and secretory defects

NEFAs are important for normal β-cell function and potentiate GSIS, possibly by generating a lipid signal positively affecting insulin secretion (53). This lipid signal is suggested to be fatty acyl-CoA, previously known to have stimulating effects on insulin secretion from the β-cell. However, prolonged exposure to increased levels of NEFA is suggested to cause the β-cell abnormalities of non-diabetic obesity which ultimately result in obesity-dependent diabetes (50). Non-diabetic obesity in Zucker rats is characterized by hypersecretion of insulin at normal fasting glucose concentrations, resulting from β-cell hyperplasia and increased glucose usage and oxidation. Once the obese Zucker diabetic fatty (ZDF) rat (a rat prone to development of diabetes) becomes diabetic, GSIS is absent and beta-cell GLUT 2 is reduced (54). Islet TG content is highly increased, correlating with an increase in plasma levels of NEFA beginning shortly before onset of diabetes. β-cell abnormalities are prevented by reducing plasma NEFAs by caloric restriction. By culture of normal islets with high levels of NEFA, loss of GSIS and TG accumulation can be induced. Prediabetic islets however, from diabetes prone ZDF rats, seem far more vulnerable to NEFA-induced functional impairment (50).

In humans, a large part of obese subjects never develop T2D despite the presence of severe insulin resistance during a prolonged time. An explanation is that in obese subjects with

normal pancreatic β-cells, NEFAs function as potent insulin secretagogues that can compensate for the insulin resistance that they produce (31). However, in first-degree relatives of patients with T2D, NEFAs are unable to provoke an adequately increased insulin secretion, suggesting that obese individuals who develop T2D have a genetic predisposition to pancreatic β-cell failure.

T2D is characterized by a progressive loss of β-cell function throughout the course of the disease, including initial defect in first phase insulin secretion, followed by a decrease in maximal capacity of glucose to potentiate non-glucose signals. Last, a defective steady-state develops, with a reduction in number and function of β-cells, leading to complete β-cell failure. Fasting hyperglycemia and the diagnostic level for diabetes is not present until β-cell function is decreased by 75% or more (7).

DIABETES MELLITUS

Diabetes mellitus commonly referred to as diabetes, is a group of disorders that lead to an elevation of glucose in the blood (hyperglycemia), resulting from defects in insulin secretion, or action, or both. Diabetes as a disease has been known for a very long time. The word "diabetes" is borrowed from the Greek word meaning "a siphon". The 2nd-century A.D. Greek physician, Aretus the Cappadocian, named the condition "diabetes". He explained that patients with it had polyuria and "passed water like a siphon. *Mellitus*, the Latin word for honey, comes from the early identification of the disease associated with “sweet urine”. Sustained hyperglycemia leads to spillage of glucose into the urine, hence the term sweet urine. A major breakthrough in diabetes research occurred at the end of the 19th century when Oskar Minkowski performed experiments on pancreatectomized dogs. He noticed that the dogs suffered from polyuria and that the urine attracted an unusual number of flies, and associated this with diabetes. When glucose was found in the urine he concluded that pancreas produces a substance that control glucose concentration and that diabetes occur without this substance. These findings were published in a pioneering article and mark a milestone in diabetes history (55). A second landmark was made by Frederick Banting around 20 years later, discovering that insulin was the active element from the pancreas (56).

Fasting blood glucose levels are normally kept within a narrow range between 4-7mM. When the fasted plasma glucose level is equal to or higher than 7mM the patient is diagnosed as being diabetic. Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) are intermediate conditions preceding type 2 diabetes, characterized by slightly elevated glucose levels.

Type 1-diabetes

Type 1-diabetes (T1D), also known as insulin-dependent diabetes mellitus, is an auto-immune disease where the insulin producing pancreatic β-cells in the islets of Langerhans are destroyed, causing a lack of insulin in the plasma. This type was previously known as juvenile-onset diabetes, because it most commonly develops in people younger than the age of 20 and patients require lifelong treatment with insulin. Around 10% of diabetic patients are diagnosed with T1D.

Type 2-diabetes

Type 2-diabetes (T2D), also known as non-insulin-dependent diabetes mellitus, is a multifactorial polygenic disease representing more than 90% of all cases. It is strongly associated with obesity (over 80% of sufferers are obese) and usually occurs in people over 40 years of age (giving the disease its former name maturity-onset diabetes) although an increased incidence in children can be seen, in association with obesity. It was estimated that more than 170 million people worldwide had diabetes in the year 2000 and that this number is likely to more than double by 2030 giving the disease epidemic proportions (57). The greatest absolute increase in the number of people with diabetes will occur in India, estimated to constitute 30% of the increased numbers. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people over 65 years of age. Taking into consideration the increasing prevalence of obesity these figures are likely to be underestimated. Diabetes and hyperglycemia is a serious condition with multiple complications, accounting for at least 10% of total health care expenditure in many countries (58). Since most individuals with diabetes most often die of cardiovascular and renal disease and not from a cause uniquely related to diabetes (e.g. hyperglycemia), reported statistics on diabetes-related deaths are often seriously underestimated. A study taking this into consideration has estimated that around 2.9 million deaths (5.2%) in the year 2000 were attributable to complications of diabetes, making it the fifth leading cause of death globally.

Gestational diabetes

A third type of diabetes is gestational diabetes, defined as glucose intolerance of various degrees that is first detected during pregnancy. During pregnancy, a progressive insulin resistance is a normal feature and appears to result from a combination of maternal adiposity and the insulin desensitizing effects of hormones produced from the placenta, securing nutrient supply for the growing fetus. Insulin resistance is normally compensated for by an increased secretion of insulin from the pancreatic β-cells, keeping glucose levels at a constant range. The hallmark of gestational diabetes is hyperglycemia, resulting from an abnormally increased insulin resistance and a simultaneous defect in pancreatic β-cell function, unable to respond to increased demands of insulin (59).

MODY

There are also a number of rare hereditary forms of diabetes mellitus collectively named maturity-onset diabetes of the young (MODY). Clinical characteristics that distinguish patients with MODY from those with type 2 diabete are the young age of presentation (onset usually before the age of 25 years) and the absence of obesity (60). They are caused by single-gene mutations that, in turn, affect the functions of the insulin producing pancreatic β-cells. The two most common variants are MODY 2 and 3 associated with mutations in the genes encoding glucokinase and the transcription factor HNF1α, respectively.

Hallmarks of T2D

The two hallmarks of type 2 diabetes are insulin resistance in liver and peripheral target tissues, i.e. skeletal muscle and adipose tissue, and perturbation of insulin secretion from the pancreatic β-cells. The pathogenesis of type 2 diabetes is complex and in most instances requires both defect in β-cell function and peripheral insulin resistance (7). It is only when

the pancreatic β-cells fail to supply an increased demand for insulin to compensate for the peripheral insulin resistance, that hyperglycemia is seen. Therefore, dysfunction of the pancreatic β-cell is an important defect in the pathogenesis of T2D. β-cell function deteriorates gradually during development of the disease and is decreased by about 75% when fasting hyperglycemia is present. In type 2 diabetes, hyperglycaemia is accompanied by abnormalities in lipid metabolism, seen by increased plasma levels of NEFA in type 2 diabetic patients (50), and are together more deleterious to islet health and insulin resistance than either alone (61), a concept called glucolipotoxicity. Speculations have been made that the release of NEFAs may be the single most critical factor in modulating insulin sensitivity, associated with the insulin resistance observed in obesity and T2D (9) (39).

Treatment

Treatment of T2D is not straight forward owing to the complexity of the disease involving interactions between a number of genetic and environmental factors. The prediabetic state is reversible and can be treated by lifestyle changes, such as increased exercise and food restriction. In the prediabetic state, treatments focusing on reversing the insulin resistance would seem legitimate in order to prevent β-cell exhaustion and diabetes. A pivotal role of NEFAs for the development of insulin resistance and T2D suggests that the optimal therapeutic intervention would be to decrease plasma NEFA levels (31).
After development of irreversible T2D, there are several possible therapeutic targets available. Anti-diabetic drugs either target the insulin resistance or the relatively insufficient insulin secretion from the pancreatic β-cells or both. Based on this anti-diabetic drugs can be divided into two major groups: The first group includes substances which enhance insulin sensitivity and regulate glucose and lipid metabolism, i.e. metformin, statin, fibrates and thiazolidinediones (TZDs). The second group includes substances that enhance insulin secretion from the pancreatic β-cells e.g. sulfonylureas, but also substances which result in an incretin effect and enhance glucose-dependent insulin secretion, i.e. GLP-1 analogues and dipeptidylpeptidase 4 (DPP4)-protease inhibitors.

ADIPOSE TISSUE

The adipose tissue is basically a form of loose connective tissue, specialized in storing energy reserves. It is highly vascularized and is innervated by the sympathetic nervous system. Like other types of connective tissues adipose tissue consists of cells and a non-cellular matrix, containing protein fibers and a ground substance, providing strength and support for the tissue as well as a medium in which substances are exchanged between cells and blood. The cells in connective tissue are derived from embryonic mesodermal cells called mesenchymal cells. Besides adipocytes, that although constituting the majority of the volume in adipose tissue only represent between one third and two thirds of the total number of cells in adipose tissue (62), several other cell types are also contained in this tissue namely fibroblasts, macrophages, endothelial cells, plasma cells, mast cells and adipocyte precursor cells (preadipocytes) in various degrees of differentiation. Fibroblasts are immature cells with a retained capacity for mitosis that help forming the matrix of the tissue. Adipocytes are believed to derive from multipotent resident mesenchymal cells.

Two types of adipose tissues exist with distinct locations in rodents, white adipose tissue (WAT) and brown adipose tissue (BAT), where the absolute majority of the adipose tissue in adults exists in the form of WAT. In mice and rats, BAT shows a prenatal development whereas WAT is mainly developed after birth (63). Although these two tissues share similarities in the expression and regulation of many genes, there are profound differences regarding gene expression, phenotype and function, showing that whereas an important purpose of WAT is to serve as an energy reserve, BAT is mainly involved in the control of body temperature through adaptive non-shivering thermogenesis.

WHITE ADIPOSE TISSUE

The two types of WAT are subcutaneous and visceral adipose tissue. Whereas the subcutaneous adipose tissue is found under the skin, the visceral adipose tissue is found within the peritoneal cavity. About 80% of body fat in lean humans is located in the subcutaneous adipose tissue and ~ 10% is located in visceral adipose tissue (64). Adipocytes in WAT contain a large unilocular lipid droplet, consisting mainly of triglycerides. In mature adipocytes, the lipid droplet constitutes the absolute majority of the cell, pushing the cytoplasm and nucleus to the periphery of the cell. The simplicity of WAT and white adipocytes in particular could be a reason to why this organ has been ignored for such a long time. With triacylglycerols constituting more than 85% of the tissue weight, and a cytoplasm in large adipocytes occupying less than 1% of the cell volume it is far from surprising that this tissue has been regarded as essentially limited in function to lipid storage and mobilization (65). Within the adipocytes, the level of fatty acids has to be tightly controlled, as fatty acids can act as detergents that rapidly dissolve the plasma membrane, causing cell lysis if allowed to accumulate (66). This makes an efficient conversion of fatty acids into TG for subsequent storage in the central lipid droplet crucial for the adipocyte. Parallel to the glucose buffering effects of muscle and liver, adipose tissue plays an important role in buffering the postprandial flux of plasma NEFAs by the uptake and incorporation of NEFA into TG (67). This prevents the ectopic accumulation of lipids in organs such as liver, muscle and pancreatic β-cells, associated with insulin resistance. This buffering capacity of the WAT is impaired in lipodystrophy as well as in obesity, showing the absolute necessity of a

functional adipose tissue for glucose and fatty acid homeostasis. An inability of the adipose tissue to expand to accommodate excess calories, seen especially in central obesity, seems to be highly connected to development of disease (68). Although adipocytes have a great capacity to accumulate lipids and increase in size, there is an upper limit after which the adipocytes show resistance to insulin and functional impairment. An intact ability to form new adipocytes thus seems crucial to prevent metabolic disease. It is suggested that a primary effect of the insulin sensitizing agents TZD, acting through PPARγ receptors, might be to stimulate the differentiation of new smaller and more insulin sensitive adipocytes, functioning as powerful buffers that absorb lipids in the postprandial state (69).

Current models recognize the adipose tissue as a highly metabolic organ with a central position in the integration of many homeostatic processes, and as an active player in the development of obesity related metabolic disorders (70). Many processes are coordinated through the release of peptide hormones from the adipose tissue, now being recognized as an endocrine organ. Quantitatively the most important product secreted from white adipocytes is NEFA, being used by peripheral tissues as fuel when glucose is limited, but the WAT also releases other lipid species including cholesterol, retinol, steroid hormones and prostaglandins (65), as well as the cytokine-like hormone leptin (71) that led to the recognition of WAT as an important endocrine organ.

Adipogenesis

Early adipocyte differentiation

In both animals and humans, the potential to acquire new fat cells appears to be permanent throughout life (62). Multipotent mesodermal stem cells have the abilities to differentiate into several different cell types, including adipocytes, chondrocytes and myocytes showing that crucial transcription factors and enzymes responsible for adipocyte development are not activated in these cells. A possible determining factor for the white adipocyte lineage is Tcf21, a transcription factor found in white preadipocytes (72). Tcf21 positively regulates the expression of bmp4, which has the ability to commit pluripotent mesenchymal cells to form white adipocytes, which suggestes a potentially important role for Tcf21in adipogenesis. Also, retinoic acid (RA) is shown to have an important role for embryonic stem cell commitment into the adipocyte lineage highlighted by the findings that treatment of stem cell-derived embryonal bodies with RA is a prerequisite for high adipogenesis (73). Development of the 3T3-L1 and 3T3-F442A preadipocyte cell lines from Swiss mouse embryos (74) (75) have been of tremendous help when investigating the molecular mechanisms controlling adipogenesis. Although committed to the adipocyte lineage, proliferating 3T3-L1 preadipocytes are morphologically similar to fibroblastic preadipose cells in the stroma of adipose tissue. Once 3T3-L1 cells have reached confluence, and are stimulated with an adipogenic cocktail, they start differentiating in a manner highly resembling the differentiation of primary adipocytes.
When the multipotent stem cells receive specific signals, and become committed to the adipocyte lineage they are called adipoblasts (62). Unipotent adipoblasts have a retained ability for proliferation and will continue to divide until they reach confluence and cell/cell interactions trigger the transformation and further commitment into preadipocytes (**Fig 1**). Preadipocytes have a very low ability to store lipids, resulting from the low expression of

lipogenic enzymes in these cells. However, they express early adipocyte markers such as LPL and the ubiquitously expressed adipocyte differentiation-related protein (ADRP) (76). ADRP is located to the small lipid droplets in 3T3-L1 preadipocytes and early differentiated adipocytes, whereas it is absent in mature adipocytes, instead expressing high levels of perilipin (77). Perilipin surrounds and protects the large lipid droplet in mature adipocytes and is absent during early differentiation. At a timepoint occurring when the preadipocytes start to accumulate lipids, there is a switch between these two proteins, suggesting that ADRP plays a role in early management of lipid accumulation in preadipocytes. The preadipocytes remain in an undifferentiated state due to autocrine Wnt signaling, which inhibits PPARγ and C/EBPα, key regulators of adipogenesis (78). Also, Wnt signaling appears to be important for the myogenic development since myocytes with a defect Wnt signaling, start developing into adipocytes. The suggestion that Wnt signaling inhibits adipogenesis in part through dysregulation of the cell cycle has also been made (79). Adipogenesis only occurs when confluent preadipocytes are treated with an adipogenic medium of hormones and mitogens (80). This triggers a second round of cell division, known as the mitotic clonal expansion, where growth-arrested preadipocytes reenter the cell cycle and undergo one or two rounds of cell division. In this phase, a morphological change of the preadipocyte into a less elongated cell shape occurs, most likely resulting from the decreased expression of cytoskeletal proteins seen prior to morphological change in differentiating 3T3-L1 cells (81). After one or two rounds of cell division, the clonal expansion slows and the preadipocytes go into a second growth arrest, losing the ability to proliferate. The early adipocytes now start to accumulate TGs into several small lipid droplets, occurring with a concomitant induction of late markers of differentiation including several genes involved in lipid metabolism such as HSL, GLUT4, C/EBPα and FAS (80). Among the first proteins to be expressed after growth arrest is C/EBPα, which due to its antimitogenic properties (82) has been implicated in the termination of clonal expansion and maintenance of the terminally differentiated state. The genes for very late markers of adipocyte differentiation, including leptin and PEPCK, are only transcribed in fully mature adipocytes with a more unilocular lipid droplet (62). Seen by the late appearance in differentiation, HSL likely plays a minor if any role in the earlier phases of adipocyte differentiation.

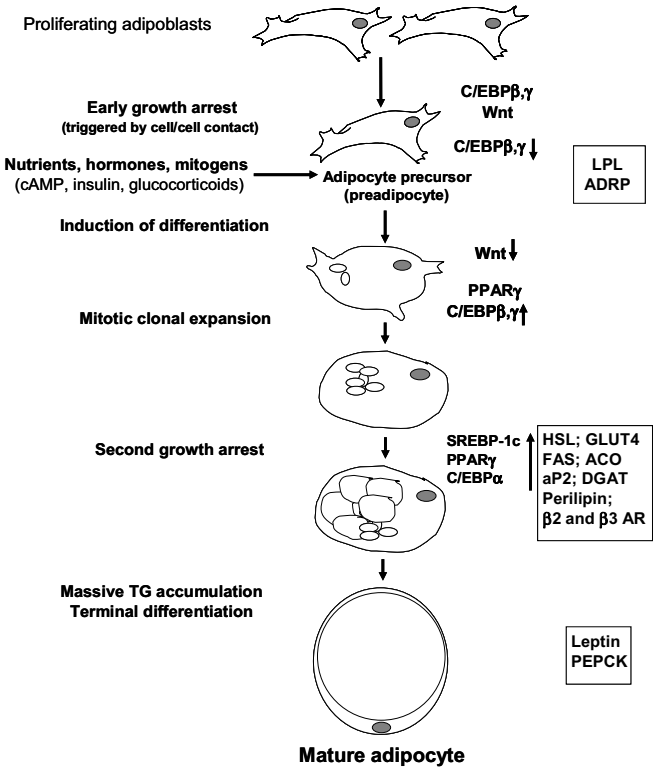

Figure 1 Differentiation of adipocyte precursor cells and the regulation of various genes during the differentiation process.

At states of energy imbalance the adipose tissue has two ways of buffering an excess in energy, either increasing the incorporation of lipids into adipocytes thereby increasing the size of the preexisting adipocytes (hypertrophy) or by increasing the number of adipocytes (hyperplasia). Whereas a moderate expansion of body fat in humans is mainly due to increase in adipocyte volume, a large expansion includes both increased adipocyte size and number (83). New adipocytes are derived from preadipocytes, present in WAT throughout adult life. Preadipocytes can proliferate and undergo differentiation to adipocytes when stimulated by certain transcription factors, a process called adipogenesis. The signal for differentiation of new adipocytes is related to nutritional state and important stimuli for differentiation include insulin and NEFAs acting through members of the PPAR family (70). Adipogenesis *in vitro* follows a highly ordered and well characterized temporal sequence and is controlled by a number of transcription factors (84). The most extensively studied are the two master regulators of adipogenesis, peroxisome proliferator activated receptor γ (PPARγ) and the CCAAT/enhancer binding proteins (C/EBPs) as well as adipocyte determination and differentiation-dependent factor 1/sterol regulatory element-binding protein 1c (ADD-1/SREBP-1c). A brief overview of the effects of these transcription factors in adipogenesis is given.

Key regulators in terminal adipogenesis

PPARγ

PPARs are members of the nuclear hormone receptor superfamily along with the receptors for retinoic acid, thyroid hormones and vitamin D that upon binding and activation by a ligand regulate the expression of genes expressing a specific response element (PPRE). Although the PPARs are shown to be activated by fatty acids (85), a high affinity endogenous ligand is still not found. Of the three isoforms of PPARs that have been cloned, PPARα, PPARδ and PPARγ, it is PPARγ that is shown to be the most adipogenic, and the only one capable of cooperating with C/EBPα to induce adipogenesis (86). PPARδ (also known as fatty acid-activated receptor, FAAR) is expressed ubiquitously and has been shown to promote adipogenesis in fiboblasts (87). However, a PPARδ selective agonist did not induce adipogenesis in 3T3-L1 cells (88), suggesting that the role of PPARδ in adipogenesis is minor.

PPARγ is expressed at high levels in white adipocytes (89) (90), and exists in two isoforms (PPARγ1 and 2) generated by alternative splicing (91), whereof PPARγ2 is shown to be the most adipocyte specific (90). In humans, two isoforms of PPARγ have also been described that are highly expressed in the adipose tissue, however, with a preferential expression of PPARγ1 (92). PPARγ has been shown to heterodimerize and form a functional complex with the retinoid X receptor (RXR), another member of the superfamily of transcription factors (93) (94), and activation of both receptors is necessary to promote maximal activity of the complex (95). In adipocytes, PPARγ2 is highly involved in the transcription of genes promoting fatty acid storage, seen from the high number of target proteins containing a PPRE, including fatty acid binding protein 4 (aP2) (96).

PPARγ is present at low levels in 3T3-L1 preadipocytes, and is induced dramatically during adipocyte conversion (89) (90). By the activation of PPARγ, the preadipocytes stop proliferating and convert to the adipocyte phenotype. Expression and activation of PPARγ is sufficient to trigger the adipocyte differentiation cascade in fibroblasts (96). The importance of PPARγ through loss of function studies has been difficult to study *in vivo* due to the fact that mice with a deletion of the gene for PPARγ die at an embryonic stage, i.e before the development of the adipose tissue. However, using different approaches to overcome embryonic lethality, the absolute requirement of PPARγ for adipocyte differentiation and adipose tissue development was shown also *in vivo* (97) (98). Adipose specific PPARγ deletion resulted in marked adipocyte hypocellularity and caused insulin resistance in adipose tissue and liver (99). The crucial role of PPARγ for adipognesis was also shown *in vitro* by the differentiation of embryonal stem (ES) cells lacking PPARγ, where no adipocyte development could be seen in homozygous ES cells and impaired adipocyte development was seen in heterozygous ES cells (97). I line with C/EBPβ and C/EBPδ being expressed before the onset of PPARγ, the expression of these transcription factors was unaltered. Even though expression and activation of PPARγ is sufficient to trigger the adipocyte differentiation cascade in fibroblasts, a marked synergy is seen when introducing both PPARγ and C/EBPα to the cells (96). Also, the ectopic expression of either transcription factor alone induces the expression of the other, suggesting a cooperative interaction of PPARγ and C/EBPα.

C/EBP

The C/EBP family of transcription factors was the first shown to be involved in adipocyte diffentiation (82) (100). All three members of the C/EBP family of transcription factors, C/EBPα, C/EBPβ and C/EBPδ are involved in the induction of adipocyte differentiation, in a typical temporal pattern. The isoforms also readily form homodimers and heterodimers with one another (101). The expression of C/EBPα is limited to the tissues with high lipogenic capacity such as the adipose tissue and the expression of the two other isoforms mostly corresponds to sites of expression of the alpha isoform. C/EBPα is expressed in the late phase of adipocyte conversion, just before the transcription of most adipocyte-specific genes is initiated, and an increase in the rate of transcription of the C/EBPα gene precedes that of several adipocyte-specific genes whose promoters are transactivated by C/EBPα (100). In contrast to the expression pattern seen for C/EBPα, the beta and delta genes are detected already in proliferating preadipocytes (101) (102). Upon confluence the levels diminish. Directly after induction of differentiation the expression of C/EBPβ and δ are increased transiently, but then decrease again sharply before the onset of C/EBPα accumulation. C/EBPβ is not antimitotic and is able to induce the expression of C/EBPα through binding to its promoter (103). However, a recent study has shown that whereas ectopic expression of C/EBPβ in fibroblasts induces PPARγ, it is incapable of inducing C/EBPα to a significant extent, unless a ligand for PPARγ is provided (104). Thus it seems like the primary target for C/EBPβ stimulation is PPARγ. The importance of C/EBPβ and C/EBPδ is seen in mice where both of these transcription factors have been deleted showing significantly reduced adipose tissue with impaired adipogenesis (105). Both C/EBPα and C/EBPβ are also independently capable of inducing the expression of PPARγ and stimulate adipogenesis in preadipocytes (106). Once transcription of the C/EBPα gene has been activated its continued expression is assured through transcriptional autoactivation of its own gene (100). Expression of C/EBPα is sufficient to induce adiopogenesis in 3T3-L1 preadipocytes without the addition of adipogenic inducers (107). Mice with a deletion of the gene for C/EBPα have a defective development of the adipose tissue (and other organs), which fails to accumulate lipids (108). Fibroblasts from these mice can undergo differentiation through expression and activation of PPARγ, but the adipocytes show several defects, including decreased lipid accumulation, failure to induce endogenous PPARγ and insulin insensitivity (109). This indicates that a cross-regulation between C/EBPα and PPARγ is a key component of the transcriptional control of adipogenesis. Whereas PPARγ is able to promote adipogenesis in C/EBPα deficient cells, C/EBPα has no ability to promote adipogenesis in PPARγ deficient fibroblasts (110). This suggests that these two transcription factors participate in a single pathway in adipocyte differentiation and that C/EBPα is entirely dependent on PPARγ.

SREBP-1c

An additional factor that is induced early during adipocyte differentiation and that converge on PPARγ at a stage downstream of C/EBPβ and C/EBPδ, is SREBP1c. ADD1/SREBP1c is a member of the basic helix-loop-helix leucine zipper family of transcription factors and is associated in adipocyte determination and differentiation (111). SREBP1c is abundantly expressed in adipose tissue and is implicated in adipogenesis. Ectopic expression of SREBP-1c in NIH-3T3 cells enhances the adipogenic activity of PPARγ, whereas the ectopic expression of a dominant negative SREBP-1c has been shown to inhibit preadipocyte

differentiation (112). However, the expression of SREBP-1c alone only leads to a minor induction of adipogenesis, and additional studies have suggested that SREBP-1c contributes to adipogenesis by the production of ligands for PPARγ, thereby facilitating the action of PPARγ (113).

In conclusion this suggests a transcriptional cascade controlling adipogenesis where a transient increase in C/EBPβ and C/EBPδ levels, and possibly also SREBP1c, contributes to initial induction of PPARγ, and possibly also C/EBPα soon after induction of differentiation. PPARγ then activates the transcription of C/EBPα, which in turn activates PPARγ, and itself, in a positive feedback loop (**Fig 2**). The synergistic effect of PPARγ and C/EBPα then drives the process of terminal adipocyte differentiation and are also important for maintaining the differentiated state of the adipocyte. The latter is in agreement with a study showing that PPARγ is required in mature white and brown adipocytes for their survival (114).

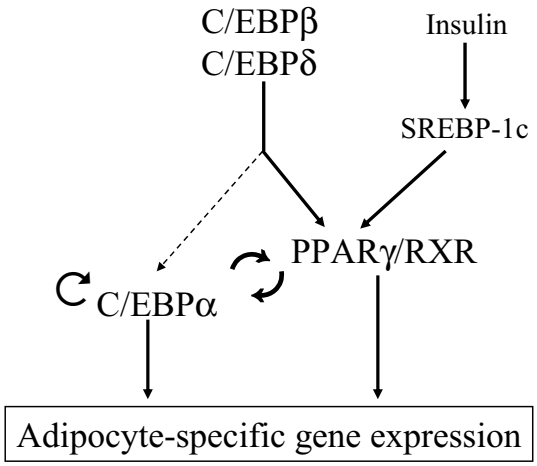

Figure 2 Induction of adipogenesis by a cascade of transcription factors.

The above is a rather simplified view of a complicated process and many other transcription factors and mediators are involved in the complex regulation of adipogenesis. An example is Krox 20, a transcription factor induced early in the adipogenic program, immediately following exposure of cells to mitogens, and that appears to contribute to induction of C/EBPβ expression (115). Another factor, Kruppel-like factor (KLF5), is suggested to mediate the stimulation of C/EBPβ and C/EBPδ on the expression of PPARγ (116). There is further a suggested role for clonal expansion and expression of cell-cycle related proteins in regulating adipogenesis, and it is generally thought that clonal-expansion is a prerequisite for the terminal differentiation into adipocytes (79) (117). One example of a cell cycle protein involved in adipocyte differentiation is the retinoblastoma protein (pRb) that has been shown to stimulate terminal adipocyte differentiation through direct interaction with C/EBPs (118).

Transcriptional coregulators

An increasing number of nuclear cofactors have been identified that are shown to contribute to the regulation of gene expression but also determination of cell fate. These factors do not bind to the DNA directly but participate in the formation of large transcriptionally active (coactivator) or inactive (corepressor) complexes that link transcription factors to the basal transcription machinery. The coregulators work through remodeling of the chromatin. An open chromatin structure allows full activation of transcription. Whereas some coactivators have the ability to directly modify chromatin, (e.g. histone acetyltransferases), others function by the recruitment of chromatin modifiers (119). Corepressors on the other hand, recruit histone deacetylases to target promoters, which block transcription by closing the chromatin structure. In general, coactivators that associate with adipogenic transcription factors are proadipogenic whereas corepressors are antiadipogenic, and only promote differentiation when their levels are decreased. PPARγ appears to be able to interact with several different coregulators, which could help explain how it functions to control the expression of numerous gene programs in the mature adipocyte. The first coactivator to be described was steroid receptor coactivator 1 (SRC-1) that was shown to interact with PPAR in solution (120). Thyroid hormone receptor-associated protein (TRAP) also known as PPAR-binding protein (PBP) is a transcriptional coactivator complex that has been shown to interact with several nuclear receptors through the TRAP220 subunit, including PPARγ2. It has been shown that TRAP220 is important for adipogensis since TRAP220 deleted fibroblasts are a less responsive to PPARγ2-stimulated adipocyte differentiation (121). However, since the absence of TRAP220 did not affect the MyoD-stimulated myogenesis, it is suggested that TRAP220 acts as a PPARγ2-selective coactivator specific for adipogenesis. Examples of corepressors include nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT). A few coregulators that are associated with determination of brown versus white differentiation will be discussed in a separate chapter. Thorough reviews on the transcriptional control of adipocyte differentiation have recently been published (122) (119).

Adipokines

The adipocyte secretes a multiplicity of protein signals and factors with endocrine functions, collectively called adipocytokines or shortly adipokines. This illustrates a new and important role of WAT as a secretory organ, highly integrated in the metabolic control systems and overall physiology. The diversity of the adipokines is considerable and includes cytokines and cytokine-like proteins (leptin, visfatin, TNFα, IL-6), chemokines (MCP-1), growth factors (TGF-β), complement and complement-related proteins (adiponectin and adipsin), proteins involved in vascular homoeostasis (PAI-1), the regulation of blood pressure (angiotensinogen) and lipid metabolism (RBP) and also resistin (123) (65). A majority of the adipokines arise from non-adipocytes within WAT. However, two adipokines that are secreted almost exclusively by the adiocytes are leptin and adiponectin, and the discovery of leptin by Zhang *et al* had a major impact on the recognition of WAT as an endocrine organ (71). A short summary of a few adipokines implicated in metabolic disease follows below. Inflammatory cytokines, e.g. TNFα, IL-6 and MCP-1 will will be included in the part of WAT inflammation.

Leptin

Leptin (from the Greek word leptos, meaning thin) is a polypeptide hormone secreted by the adipocytes, in direct proportion to adipose tissue mass and adipocyte size. Leptin plays an important role in maintaining energy homeostasis, acting both centrally and peripherally to regulate food intake and energy expenditure. The most well known effect of leptin is to decrease appetite by the combined effects of decreasing orexigenic while increasing anorexigenic peptide expression in the hypothalamus region, leading to a powerful suppression of food intake (124). However, *ob/ob* mice lacking functional leptin have a greatly reduced capacity for diet-induced themogenesis, suggesting an important role of leptin in energy expenditure besides regulation of appetite. This is confirmed by leptin administration to *ob/ob* mice, resulting in increased energy expenditure as well as reduced food intake (125), suggested to be the result of increased sympathetic activity on BAT associated with an increase in UCP-1 mRNA (126). Stimulation of WAT lipolysis is coupled to reduction of the adipose tissue mass after leptin administration (124). Administration of leptin in normal rats leads to reduced levels of intracellular TGs in both adipocytes and most importantly in non-adipocytes e.g. β-cells, skeletal muscle and liver (127), and occurs without increased plasma NEFAs or ketones, suggesting increased intracellular FFA oxidation.

In humans, plasma leptin levels are highly correlated with BMI, and obesity is associated with increased plasma levels of leptin in both mice and humans, reversible by weight loss due to food restriction (128). This suggests that obesity is associated with leptin resistance, as reported in obese mouse models (129). Leptin also reverses insulin resistance and complications rising from lipodystrophy in both mice (49) and humans (130). However, the role of leptin in obesity-related insulin resistance is still under debate.

Adiponectin

Adiponectin or adipocyte complement-related protein of 30 kDa (Acrp30) was first described by Scherer *et al*., as a secretory protein found exclusively in differentiated adipose tissue (131). Adiponectin is an abundant plasma protein and the secretion of adiponectin is stimulated by insulin. The mRNA and plasma levels of adiponectin are decreased in obese/diabetic mice (132) and humans (133) and this obesity-associated hypoadiponectinemia is reversed by weight loss (134). Decreased expression of adiponectin correlates with insulin resistance in mouse models (135). The administration of adiponectin decreases insulin resistance by lowering TG content in muscle and liver of obese mice, the most important effects occurring in muscle by the increased expression of proteins involved in fatty acid transport, β-oxidation and energy dissipation, leading to reduced plasma levels of NEFAs. The glucose lowering effects by adiponectin is completely accounted for by a decreased rate of endogenous glucose production, possibly by the inhibition of hepatic gluconeogenic enzymes (136). In humans, adiponectin levels are more correlated to insulin-induced suppression of hepatic glucose production than with β-oxidation (137). On a molecular level, adiponectin has been shown to enhance insulin-stimulated tyrosine posphorylation of IRS-1 and PKB phosphorylation as well as to activate AMPK. These *in vitro* and *in vivo* studies strongly demonstrate the insulin-sensitizing effects of adiponectin, which is further illustrated in mice lacking adiponectin, exhibiting diet-induced insulin resistance (138). Adiponectin also has anti-inflammatory properties and is able to suppress

TNFα secretion from macrophages (139), demonstrating another possible mechanism by which adiponectin increases insulin sensitivity.

Resistin

Resistin (resistance to insulin) is a polypeptide hormone specifically expressed in adipocytes (visceral in particular), whose levels are induced during adipogenesis but suppressed by TZD treatment (140). Resistin levels are increased in plasma from diet-induced and genetically obese mice. Initial studies suggested that resistin had negative effects on insulin action, potentially linking obesity to insulin resistance. However, conflicting studies reporting increased resistin levels after TZD stimulation and decreased levels of resistin in obesity exist (123). Also, resistin is expressed at very low levels, if at all, in human adipocytes. In fact, it appears that in humans, macrophages are the major source of resistin (141). Further studies in humans are needed to confirm a relevant role of resistin in obesity related insulin-resistance in man.

Visfatin

Visfatin was recently reported to be an adipocyte-derived hormone (142). Although not specifically expressed in adipose tissue, visfatin is highly enriched in visceral fat of both mice and humans and is released abundantly into culture medium from 3T3-L1 adipocytes (142) (143). Plasma levels of visfatin are increased during the development of obesity (142). Visfatin was described to have putative antidiabetic properties, with insulin-mimetic effects in cultured cells and a capacity for lowering plasma glucose in both insulin-resistant and insulin deficient mice. A surprising finding was that visfatin binds to and activates the insulin receptor, suggesting a direct regulation of this pathway. However, a recent retraction of the paper by Fukuhara *et al* was made after investigation of the study by the local Committee for Research Integrity. Apparently, not all preparations of visfatin bind to and activate the insulin receptor. The current opinion of the role of visfatin in obesity and insulin resistance is uncertain due to opposing reports.

Retinol binding protein 4

Retinol binding protein 4 (RBP4) is a specific transport protein for retinol in the circulation that was recently discovered to be an adipocyte derived protein capable of impairing insulin action throughout the body (144). Circulating levels of RBP4 are substantially increased in obese and insulin resistant mice and humans and is reversed in mice by TZD treatment. RBP4 is suggested as a circulating factor released by the adipose tissue to negatively affect insulin sensitivity in muscle, thereby sparing glucose to other tissues. This could help explain the paradox that obese and insulin resistant subjects have decreased expression of GLUT4 only in adipocytes, accounting for a relatively small part of glucose uptake.

Although a number of the adipokines were detected over a decade ago, many questions still remain regarding their physiological roles and association to disease. The lack of correlation between studies in rodents and humans suggests species-related differences in the function of at least some of the adipokines. Also, the many contradictory results from studies within the same species, show the complexity of the systems where many of the adipokines are likely to interplay. Additional studies are needed to further understand the physiological roles of the different secretion factors discussed.

White adipose tissue inflammation

Obesity, in particular abdominal, is associated with a state of low-grade chronic inflammation, characterized by abnormal production of proinflammatory cytokines, acute phase reactants and activation of inflammatory pathways. WAT is known to release a number of inflammation-related proteins, including the cytokines TNFα, IL-1β, IL-6, IL-8, and IL-10, monocyte chemoattractant protein-1 (MCP-1), iNOS, C-reactive protein and PAI-1 (65). In obesity the expression and secretion of all of these inflammatory mediators are increased, associated with the development of insulin resistance, atherosclerosis and CVD. A focus on TNFα will be made since this is an extensively studied cytokine, with an important role as an inflammatory mediator in the development of insulin resistance.

TNFα

Tumor necrosis factor α (TNFα) is produced by the adipose tissue and is synthesized as a 26-kDa transmembrane protein, which undergo proteolytic cleavage to yield a 17-kDa soluble TNFα molecule. Since both forms are able to mediate biological response, TNFα is capable of acting both locally and systemically. The multiple effects of TNFα on adipose biology include the ability to alter glucose homeostasis and lipid metabolism, central mechanisms in the development of insulin resistance (145). Effects on adipocyte lipid metabolism include inhibition of fatty acid transport and lipogenesis and stimulation of lipolysis, resulting in increased secretion of NEFAs from the adipocyte. The decrease in fatty acid transport and lipogenesis seems to be mediated by decreased expression of key enzymes for these processes, e.g. LPL, FATP, GLUT 4 and aP2. The increase in lipolysis seems to be mediated by downregulation of perilipin. TNFα negatively affects adipogenesis by downregulating the expression of the key adipogenic transcription factors PPARγ and C/EBPα, and can also be cytotoxic to both adipocytes and preadipocytes (145). These two mechanisms in combination with effects on lipid metabolism could be a way for TNFα to limit adipose expansion and cellular stress.

It is firmly established that TNFα is an important mediator of obesity-related insulin resistance, and direct exposure of healthy subjects and rodents to TNFα induces a state of insulin resistance (145). In obese individuals and rodent models of obesity, TNFα is overexpressed in adipose tissue (146) (147). The levels of TNFα expression strongly correlate with hyperinsulinemia and insulin resistance, and there is a negative correlation between TNFα and adiponectin release from mouse WAT (148). In cultured murine adipocytes TNFα induces insulin resistance by triggering serine phosphorylation of IRS-1, leading to an IRS-1 mediated inhibition of the insulin receptor kinase (149). TNFα has also been reported to interfere with insulin signaling in many other cell types including hepatocytes, fibroblasts and myocytes (145). Weight loss improves insulin sensitivity, and correlates with decreased production of TNFα in humans (147). Deletion of TNFα improves insulin sensitivity in both diet-induced obesity and obesity from leptin depletion, possibly explained by lower levels of circulating NEFA and TG in these mice (150). However, another study where the TNFRs have been deleted, report the opposite results (151). Neutralization of TNFα with an antibody in obese type 2 diabetics had no effect on insulin sensitivity, questioning TNFα as a therapeutic target (152). The possibility exists that TNFα under normal conditions serves to regulate adipocyte size by its own effects on lipid metabolism and by inducing a local insulin resistance making the adipocyte less responsive to the lipid storing actions of insulin. In the abnormal obese state, increasing amounts of NEFAs and TNFα in combination with

adipocyte cell stress provoke an uncontrollable insulin resistance that eventually spreads systemically.

Since very low secretion of TNFα has been reported in mice (146) and none in humans (153), it is possible that systemic inflammation is mediated by other cytokines. Interleukin (IL)-6 is a candidate being abundantly secreted into plasma of mice (148) and humans (153), leading to hepatic insulin resistance in mice (154). One detrimental effect of IL-6 in human obesity could be the stimulation of C-reactive protein. However, beneficial effects of IL-6 are also reported, where infusion of IL-6 in humans increases glucose disposal (155).

Non-adipocyte origin of proinflammatory mediators

Whereas the connection between obesity and insulin resistance, and an increased release of proinflammatory mediators from WAT is firmly established, up until recent years little was known about the origin of the inflammatory signals. When the adipose tissue is separated into an adipocyte and a non-adipocyte fraction (stromal-vascular fraction (SVF)), only a fraction of the proinflammatory adipokines are produced by the adipocyte fraction (148). It is shown that over 95% of TNFα released to the medium in human adipose tissue explants are from non-fat cells present in adipose tissue. Numerous factors involved in inflammatory response are secreted from adipocytes, preadipocytes and macrophages. There is a striking overlap between the biology of adipocytes and macrophages, and many genes that are critical to adipocytes are also expressed in macrophages and have important roles in macrophage biology, e.g. PPARγ that promote differentiation of both adipocytes and monocytes (156). It has been shown that proliferating preadipocytes have phagocytic activity, and express markers of a monocyte-macrophage lineage, traits that are lost when they stop proliferating and start to differentiate into adipocytes (157). In fact, the preadipocyte phenotype is closer to the macrophage phenotype than to that of mature adipocytes, and in a macrophage environment the preadipocytes can be converted to macrophage-like cells (158).

Obesity and macrophage infiltration in WAT

In 2003 two independent studies reported an increased infiltration of macrophages into adipose tissue of obese mice (15) (159) and humans (15) and concluded that this could contribute to the pathogenesis of obesity-induced insulin resistance. Both of these studies started with large-scale gene expression analysis between obese and lean mouse models, and found that the largest class of genes significantly regulated in obesity consists of inflammation and macrophage specific genes in WAT (15) (159). The progressive upregulation of these genes in WAT precedes a dramatic increase in circulating insulin levels. Also, treatment with TZDs downregulates the expression of the inflammatory genes (159). Histological, staining for F4/80, a macrophage specific protein, revealed an increased staining in WAT from obese mice, correlating with both BMI and adipocyte size (15). Further investigations showed that following a HFD, 85% of the F4/80 positive cells in the adipose tissue were bone marrow derived demonstrating a substantial infiltration of macrophages into WAT in obesity. Macrophage infiltration was also seen in obese humans, where it correlated to BMI and adipocyte size. It has been estimated that whereas in lean mice and humans around 10% of the cells in WAT are macrophages, this number increases to nearly 40% in obese humans (15). Also, macrophages are responsible for almost all TNFα and significant amounts of IL-6 expression in WAT from obese mice. Increased infiltration of macrophages

is also seen in adipose tissue that surrounds and infiltrates muscle, and the production of inflammatory cytokines by these macrophages may contribute to the decreased insulin sensitivity of muscle, characteristic of obesity. As a consequence of increased macrophage infiltration, increased amounts of TNFα and other inflammatory mediators are produced by infiltrating monocytes, resident macrophages or converted preadipocytes, amplifying the inflammatory state.

A positive correlation between BMI and number of resident macrophages in human adipose tissue is suggested to involve adipokines, most likely leptin, derived from mature adipocytes (160). Leptin is besides promoting inflammation also shown to activate microvascular endothelial cells that could stimulate monocyte diapedesis and the accumulation of macrophages within adipose tissue. Increased macrophage infiltration into visceral fat is observed in lean subjects and is exaggerated in obesity, particularly in abdominal obesity, demonstrating a possible link between central adiposity and increased risk of developing insulin resistance (161).

MCP-1 and chemotaxis

MCP-1 is an important chemokine that attracts inflammatory cells to the site of inflammation. The gene for MCP-1 is insulin-responsive and remains sensitive to insulin even in insulin resistant states, and high levels of MCP-1 are found in obese mice, a major source being WAT (162). A marked stimulation of TNFα on the production of the chemokines MCP-1 and IL-8 is seen in human adipocytes (163), which is consistent with reports that monocytes infiltrate WAT in obesity. Also, elevated levels of circulating MCP-1 and IL-8 are seen in obese subjects (164). Targeted deletion of MCP-1 reduces macrophage infiltration of fat depots and improves insulin sensitivity (165) whereas overexpression of MCP-1 has the opposite effect (166). Expanding adipocytes and preadipocytes might thus be stimulated to produce and release chemotactic signals that attract macrophages to the WAT.

Inflammatory signaling pathways

Potential cellular mechanisms for the activation of inflammatory signaling include stress/inflammatory kinase c-Jun N-terminal kinase (JNK) and I kappa B kinase beta (IKKβ)/nuclear factor kappa B (NF-κB), both playing important roles in inflammation-induced insulin resistance. JNK has been shown to directly interfere with insulin signaling through the phosphorylation of serine residues on IRS-1 (167). IKKβ on the other hand acts specifically by phosphorylating the NFκB inhibitor Inhibitor κB (IκB) leading to its degradation, in turn liberating the transcription factor NFκB that enters the nucleus and stimulates the gene expression of a majority of mediators involved in inflammatory pathways (45). PKC is another important mediator between inflammatory and metabolic pathways and is implicated as a kinase relaying lipid signaling (168). Most likely, these pathways are activated at the same time in the development of inflammation and insulin resistance, each one relaying signals from specific mediators.

TNFα, NEFAs and endoplasmic reticulum (ER) stress, all of which are increased in the obese state, can activate the JNK signaling pathway and cause insulin resistance. In 3T3-L1 adipocytes, NEFA-induced insulin resistance is suggested to be mediated by the activation of JNK, IKKβ and by the subsequent increased secretion of TNFα (169). By blocking JNK,

most effects of NEFAs on insulin actions were abolished. The JNK activity in adipose tissue, liver and muscle is abnormally elevated in both dietary and genetical (*ob/ob*) obesity in mice (167). Deletion of JNK1 in mice results in decreased adiposity, improved insulin sensitivity and enhanced insulin receptor signaling capacity, through effects on phosphorylation of IRS-1, suggesting JNK as a crucial mediator of obesity and insulin resistance. Mice heterozygous for a deletion of IKKβ are partly protected from HFD and genetically-induced insulin resistance, and inhibition of IKKβ by administration of high concentrations of salicylate or aspirin improves insulin actions in obese rodents (170) and type 2 diabetics (171). Also, a deletion of IKKβ in myeloid cells leads to global protection from insulin resistance, showing a central role for myeloid cells in development of systemic insulin resistance (172). In certain conditions, e.g. accumulation of misfolded proteins and energy and nutrient fluctuations, the ER lumen is perturbed creating stress. The two principal pathways that disrupt insulin action, JNK and IKKβ/NFκB are both activated by ER stress, and in both dietary and genetic obesity ER stress is increased in adipose tissue and liver (173).

Working model of WAT inflammation

A possible working model where the inflammation originates from the adipocyte, and the macrophages serve to amplify the signal is as follows (**Fig 3**). Excess fat accumulation in adipose tissue causes cellular stress of the adipocytes leading to activation of JNK and NFκB (45). Increased amount of NEFAs can also directly activate these signaling systems. The JNK and NFκB signaling pathways regulate protein phosphorylation and transcriptional events that lead to increased adipocyte production of proinflammatory mediators including TNFα, IL-6 and chemokines such as MCP-1. This attracts monocyte-recruitment into the adipose tissue, where the monocytes subsequently differentiate into macrophages and start producing more proinflammatory cytokines and chemokines. This further promotes the local inflammation and also leads to systemic inflammation.

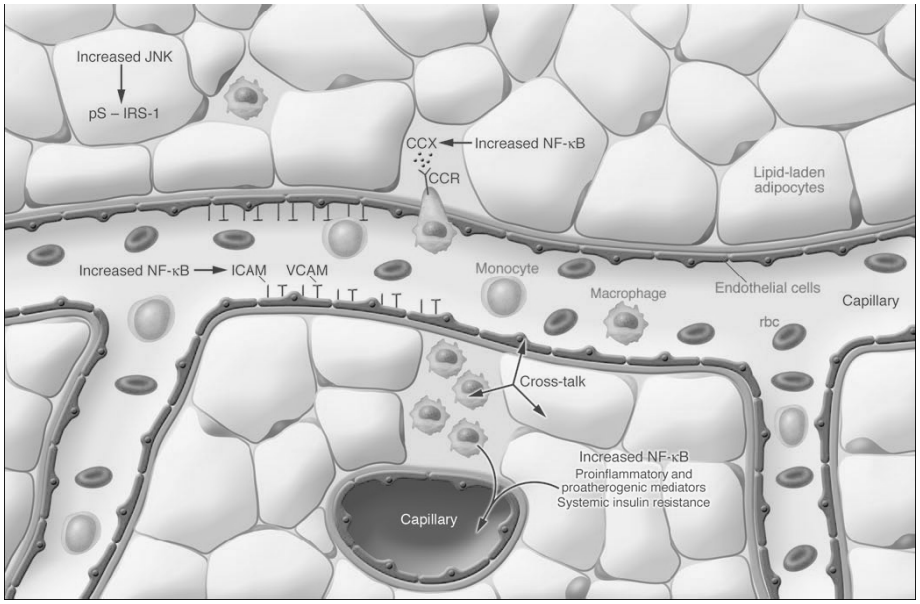

Figure 3 Potential mechanisms for activation of inflammation in WAT (45).

BROWN ADIPOSE TISSUE

Morphologically, brown adipocytes have multilocular lipid droplets and contain less overall lipid than white adipocytes, and are particularly rich in mitochondria (3). The high density of mitochondria, containing colored cytochrome pigments that participate in cellular respiration, together with a very rich blood supply, is what gives the tissue its brown color. Although BAT is widespread in the human fetus and surrounds the heart and great vessels in infancy, it disappears shortly after birth and in adults only small amounts are present, dispersed within the WAT. In rodents however, a distinct brown fat pad remains in adult years, which lies in the interscapular region. Brown adipocytes are found only in mammals and express uncoupling protein 1 (UCP-1, thermogenin), a protein expressed exclusively in brown adipocytes. UCP-1 dissipates the proton gradient across the inner mitochondrial membrane generating heat at the expense of ATP. BAT generates considerable heat through this process of nonshivering thermogenesis, which helps to maintain proper body temperature in the newborn, and in hibernating rodents. The heat generated is transported by the extensive blood supply to other tissues. It has been estimated that BAT accounts for at least 60% of nonshivering thermogenesis in rat (174). However, the contribution of BAT to total energy balance in humans is largely unexplored. Mice with a genetic ablation of BAT develop obesity without hyperphagia, demonstrating a crucial role for BAT energy dissipation in the control of body weight in this species (175).

Thermogenesis and sympathetic control

BAT is highly innervated by the sympathetic nervous system that regulates many functions of this tissue (3). Norepinephrine (NE) controls the thermogenic process through binding of β_3-adrenergic receptors stimulating the activation of UCP-1 mainly through activation of p38 MAPK (176) ultimately leading to increased uncoupling and heat production (3). However, NE can also regulate the expression of the UCP-1 gene. Inhibition of thermogenesis is regulated by signaling through inhibitory α_2-adrenergic receptors. In addition, NE also stimulates proliferation of brown preadipocytes and differentiation of mature brown adipocytes. Feeding increases, and starvation and caloric restriction inhibits adaptive thermogenesis, showing the regulatory function of diet on this process. Besides BAT, skeletal muscle is also a major organ involved in adaptive thermogenesis, although in muscle this is accomplished through shivering thermogenesis, resulting from involuntary muscle contractions. When mice are subjected to acute cold, initial thermogenesis results from shivering. After acclimatization, adaptive nonshivering thermogenesis occurs stimulated by NE. Nonshivering thermogenesis is entirely dependent on brown fat, during which BAT is the major oxygen-consuming organ. Also, adaptive nonshivering thermogenesis in response to cold is entirely UCP-1 dependent, seen in studies where the gene for UCP-1 has been deleted (177). UCP-1 deficient mice can still acclimatize to cold, but only through the process of shivering thermogenesis. Also, NE and FFA induced thermogenesis is fully UCP-1 dependent in brown fat cells (178). Another potent stimulator of thermogenesis in BAT is thyroid hormone acting through its receptor (TRβ) to increase mitochondrial respiration rates. Unexpectedly, UCP-1 deficient mice do not develop hyperphagia or obesity, the latter possibly resulting from compensatory upregulation of the ubiquitously expressed UCP-2, seen in these mice (179). The adaptive thermogenic program in both BAT and muscle involves the stimulation of mitochondrial biogenesis, expression of the mitochondrial enzymes of the respiratory chain, increased fatty acid oxidation and the uncoupling of oxidative phosphorylation (180).

Brown adipocyte differentiation

Relatively little is known about the determining factors driving differentiation towards the brown adipocyte lineage. The transcriptional cascade of PPARγ and C/EBPs has been shown to be important also for brown adipogenesis. Although C/EBPα is not required for differentiation of BAT (181), mice with a deleted C/EBPα gene have decreased expression of UCP-1, indicating a role for C/EBPα also in the brown adipogenesis (108). C/EBPβ and C/EBPδ are also important for brown adipogenesis seen in mice where both of these transcription factors have been deleted showing significantly reduced accumulation of lipid droplets and UCP-1 levels in brown adipocytes (105). The levels of PPARγ and C/EBPα were normal however, showing that these factors are not solely regulated by C/EBPβ and δ, but also that expression of PPARγ and C/EBPα is insufficient to complete brown adipocyte differentiation in the absence of C/EBPβ and δ. Similar to white adipocytes, brown adipocytes are also under transcriptional control by PPARs. However, unlike white adipocytes brown adipocytes also express PPARα. The level of PPARα rises dramatically during differentiation of brown adipocytes (182). The amount of PPARγ being particularly high in brown adipocytes, is high already in undifferentiated proliferating brown preadipocytes, and increases even more during differentiation, paralleled with an increase in C/EBPα. Although not crucial for brown adipocyte differentiation or UCP-1 expression (99),

PPARγ is still likely to be important for brown adipogenesis (183), and for the survival of mature brown adipocytes (114). A PPARγ response element (PPRE) has been found in the enhancer of the UCP-1 gene (184), and PPARγ binding to this PPRE of UCP-1 is seen in BAT of cold-exposed mice. However, forced expression of PPARγ and RXRα is only able to activate a UCP-1 reporter in brown adipocytes and not in fibroblasts, suggesting that additional cofactors exist in brown adipocytes, required for UCP-1 expression. This factor is now identified as PGC-1α, a coactivator, which is dramatically induced upon cold exposure and β-adrenergic stimulation of mice in both BAT and muscle, the two tissues responsible for adaptive thermogenesis (185).

PGC1 functions in BAT

Phosphorylation of PGC-1α by p38 MAPK results in an increased stability and transcriptional activity (186). PCG-1α greatly increases the transcriptional activity of PPARγ and TRβ on the UCP-1 promoter indicating a key role in linking nuclear receptors to the transcriptional control of thermogenesis. However, PGC-1α is not only involved in adaptive thermogenesis and is now more generally viewed as an activator of a more oxidative phenotype regardless of tissue, promoting mitochondrial biogenesis, that based on cell type have different outcomes (183). Ablation of PGC-1α in mice leads to a decrease in mitochondrial fatty acid oxidation and respiratory chain enzymes in BAT (187). Also, these mice have a seriously decreased ability to increase UCP-1 expression in response to cold, showing a crucial role of PGC-1α for adaptive thermogenesis in brown adipocytes, however with preserved basal UCP-1 levels. No compensatory increase in PGC-1β was seen. Unexpectedly, the mice are lean and resistant to diet-induced obesity due to a compensatory hyperactivation. It should be mentioned that an independent study of PGC-1α deleted mice did not show an altered phenotype in BAT (188). PGC-1α is expressed at high levels in brown adipocytes (unlike white adipocytes) and can be further induced by NE stimulation (183). Despite its name (PPARγ coactivator), PGC-1α is not specific for PPARγ, but also mediates the effects of many other transcription factors including PPARα, that collectively participate in energy balance (189). PPARα also binds the PPRE on the UCP-1 enhancer and induces its gene expression (190), demonstrating a role for PPARα-stimulated UCP-1 regulation. Also, the UCP-1 inducing effects of PPARα are enforced by PGC-1α. PGC-1β is the second PGC-1 isoform to be discovered and has similar tissue distribution as the alpha isoform, i.e. high levels in BAT and heart, tissues rich in mitochondria (191). Unlike PGC-1α, PGC-1β has no ability to stimulate cold induced thermogenesis in brown adipocytes. However, during differentiation of brown adipocytes, a rise in PGC-1β parallels a decrease in PGC-1α. Stimulation with forskolin rapidly induces the expression of PGC-1α and UCP-1, while decreasing PGC-1β levels. This implies that PGC-1α and β are likely to have distinct functions both in brown adipogenesis and in the mature adipocyte. A recent study has shown that brown preadipocytes lacking PGC-1α have unaltered adipogenic capacity, but have severely reduced induction of thermogenic genes (192), showing that PGC-1α is not essential for brown adipogenesis. The expression of UCP-1 can still be induced in cells lacking PGC-1α, by retinoic acid and insulin, indicating that induction of UCP-1 is not entirely dependent upon PGC-1α. Deficiency of both PGC-1α and PGC-1β causes a total loss of differentiation-linked mitochondrial biogenesis and mitochondrial respiration. Thus, it seems likely that other factors function upstream of PGC-1α to control the determination of brown fat cells.

Ectopic expression of PPARγ and C/EBPα in fibroblast as a default leads to formation of adipocytes with the characteristics of white fat cells, having little or no UCP-1 expression. This suggests that the involvement of transcriptional cofactors play an important role in determining the brown adipocyte cell fate. Several possible cofactors have been described including, PGC-1α (185), SRC-1 and TIF-2 (193), pRb (194) and RIP140 (195) that seem to be involved in the determination of brown versus white cell determination. These cofactors will be discussed in more detail in a following chapter.

Brown adipocytes are in many ways more similar to myocytes than to white adipocytes. They contribute to energy expenditure and have high oxidative capacity due to an abundance of mitochondria, with high expression of PPARα and PGC1s, activators of fatty acid oxidation and mitochondrial biogenesis, respectively. The recent finding of an interlinkage between the myocyte and the brown preadipocyte, suggests a distinct origin of the brown versus white adipose tissue (72). This could explain why brown adipocytes much like myocytes ultimately specialize in lipid catabolism, rather than storage.

TRANSCRIPTIONAL REGULATORS

Little is known about the regulatory systems controlling white versus brown adipocyte differentiation. Since the key adipogenic regulators in white adipogenesis i.e. PPARγ and C/EBPs, seem to play similar roles also in brown adipocyte differentiation (84), the coregulators of the transcription factors involved in adipogenesis seem to play important roles. Several studies using genetically modified mice have made valuable contributions to our understanding of coregulators involved in brown versus white adipogenesis. A few important and relevant coregulators and factors will be mentioned here.

FOXC2

Although not a transcriptional cofactor, forkhead transcription factor (FOXC2) is still mentioned in this part due to its ability to affect adipocyte phenotype. FOXC2 is a human winged helix/forkhead transcription factor gene, expressed in both WAT and BAT. Mice overexpressing FOXC2 in WAT and BAT have reduced intra-abdominal WAT depots with acquirements of a brown fat-like histology and an induction of UCP-1 and PGC-1α, whereas the interscapular BAT (iBAT) is hypertrophic (196). The mice are further lean and resistant to diet-induced obesity. FOXC2 can be induced by a HFD in WT mice. Disruption of FOXC2 in mice leads to decreased iBAT cell mass. These observations underscore the important role of this transcription factor in regulating adipocyte metabolism with the ability to alter metabolic efficiency in response to dietary changes. FOXC2 affects adipocyte metabolism by increasing the sensitivity of the β-adrenergic PKA signaling pathway, which maintains the BAT phenotype. This effect is mediated by stimulating the expression of RIα, a regulatory subunit of PKA with high affinity for cAMP. This renders the cell more susceptible to PKA mediated phosphorylation and activation of MAPK, in turn activating PGC-1α. However, the physiological role of FOXC2 is still not fully understood.

PGC1

The PGC-1 (PPARγ coactivator 1) coactivators include PGC-1α, PGC-1β and PRC (PGC-1-related coactivator). Important roles of PGC-1 coactivators include regulation of

mitochondrial oxidative metabolism and the maintenance of glucose, lipid and energy homeostasis (189). Effects of PGC-1α in BAT have been described above (see BAT chapter). A mechanism for PGC-1α mediated mitochondrial biogenesis was presented in myoblasts, where PGC-1α acts through induction of the nuclear respiratory factor (NRF) 1 and NRF-2α/GABPα (GA-binding protein α) genes, but also through binding and coactivation of NRF-1 to the promoter for mitochondrial transcription factor A, a direct regulator of mitochondrial DNA replication/transcription (197). To remodel chromatin and initiate transcription, PGC-1α recruits proteins with histone acetyltransferase activity (HAT) including cAMP responsive element binding protein (CREB) binding protein (CRB) CRB/p300 and SRC-1 (198). The docking of PGC-1α to PPARγ leads to a conformational change in PGC-1α that permits binding of SRC-1 and CBP/p300, resulting in transcriptional activity.
Ectopic forced expression of PGC-1α in white adipocytes from mouse (185) and humans (199), normally containing limited amounts of PGC-1α, has major effects on the induction of a brown adipocyte phenotype in these cells. It induces the expression and activity of UCP-1, increases several key mitochondrial enzymes of the respiratory chain and increases mitochondrial DNA. This leads to an increased fraction of uncoupled respiration in these cells, a key characteristic of brown adipocytes. Conversely, BAT in mice lacking PGC-1α appears morphologically abnormal, with abundant accumulation of large lipid droplets and a decreased function (187). However, a recent study has showed that PGC-1α is not essential for brown adipogenesis, and that it is mainly the expression of genes involved in thermogenesis that are defective (192). Thus it is highly probable that the morphological changes in brown fat of PGC-1α deficient mice are due to decreased lipid oxidation and not due to a defect in brown adipogenesis.

p160/SRC family

The first coactivator to be described was the steroid receptor coactivator 1 (SRC-1) that was shown to interact with PPAR in solution (120). SRC-1 belongs to a protein family, currently consisting of three members, SCR-1, SRC-2 and SRC-3, that are widely expressed and coactivate many if not the majority of nuclear receptors (200). Of the SRC members, SRC-1 and SRC-3 possess intrinsic HAT activity and can thus directly remodel the chromatin structure (201). The SRCs usually also recruit other coactivator proteins with HAT activity, such as CBP/p300 to a larger complex where all cofactors participate in chromatin modification. Mice where the gene for SRC-1 has been deleted show partial resistance to steroid hormones and a reduced growth and development of various steroid target organs (202).
Deletion studies have illustrated the importance of a correct balance between SRC-1 and SRC-2 (also known as transcriptional intermediary factor 2 (TIF2)) in the regulation of transcription. Lack of TIF2 facilitates the interaction between PGC-1α and SRC-1 in BAT which promotes thermogenesis, leading to increased energy expenditure and resistance to diet induced obesity in these mice (193). TIF2 deleted mice are lean, contain less WAT and have a more active BAT, containing increased levels of UCP-1 and PGC-1α. Mice with a deletion of SRC-1 in contrast, have reduced energy expenditure and are prone to obesity. TIF2 deleted MEFs have impaired adipogenic potential and in 3T3-L1 cells, a forced expression of TIF2, unlike SRC-1, enhanced adipogenesis. Interestingly different effects of TZDs on PPARγ recruitment can be seen where roziglitasone induces a better interaction between PPARγ and

TIF2 than between PPARγ and SRC-1, whereas another TZD, FMOC-L-leucine promotes recruitment of SRC-1 (203). HFD induces the changes in TIF2/SRC-1 ratio only in WAT and BAT showing the adipose tissue specificity for this regulatory system. An altered balance of TIF/SRC-1 could be of relevance to the pathogenesis of obesity. High-energy intake increases TIF2 in WAT leading to fat accumulation, whereas an increased TIF2/SCR-1 ratio in BAT leads to reduced thermogenesis. This leads to inability to compensate for higher energy intake by adaptive thermogenesis, promoting development of obesity and insulin resistance.
PKA, mimicking the effects of β-adrenergic stimulation, directs TIF2 to proteasome degradation (204), which enhances the activity of the PPARγ/PGC-1α complex. Recently it was shown that a double knockout of SRC-1 and p/CIP resulted in a defective development of BAT, occurring early in development, but after the induction of PPARγ (205). Also, the effect on gene expression was BAT specific and mainly UCP-1 was downregulated. Decreased binding of PPARγ and increased repressor binding in the form of RIP140 and NCoR to the UCP-1 promoter was shown. A decreased adaptive thermogenesis is however compensated with an increased basal metabolic rate and higher physical activity of the mice keeping them lean.

pRb family

The retinoblastoma protein (pRb) is encoded by the retinoblastoma gene (Rb) and belongs to a family of pocket proteins, which also include p107 and p130 (206). pRb, is widely known as a tumor suppressor protein, regulating entry into the cell cycle. These proteins control cell cycle entry by interrupting the transcriptional signaling from E2Fs, key transcription factors that regulate the levels of many genes involved in cell cycle regulation and progression. Pocket proteins can either directly inhibit the positive transcriptional activity of bound E2F factors, or recruit transcriptional repressor complexes to the promoters of T2F target genes. pRb has been shown to directly interact with histone deacetylase. The inhibiting effect of pRb is relieved upon phosphorylation by specific cyclin-dependent kinases triggering a release of pRb from E2F.
However, retinoblastoma protein is shown to positively regulate terminal adipocyte differentiation of mouse embryonic lung fibroblasts through direct interactions with C/EBPs (118). Rb negative cells were unable to differentiate unless ectopic expression of normal Rb was administered. Rb also enhanced the transactivation of a C/EBPβ-responsive promoter in cells. This shows a direct and positive role for Rb in terminal adipogenesis, which actually is in contrast with its function in arresting cell cycle entry, since preadipocytes upon adipogenic stimulation reenter the cell cycle for a clonal expansion before permanent cell cycle removal and differentiation. It was subsequently shown that the defective differentiation seen in Rb negative fibroblasts can be bypassed by administration of a PPARγ ligand or by suppression of ERK1/2 activation (207) (208).
Another major finding is that pRb regulates brown versus white adipocyte differentiation and that functional inactivation of pRb in mouse embryo fibroblasts (MEFs) and in white preadipocytes results in the expression of UCP-1 (194). It was further shown that Rb gene deficient MEFs and stem cells differentiate into adipocytes with brown characteristics. These MEF cells also increased FOXC2 and its target gene PKA regulatory subunit RIα, resulting in increased cAMP sensitivity, and PKA inhibition blocked the brown adipocyte-like gene expression. In normal cells, pRb is present in the nuclei of white but not brown

preadipocytes, at a stage when the cells start to accumulate lipids and brown adipocytes express UCP-1. It thus appears that pRb has opposing roles in white and brown adipocyte differentiation, promoting white and inhibiting brown adipogenesis. Also, pRB rapidly undergoes phosphorylation and inactivation in response to cold, resulting in upregulation of UCP-1 and induction of neodifferentiation in BAT. A transdifferentiation of white into brown adipocytes, seen by a multilocular appearance, in response to β3-adrenergic stimuli is shown as a result of downregulation of pRb expression. A proposed model for brown adipocyte development in the absence of Rb is that an increase in FOXC2 early in differentiation, results in sensitization of the cells to cAMP which activates CREB, in turn assisting in the elevation of PGC-1α (209) which coactivates BAT conversion. This establishes pRb as an inhibitor of brown adipocyte differentiation and as a molecular switch involved in adipocyte lineage determination *in vitro*.
It was recently shown that WAT mass is severely decreased whereas the iBAT mass is unchanged in mice where the gene for p107 has been deleted (210). The white adipocytes are multilocular and contain less lipids, they have more mitochondria and express high levels of UCP-1 and PGC-1α, all features of brown adiopocytes. There was a markedly increased number of preadipocytes in WAT depots suggesting that p107 is important for white adipogenesis. However, when culturing p107 deficient preadipocytes in vitro, there was no decreased adipogenic potential. The finding that preadipocytes isolated from WAT depots contain severely decreased levels of pRb, suggests that a lack of pRb is the cause of the reduced adipogenic potential in these cells. This was confirmed when a cre-mediated deletion of Rb in adult primary preadipocytes blocked white adipogenesis. An important finding was that pRb was shown to bind the PGC-1α promoter and repress transcription. This demonstrates an *in vivo* role for pRb and p107 in the control of differentiation into white or brown adipocytes, through repression of PGC-1α.
A recent study has shown that mice with a specific inactivation of Rb in adult adipose tissue, show resistance to HFD-induced obesity due to increased energy expenditure, by the activation of mitochondrial activity in white and brown fat, confirming a role for pRb in determination of adipocyte cell fate *in vivo* (211).
In conclusion, pRB and p107 are potent regulators of white versus brown adipocyte differentiation both *in vitro* and *in vivo*, most likely through the regulation of PGC-1α.

RIP140

Receptor interacting protein (RIP140) is a nuclear corepressor that was first identified and characterized as a hormone dependent estrogen receptor interacting protein (212). It is an unusual corepressor due to the fact that it is recruited to most nuclear receptors in a ligand-dependent manner (213). RIP140 seems to work mainly as a scaffold protein that links nuclear receptors to chromatin remodeling enzymes involved in chromatin condensation leading to transcriptional repression. Depending on cell type, the expression of RIP140 may be regulated by a variety of hormones including retinoic acid (214). It has a wide tissue distribution, but is often restricted to specific cell types within the tissues. High levels of RIP140 are seen in metabolic tissues including liver, muscle and WAT, whereas BAT levels are lower. RIP140 deleted mice are viable, but females are infertile (215). The RIP140 mice are lean and show increased resistance to diet-induced obesity, with highly decreased body fat content, in particular the subcutaneous (195). The decrease in fat mass is due to decreased triacylglycerol storage in the adipocytes, as the number of adipocytes is unaltered. The lean

phenotype could not be explained by decreased food intake or increased physical activity since neither of these parameters were altered. No increase in ectopic fat accumulation was obvious, but rather a protection from diet-induced hepatic fat accumulation could be seen, with normal insulin sensitivity. Although the process of adipogenesis was unaltered, the expression of certain lipogenic enzymes was decreased. These findings suggested an increased energy expenditure of the mice, and accordingly, the expression of genes involved in mitochondrial β-oxidation, e.g. carnitine palmitoyl-transferase 1b (CPT1b) was upregulated in RIP140 deleted mice together with a massive increase in UCP-1 expression. Surprisingly there was no increase in PGC-1α mRNA expression, and the levels of TIF2 and SRC-1 were unaltered.
Cells devoid of RIP140 show elevated energy expenditure, seen by an increase in β-oxidation, and express high levels of UCP-1 and CPT1b, similar to what has been seen *in vivo* (216). Conversely, these changes are abrogated by the reexpression of RIP140. Analysis of the UCP-1 promoter showed RIP140 recruitment to a key enhancer element, demonstrating a direct role for RIP140 in repressing gene expression. Consequently, by reducing the levels of RIP140 or preventing its recruitment to nuclear receptors, energy expenditure in adipocytes can be regulated. A recent study supported the role of RIP140 as a major suppressor of adipocyte oxidative metabolism and mitochondrial biogenesis and further showed a role of RIP140 as a negative regulator of whole-body glucose tolerance and energy expenditure in mice (217). It should finally be mentioned that RIP140 is one of the few regulators that induce UCP-1 without affecting the levels of PGC-1α. In the RIP140 deleted mouse it appears that an absence of RIP140 allows ectopic activation of UCP-1 by low levels of PGC-1α (possibly PGC-1β) in WAT.

Several other coregulators and factors besides the few dealt with here have been implicated in the complex network regulating adipocyte determination. For a more complete overview of the area, a recent thorough review dealing with regulatory circuits controlling white versus brown adipose tissue by Hansen and Kristiansen is available (218).

PLASTICITY OF THE ADIPOSE TISSUE

Two key components of energy expenditure are physical activity and adaptive thermogenesis. With a constant increase in the prevalence of obesity a highly interesting area of research would be how to increase non-exercise related energy expenditure in the body. In mice, the thermogenic and antiobesity properties of BAT are conclusively established. However in adult humans, the role of BAT for energy expenditure is less clear. And the question is if white fat storing cells can be transdifferentiated into brown fat. Expression of UCP-1 in WAT of adult humans has been reported suggesting the occurrence of brown adipocytes in white depots that can be recruited in response to adrenergic stimulation (219). However it is only in infants or adults with diseases like pheochromocytoma (a disease leading to overproduction of catecholamines from the adrenal medulla) that show a well developed BAT. It has been estimated that only about 1 in 100-200 adipocytes in a human visceral depot express UCP-1, but the functional consequences of this in the context of energy expenditure is largely unknown (220).

Since WAT mainly starts developing after birth when brown adipose tissue is already present, the possibility of a conversion of brown into white adipocytes exists. However, it has been shown that most white adipocytes do not derive from brown adipocytes during postnatal development (221). Also, preadipocytes from SVF of WAT and BAT develop mainly into white and brown adipocytes, respectively (222). This would argue against a brown to white transdifferentitation.

A mutation in the ligand binding domain of PPARγ reduces UCP-1 expression in "minor" BAT depots such as inguinal and epididymal, whereas UCP-1 expression in the "major" interscapular BAT depot was unaltered (223). This suggests that PPARγ has a more important effect on UCP-1 expressing cells in white depots and that the UCP-1 expressing cells differ based on location. UCP-1 gene expression can be induced with PPARγ agonists in both mesenteric and subcutaneous fat whereas UCP-1 in interscapular depots is not affected (224). However, it is not known whether it is genuine white adipocytes that transform into brown adipocytes of if it is resident brown adipocytes that are stimulated to increase the expression of UCP-1.

In all larger mammals, there is a regional difference in UCP-1 expression with the highest level of expression in visceral adipose tissue, especially perirenal. There is also a regional difference of UCP-1 mRNA levels in fat tissues between mouse strains after stimulation of adrenergic signaling by cold exposure or treatment with a β3 receptor agonist, especially in retroperitoneal WAT (225). If this regional difference is also seen between humans, there might be a large inter-individual difference in thermogenic capacity in WAT depots. Also, this suggests that the appearance of brown adipocytes is under genetic control. It was further shown that a high capacity to induce brown fat in traditional white fat correlates with the loss of weight in response to treatment with a β3-adrenergic agonist. There was no effect on weight gain. This stresses an important issue that an increase in UCP-1 protein levels does not necessarily reflect or lead to increased UCP-1 activity, and high expression of UCP-1 provoked by PPARγ stimulation does not automatically have any metabolic effect (226). In these studies it was only after sympathetic stimulation that the increased thermogenesis was revealed.
A study has suggested that a few multilocular fat cells in WAT after chronic β3-adrenergic stimulation of rats derive directly from white adipocytes (227). However, only a small fraction of the multilocular cells contained UCP-1. A suggestion was made that the multilocular cells had two origins, non- UCP-1 expressing cells originated from transdifferentiation of white adipocytes whereas UCP-1 expressing cells arise from another cell type, giving rise to a more brown phenotype. This would provide some evidence that white adipocytes can acquire characteristics of brown in conditions of cold exposure or catecholamine excess. The presence of UCP-1 independent thermogenesis in WAT has been suggested by the fact that activation of β_3-adrenergic receptors increase metabolic rate and body temperature in UCP-1 deficient mice (228).

If it would be possible to convert white energy storing adipocytes into energy consuming brown adipocytes this would be of possible significance in the efforts to combat obesity. Many models have now demonstrated plasticity of white and brown adipocyte development, and by regulating the expression of certain cofactors, it is possible to transform brown

adipocytes to white and vice versa. One example is the overexpression of PGC-1α in white preadipocytes, leading to a much "browner" phenotype of the adipocyte (185). This PGC-1 overexpression was subsequently repeated in human subcutaneous white adipocytes, with the results of increased BAT characteristics in these cells (199). The coordinated regulation of gene expression was functionally reflected in increased palmitate oxidation of these cells. Rosiglitazone, a PPARγ agonist further stimulated UCP-1 expression and palmitate oxidation in these cells. Injection of PGC-1α adenovirus in white fat of mice induced UCP-1 expression in this tissue, demonstrating an effect *in vivo*. A question is if there is a physiological significance of this. Another agent highly capable of inducing UCP-1 expression and a more oxidative metabolism in white adipocytes both in vitro (229) and in vivo (230) is retinoic acid.

Adenovirus-induced hyperleptinemia in rats promotes fat depletion and profoundly downregulates PPARγ and lipogenic enzymes in epididymal fat (231). A simultaneous increase in the expression of PPARα and enzymes involved in fatty acid oxidation can be seen, together with increased UCP-1 levels. The transformation into a more oxidizing cell is accompanied by loss of adipocyte differentiation markers and by the appearance of a preadipocyte marker, suggesting that leptin promotes a less differentiated state of the adipocyte. The same approach by another group found small fatless adipocytes crowded with mitochondria that were much smaller than those of brown adipocytes (232). There was a striking upregulation of PGC-1α, increased UCP1 and fatty acid oxidation and a downregulation of lipogenic enzymes. The ability to transform adipocytes to fat-burning cells was suggested as a novel therapeutic strategy.

All in all, it appears that some form of plasticity exists between white and brown adipocytes. However, the extent of this plasticity and the molecular mechanisms governing it remain largely unknown.

HORMONE-SENSITIVE LIPASE

Hormone-sensitive lipase (HSL) was for a long time regarded as an enzyme specifically expressed in adipose tissue, controlling the rate limiting step in hormone-stimulated lipolysis. However, current models put HSL in a much wider perspective as a multifunctional enzyme that is expressed in several tissues capable of hydrolyzing a wide range of substrates.

Tissue expression and isoforms

HSL has quite the opposite to previous views been shown to have a wide distribution in mammalian tissues, and although the highest levels are found in WAT (233), HSL is also expressed and active in BAT (234), heart and skeletal muscle (233) (235) (236), macrophages (237) (238) (239), intestinal mucosa (240), steroidogenic tissues including adrenals, ovaries and testis (233) (241) and pancreatic β-cells (242).
HSL exists in three active isoforms differing in size from 84-130 kDa. All isoforms arise from the same gene through the use of alternative promoters and alternative splicing. Sequences encoded for by exons 1-9 are common to all isoforms. The most common one is the 84 kDa isoform (243), highly expressed in adipocytes and also in most other tissues that display HSL expression. A slightly larger isoform of 89 kDa was found in the pancreatic β-

cell, that also express 84 kDa variant (242). Compared to the 80 kDa isoform, the 89 kDa isoform contains an N-terminal addition of 43 amino acids transcribed from a specific exon A, and is also found in other tissues such as adipose tissue, adrenal glands and ovaries (244). A large testis-specific isoform of HSL exists with a size of 130 kDa is generated through the use of the specific exon T (241).

Substrate specificity

Compared to other lipases, HSL has a unique ability to hydrolyze a vide variety of substrates. Besides the commonly described ability to hydrolyze all forms of acylglycerols (TG, DG and MG) (245), HSL is also able to hydrolyze cholesteryl esters (245) (246), steroid esters (247), para-nitrophenyl esters (248) and retinyl esters (249).
Adipocyte lipolysis is the sequential breakdown of TG to FFA and glycerol via generation of the two intermediary forms DG and MG. The first reaction (TG to DG) is the slowest and constitutes the rate limiting step. Although HSL has the capacity to completely hydrolyze TG *in vitro*, the final step in the reaction (MG to FFA and glycerol) is catalyzed by monoacylglycerol lipase (MGL) *in vivo*, whose activity for MGs is much greater than that of HSL. The presence of both HSL and MGL are thus required *in vivo* for the complete degradation of TG (250). HSL has for a long time been considered as a TG lipase and the rate-limiting enzyme in adipocyte lipolysis. However, when comparing the relative maximal rates of hydrolysis *in vitro*, in purified non-phosphorylated HSL, it is clear that HSL has a strong preference for the hydrolysis of DGs (245). It has further been shown that mice lacking HSL, have an accumulation of DG in both adipose tissue and muscle (251), suggesting that the main role of HSL in lipolysis is to function as a DG lipase. Considering the wide tissue distribution and the broad substrate specificity it is likely that HSL has different main functions in different tissues.

Regulation by reversible phosphorylation

Adipocyte lipolysis is acutely regulated by hormones, neurotransmitters and other affector molecules, and HSL is one of the major targets of this regulation (252). A key feature of HSL is its regulation by reversible phosphorylation, which mediates its activation by lipolytic stimuli. The adipose tissue is innervated by the sympathetic nervous system and in states of increased energy demand, lipid mobilization is stimulated by the release of the catecholamine noradrenaline, triggering lipolysis and the release of fatty acids and glycerol into the plasma through binding of β-adrenergic receptors expressed on the surface of the adipocyte. The stimulation of fat cell lipolysis by catecholamines involves activation of three β isoforms (β_1, β_2 and β_3) (253). Binding of any of the β-receptors, leads to activation of adenylyl cyclase (AC), mediated by a stimulatory G protein, resulting in an increase in intracellular cAMP levels. This in turn activates protein kinase A (PKA) which phosphorylates and activates HSL. It was initially thought that the only effect of PKA phorphorylation of HSL was to increase the lipolytic activity of the enzyme. Although this is true when stimulating purified HSL, the effect of HSL activation in cells is much greater and also more complex. Whereas HSL is found almost exclusively in the cytosol of the adipocyte under basal conditions, upon phosphorylation and activation by PKA, HSL translocates from the cytosol to the surface of the lipid droplet, which is suggested to be the most critical event for HSL mediated lipolysis (254). The translocation of HSL involves the protein perilipin, which is another main target for PKA mediated phosphorylation in lipolytic signaling, besides HSL. In the basal state,

perilipin is located on the surface of the lipid droplet where it is suggested to function as a physical barrier, suppressing basal lipolysis by protecting the droplet from cytosolic lipases. When perilipin is phosphorylated by PKA it allows access for HSL to its substrate. From studies in mice where perilipin has been deleted it has been suggested that perilipin is not only involved in the suppression of basal lipolysis, but that it is also important for stimulated lipolysis (255). This is supported by a study showing that phosphorylation and activation of perilipin is essential for the translocation of HSL to the lipid droplet to occur, during lipolytic activation (256). In the postprandial state, increased levels of insulin prevents the phosphorylation and activation of HSL by causing a reduction of the cAMP levels, which inhibits PKA activation of HSL and perilipin (252). In adipocytes this is mainly the result of insulin-mediated activation of phosphodiesterase 3B, which catalyzes the breakdown of cAMP (257). The identity and the regulation of the phosphatases responsible for dephosphorylation and inactivation of HSL, are incompletely understood. It is suggested that whereas acute activation of lipolysis, via HSL and perilipin phosphorylation, is mediated mainly by catecholamines acting on the β-adrenergic receptors, the most important factor stimulating lipolysis after an overnight fast, is the progressive removal of insulin inhibition (70).
PKA-mediated phosphorylation and activation of HSL in rat adipocytes occurs at three serine residues, Ser-563, 659 and 660. Phosphorylation of Ser-659 and 660 are required for the *in vitro* activation and translocation of HSL from the cytosol to the lipid droplet (252). The role of Ser-563, although initially thought to be the most important site for activation, remains unknown. Catecholamines have also been shown to mediate signals via the mitogen-activated protein kinase (MAPK) and through extracellular-signal-regulated kinase (ERK) (258). Another kinase that has been shown to phosphorylate and inactivate HSL is the AMP-activated protein kinase (259).

Transcriptional regulation

Whereas acute regulation of HSL involves PKA-mediated reversible phosphorylation of the enzyme, long-term regulation usually means altered gene expression. One positive regulator of HSL is glucose. HSL mRNA levels are induced by glucose in an adipocyte cell line and the proximal region of the HSL promoter contains a glucose-responsive region (260). Conversely HSL mRNA expression decreases when adipocytes are deprived of glucose (261). Positive regulation of HSL is also seen in primary white adipocytes from rat (262). In clonal β-cells and rat islets, HSL mRNA and protein levels are positively regulated by long-term exposure to glucose, suggesting that HSL is involved in the β-cell responses to hyperglycemia (263). Expression of the HSL gene is upregulated by PPARγ and its agonists in differentiating preadipocytes (264), suggesting that an important effect of TZD treatment may be to activate HSL to restore catecholamine induced lipolysis which is usually impaired in insulin resistant states. Recently, the discovery of a functional PPRE in the mouse HSL promoter was made (265) and binding of the PPARγ/RXRα heterodimer to the PPRE sequence was confirmed. The WAT secreted cytokine TNFα is known to induce lipolysis in adipocytes. However, this is not mediated by increased expression of HSL, but rather seems to be by downregulation of perilipin (145). Of the hormones, adrenaline, glucagon, growth hormone and dexamethasone only the latter was seen to regulate HSL mRNA expression in rat adipocytes (266). HSL levels have also been assessed in whole body nutritional states. After a prolonged fasting of rats, HSL protein levels increase (267). Mice fed a HFD show an

initial increase in HSL expression in pancreatic β-cells (268). After a prolonged HFD, a reduction in HSL expression is seen in β-cells, which was reversed when switching to a low fat diet.
HSL mRNA levels are reduced in WAT of patients with T2D, which might be a consequence of increased plasma levels of insulin (269). A decreased expression and function of HSL in subcutaneous adipocytes from obese subjects is reported (270). Isoproterenol and NE induced glycerol release is decreased in differentiated preadipocytes from obese humans, associated with markedly reduced HSL protein levels (271).

HSL mouse models

In recent years, several groups have generated and characterized HSL knockout mice (272) (273) (251) (274) and transgenic mice (275) (276) (277) (278). Although differences exist between the models, there are a several common features that have been reported. An unexpected finding was that HSL null mice are lean. When fed a normal chow diet these mice have unchanged or even reduced adiposity (272) (273) (274), and when challenged with a HFD they are resistant to diet-induced obesity (279) (280) (281). Another common feature is the infertility seen in the males due to oligospermia and hypotrophy of the testis, suggesting that HSL is crucial for normal spermatogenesis and testicular growth. A speculation that the male sterility might arise from a disturbance of retinol metabolism was made (272) since HSL is known to mediate retinol ester hydrolysis (249). However, this was never investigated. Besides these two major findings that existed in all models, other characteristics have differed more or less between the different mouse models.

Lipolysis studies performed on isolated adipocytes show that catecholamine-induced glycerol release is almost completely blunted and catecholamine-induced FFA release is significantly reduced in HSL null compared with WT adipocytes (274) (251). Basal lipolysis on the other hand is unaffected, suggesting the presence of another TG lipase in adipose tissue capable of at least partly compensate for the lack of HSL. This was also suggested by experiments where mouse embryonal fibroblasts (MEF) from HSL null WAT had been allowed to differentiate to adipocytes (282). Extracts from HSL null MEF adipocytes, were able to hydrolyze TG but not cholesteryl esters, indicating that the residual activity is mediated by a TG specific lipase. Another feature common between the different models is a severely altered morphology of WAT and a marked heterogeneity in cell size seen in the HSL null mice. The DG lipase activity was almost absent in white adipocytes from HSL null mice (274), supporting the view of HSL as responsive for the hydrolysis of DGs. An accumulation of DG is also reported in several tissues including adipose tissue (WAT and BAT), skeletal and heart muscle, and testis (251).

In the basal state, HSL null mice show a mild hyperglycemia and hyperinsulinemia, suggesting that lack of HSL leads to impaired insulin sensitivity (274) (283). This is further suggested by the increased release of insulin during glucose tolerance test and by the slower rates of glucose elimination during insulin tolerance test (274). Insulin resistance is observed in multiple tissues including adipose tissue (decreased insulin-stimulated lipogenesis), skeletal muscle (decreased insulin-stimulated glucose uptake) and liver (impaired ability of insulin to suppress endogenous glucose production), which is probably the underlying cause of the increased levels of plasma insulin seen in this model. However, in contrast to the liver

insulin resistance reported in our strain, two other groups have reported increased insulin sensitivity of the liver (284) (285). The reason behind these discrepancies is not known but differences in nutritional state, gender, age, experimental setup (differences in insulin clamp level in the hyperinsulinemic euglycemic clamp, where insulin levels were high (~ 5000 pM) in our experiments whereas the other two studies have used lower insulin levels (400-800 pM) and perhaps most importantly in genetic background of the mice, are probably important factor. Also, differences in the intestinal microflora of the mice cannot be ruled out as a reason behind the discrepancies, as a role of the gut microflora in regulating body weight has recently been recognized (286). In perifusion experiments on isolated pancreatic islets there is an exaggerated response to glucose, suggesting an adaptation to the insulin resistant state, further supported by a 2-fold increase in the mean volume of the pancreatic β-cells from HSL null mice (274). However, there were no secretory defects seen in the β-cells from HSL null mice, extensively confirmed in a later study (287). This is in conflict with another HSL model showing increased basal insulin secretion and decreased GSIS in pancreatic β-cells from HSL null mice (283).

Lower plasma levels of NEFA and TG seen in the fasted state, are possibly explained by an increased expression of lipoprotein lipase (LPL) in WAT and muscle (288). Besides DG accumulation in peripheral tissues, a factor highly associated with insulin resistance (42), there are also reports of a lack of induction of LPL in WAT from HSL deficient mice in response to feeding, which indicates a defect in insulin action, since the postprandial upregulation of LPL is mediated by insulin (288). In one model, the lean phenotype has been explained by a compensatory decreased in fatty acid esterification in WAT, as a result of decreased expression of several lipogenic genes in this tissue (289). In another model, resistance to high fat diet-induced obesity in HSL null mice was reported to be secondary to increased energy expenditure, as revealed by raised body temperature, possibly linked to alterations in sterol metabolism (279). Also, impaired lipolysis was suggested to be the underlying mechanism to the decrease in white adipose differentiation seen in these mice, leading to altered levels of adipose-derived hormones. Two other HSL models have confirmed a reduction in the levels of white adipocyte differentiation markers (289) (281). Reduced WAT mRNA levels and plasma levels of adiponectin in HSL null mice (279) (281) could have a role in the development of the insulin resistance observed in our model. Two models display increased inflammation in WAT of HSL null mice, showing increased macrophage infiltration and TNFα expression in this tissue (290) (291).

A few rescue models have been generated, where HSL have been expressed in specific tissues in the HSL null mice including testis (292) (293), and WAT (294). These rescue models have been partly successful in restoring the phenotype of the specific tissue where HSL was reintroduced. For example through the expression of human HSL in WAT of HSL null mice, a restored body weight and adiposity was seen, as well as normalized plasma leptin levels and a reversal of the DG accumulation in WAT (294).

The other TG lipase

HSL has for a long time been regarded as the rate-limiting enzyme in the breakdown of TG. However, the observation that mice where HSL has been deleted have a lean phenotype and accumulate DGs in various tissues suggested that HSL was not the sole enzyme capable of

TG hydrolysis but rather had an important role as a DG hydrolase. A considerable amount of TG hydrolase activity in WAT remains in HSL null mice (251) (279) and several studies report the presence of a hormone-inducible lipolysis in adipocytes lacking HSL (251) (280), which also suggests the presence of another TG lipase in these cells. The subsequent identification of a novel TG lipase termed adipocyte triglyceride lipase (ATGL), desnutrin or iPLA2ζ (295) (296) (297), as an enzyme highly expressed in WAT with a specificity for TG hydrolysis led to the suggestion that this could be the other lipase that besides HSL is responsible for the hydrolysis of TGs in the adipocyte (295). Indeed, the generation of mice where ATGL had been inactivated, showed defective lipolysis, increased adipose mass and ectopic incorporation of TG in other organs, thus confirming an important role of ATGL for adipocyte lipolysis and energy homeostasis (298). Interestingly, although the accumulation of TG in the heart caused cardiac dysfunction, the mice had improved glucose tolerance and insulin sensitivity due to increased glucose usage, showing a beneficial effect of lowering lipolysis. ATGL specifically removes the first FFA from the TG generating FFA and DG (295). It is not a target for PKA-mediated phosphorylation and is located on the surface of the adipocyte lipid droplet in both the basal and the hormone-stimulated state at similar amounts in either state. It was initially believed that ATGL was important only for basal lipolysis. However, a crucial activator protein for ATGL, CGI-58 was subsequently found (299). CGI-58 is located at the surface of the lipid droplet in the basal state, where it reversibly interacts with perilipin (300). In response to phosphorylation of perilipin by PKA, CGI-58 is released from perilipin allowing it to activate ATGL at the lipid droplet. This would mean that both ATGL and HSL participate in the regulation of catecholamine stimulated lipolysis (301) (**Fig 4**). It has further been estimated that ATGL and HSL are responsible for more than 95% of the TG hydrolase activity in mouse WAT, indicating that other lipases appear to play only a quantitative minor role in adipocyte lipolysis. The suggestion that perilipin A mediates hormone stimulated lipolysis, directly by regulating the access of HSL to substrate via close, if not direct interactions, but also indirectly by regulating accessibility of ATGL to its coactivator, CGI-58, has been made (302).

A recent study has reported that in obese subjects, increased insulin resistance correlates with lower mRNA and protein levels of ATGL and HSL in WAT (303), suggesting an important role of dysregulation of these lipases for the development of disease.

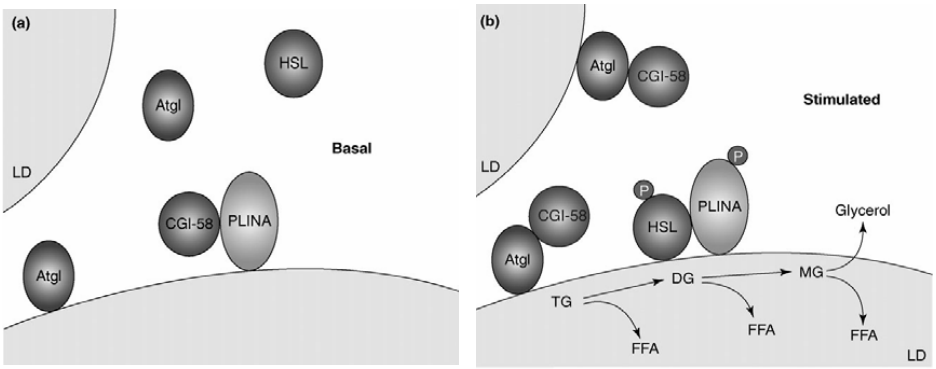

Figure 4 Model of protein trafficking during initiation of lipolysis (304). (a) Under basal conditions, perilipin (PLIN) and CGI-58 form a complex on the surface of the lipid droplet (LD). HSL is mainly cytosolic, whereas some ATGL is located to LDs. (b) Stimulation leads to the phosphorylation (P) of HSL and PLIN by PKA. Phosphorylation of PLIN releases CGI-58, which then activates ATGL. Because ATGL acts only on TG, it is likely that ATGL initiates generation of FFA. Phosphorylation of HSL promotes its translocation and tight association with PLIN. Likely, a major component of HSL activity depends on generation of DG substrate from the action of ATGL, whereas monoglyceride lipase acts to liberate glycerol and the final FFA.

VITAMIN A

Vitamin A (retinol) and its derivatives are essential dietary compounds needed in a variety of physiological processes e.g. embryonic development, reproduction, cell differentiation, postnatal growth, maintenance of the immune system and vision. Retinol deficient rats have impaired glucose stimulated insulin secretion, which can be improved by administration of RA in the diet, suggesting the importance of RA for adequate insulin secretion (305). Retinoic acid has also been shown to possess several anti-inflammatory abilities (306). In humans, retinol supplementation lowers plasma levels of the proinflammatory cytokine TNFα. Mice fed a retinol deficient diet display an elevated NFκB activity that was repressed by an oral high dose of RA (307).

Absorption of retinol

The main dietary sources of retinol are provitamin A carotenoids from vegetables, preformed retinyl esters (REs) and to a lesser extent retinol from animal sources (308). Carotene is absorbed from the intestines by passive diffusion and is converted to retinol in the enterocyte, although in humans, a significant fraction of the carotenoids is absorbed intact. REs are hydrolyzed in the intestinal lumen by a pancreatic enzyme, and mixed micelles containing retinol deliver retinol to the enterocytes, apparently by facilitated diffusion. The majority of retinol is transported to the liver as RE in chylomicrons via the lymph. Two enzymes are identified as important for the esterification of retinol into RE in the enterocytes, acyl-CoA:retinol acyltransferase (ARAT) and lecithin:retinol acyltransferase (LRAT). Whereas ARAT has a preference for unbound retinol, LRAT primarily reesterifies retinol bound to its intracellular carrier protein cellular retinol binding protein (CRBP). Constituents of

chylomicron remnants including RE are subsequently taken up by hepatocytes. Inside the hepatocyte, RE is rapidly hydrolyzed to retinol which binds to retinol binding protein (RBP) and is secreted to another cell type that resides within the liver, the stellate cell. In mammals, 50-80% of the body's total retinol is normally present in the liver, where about 90% are found in the stellate cells, almost exclusively in the form of RE. An enormous storage capacity is seen by the fact that RE stored in the liver is sufficient to sustain life for up to 75 weeks in WT mice (309). Seen from deletion studies, LRAT is the major enzyme responsible for the reesterification of retinol in the liver (310). The retinol stores in the liver are mobilized according to homeostatic need, and an absolute majority is transported in blood as retinol, primarily by RBP4, but also by albumin and lipoprotein particles. A small fraction of retinol is however transported in plasma as retinoic acid bound to albumin, which can be readily taken up by target tissues such as WAT (311). By the control of storage and release, the liver ensures a relatively steady plasma level of retinol. The mechanism by which retinol is absorbed by cells from the retinol-RBP complex in plasma in not clearly understrood. Recently, the discovery that STRA6 is a specific membrane receptor for RBP that binds RBP with high affinity and robustly increase retinol uptake from the plasma complex, was made (312). Muscle is known to express STRA6, and binding of RBP4 to this receptor might be the mechanism by which RBP4 provoke insulin resistance in this tissue. Inside the target cell retinol is bound to one of several isoforms of CRBP, differing in tissue distribution. High levels of CRBP2 are found in the enterocytes whereas CRBP1 is the predominant form expressed in stellate cells (308). Retinol bound to CRBP is readily oxidized to retinaldehyde (RALD) or esterified by LRAT to form RE.

Retinol signaling

With the exception of vision, most effects of retinol occur through RA, the main active metabolite derived from oxidation of retinol. The first step of RA synthesis, oxidation of retinol to RALD is catalyzed by Adh3, expressed ubiquitously throughout the development, with additional contributions by Adh1 and 4, having a more tissue specific expression (313). Recently, it was reported that RALD repress adipogenesis and diet-induced obesity (314). Retinaldehyde dehydrogenase (Raldh) 1 and 2 catalyze the terminal and irreversible step of RA biosynthesis. Raldh2 has been shown to be functionally more important and also more efficient and selective for RALD than Raldh1 (315).

Two isomers of RA, all-trans and 9-cis, are biologically active and most of the cells studied to date can convert either of the RA isomers to the other (316). Retinoid signaling is dependent on the presence of retinoic acid receptors (RAR) and retinoid X receptors (RXR) and their endogenous ligands (317) (318). Retinoid receptors function as ligand-dependent transcription factors. Members of the RAR family are activated by a number of physiologically occurring retinoids, including all-trans retinoic acid (atRA), 9-cis retinoic acid (9-cis RA) and many other isomers. Members of the RXR family are efficiently activated by 9-cis RA and its derivatives, although the role of 9-cis RA as an endogenous ligand for RXR has been questioned due to its low levels. Each retinoid receptor subfamily consists of several receptor isoforms termed RARα, β and γ and RXRα, β, and γ. The specific isoforms have different tissue distribution and may be involved in different physiological processes. To activate transcription, RAR forms a heterodimer with RXR and control gene expression by binding to specific DNA sequences in the target gene promoter, termed RA response elements (RARE). RXR specific response elements (RXRE) are also found in the

promoters of specific genes e.g. CRBP2, allowing receptor specific gene regulation. Although the usual way for retinol to exert its effects is through the metabolite RA binding to RARE on target genes, retinol has been shown to act through RAR-independent mechanisms (319). Unlike RAR, RXR has the ability to form heterodimers with several other nuclear receptors, including the thyroid receptor (TR), liver X receptor (LXR) and PPAR (320). Whether RXR in the heterodimer remains sensitive to ligand activation depends on the heterodimerization partner. It has been shown that when RAR and RXR form a heterodimer complex, RXR works as a silent partner, and ligand binding to RXR have little effect on transcriptional activity of the heterodimer (321). However when RXR forms a heterodimer with PPAR, it retains the responsiveness to ligands. PPARγ/RXRα is a common heterodimer combination that promotes adipogenesis, and ligand binding of both receptors in the heterodimer will allow maximal transcriptional activity of the complex (322). Recently, RXR has been shown to form active homodimers with the ability to bind PPRE sites on target genes and initiate transcription independently of PPARγ (323).

Retinol metabolism in WAT

A considerable amount of retinol is also stored in the adipose tissue, being second only to the liver as a retinol depot. About 15-20% of retinol in rat is stored in the adipocytes (324), the majority (~60-70%) in the form of retinyl esters (325) (310). The mechanism of uptake of retinol into the adipose tissue differs from that of other tissues (326). It is appears that adipocytes acquire their retinol directly from chylomicron-bound retinyl esters, and not from retinol bound to RBP. The chylomicron-bound retinyl esters are hydrolyzed by LPL localized on the surface of the endothelial cells. Inside the cell, retinol can either be oxidized to form RA or become reesterified and stored as retinyl esters. Since the capacity for RE storage in WAT is intact in LRAT deficient mice, this is apparently not the enzyme responsible for the generation of RE in adipocytes (310). However, diacylglycerol acyltransferase-1 (DGAT1) is highly expressed in adipose tissue and has been shown to catalyze acyl-CoA-dependent esterification of retinol (327), making it possible that DGAT1 is the main enzyme responsible for the esterification of retinol in adipocytes. The mechanism of release of retinol from adipocytes apparently also differs from that of liver stellate cells. Similar to the release of NEFA, increased retinol release from differentiated BFC-1β adipocytes was seen after cAMP stimulation (249). A concurrent decrease in adipocyte RE and intracellular increase in retinol, suggested that hydrolysis of RE is responsible for the generation of the released retinol. A preference for retinyl palmitate was seen compared to retinyl oleate. Since HSL is an important mediator of cAMP stimulated lipolysis, the ability of HSL to hydrolyze RE was investigated. Fractions of partially purified HSL from adipocytes were able to hydrolyze RE to retinol, in contrast to liver where a specific RE hydrolase performs this function. Further, overexpression of HSL in CHO cells resulted in a considerable RE hydrolase activity in these cells. A further confirmation of the role of HSL for RE hydrolysis is seen in our studies where the RE hydrolase activity is severely decreased in WAT from HSL null mice (291) (present investigations, **Fig 11C**). Another difference compared to hepatocytes is that adipocytes secrete free retinol, not retinol bound to RBP (249), and, in contrast to adipocytes, primary mouse hepatocytes exposed to cAMP show no increase in intracellular or released retinol. A functional role of retinol and RE in WAT can be seen when challenging mice with a vitamin A deficient diet (VAD), where a sharp reduction in retinol and RE levels is seen in

WAT, indicating that these retinoids are stores that can be mobilized in times of dietary insufficiency (310).

Retinoid receptors in adipocytes

In rodents, WAT expresses all subtypes of retinoid receptors but to a varying extent (328). White adipocytes have an abundant expression of RARα, RARγ, RXRα and RXRβ. Brown adipocytes on the other hand express high levels of RARβ and RXRγ. It is suggested that the characteristic profile in the expression of different receptor subtypes in WAT versus BAT indicates specific functions of RA in these tissues. Changes in retinoid receptor expression occur during differentiation of preadipocytes to white adipocytes, where, RXRα and RXRγ are induced, and RARα and RARγ are decreased in parallel with adipocyte conversion. The importance of RXR in adipocytes can be seen from a study in mice where RXRα has been deleted specifically in WAT, resulting in impaired adipogenesis, resistance to diet-induced obesity and impaired lipolysis in response to fasting (114).

Retinoic acid and adipogenesis

The molecular events involved in the commitment of mesenchymal stem cells to adipoblasts remain to be elucidated. However, a critical role for RA in adipocyte determination has been highlighted by the findings that treatment of stem cell-derived embryonal bodies with RA is a prerequisite for high adipogenesis (73). The role of RA in terminal adipogenesis appears to be concentration dependent (**Fig 5**). Whereas lower concentrations of RA are reported to stimulate adipogenesis (329) a higher concentration of RA, administered at the earlier stages of adipocyte differentiation, blocks adipogenesis at least in part by interfering with C/EBPβ mediated transcription (106). Retinoids selectively activating RXR promote adipogenesis. It appears that the resulting effects of retinoids on adipocyte differentiation depend on a complex balance between RA metabolism and relative RAR and RXR availability in the cell (330).

RA has also been shown to affect the transcription of factors involved in white versus brown adipocyte determination including pRb and RIP140 (331) (214) (229), and this effect of RA could also be concentration dependent. RA has recently been shown to directly favor a remodeling of WAT *in vivo* (230) and of mature 3T3-L1 adipocytes (229) towards increased oxidative metabolism.

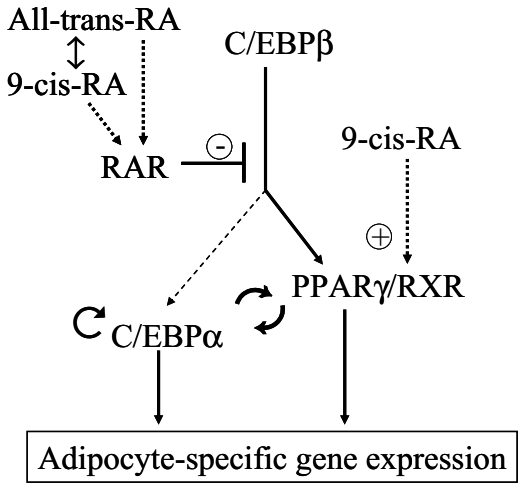

Figure 5 Dual action of retinoic acid on adipogenesis.

Retinoic acid effects on UCP-1

The activation of RAR in brown adipocytes by RA differs from that in white owing to the direct and potent action of RA on the induction of UCP-1 expression (332). RA stimulates UCP-1 gene expression through a retinoid responsive region in the distal enhancer of the UCP-1 gene (321). However, a direct action of RA on the UCP-1 protein in the mitochondria, activating the proton conductance activity of this protein is also reported (333). The *in vivo* action of RA to promote UCP-1 expression has been confirmed by treatment of mice with pharmacological doses of atRA (334). In contrast, vitamin A deficiency results in decreased UCP-1 expression in BAT. By the use of receptor-selective retinoid analogs, it was established that both RAR and RXR-dependent signaling pathways are involved in the induction of the UCP-1 gene by retinoids.

Rexinoids

A major breakthrough in the use of retinoid-related pathways to influence metabolic disturbances, especially T2DM, has been achieved by the use of RXR activators or rexinoids. In mouse models of T2DM and obesity, RXR agonists function as insulin sensitizers with the capacity to decrease hyperglycemia, hypertriglyceridemia and hyperinsulinemia (322). The antidiabetic activity can be further enhanced by combination treatment with PPARγ agonists such as TZDs. Studies in vitro support the view that RXR ligands stimulate the RXR/PPARγ heterodimer, seen by the promotion of white adipocyte differentiation by the respective ligands for RXR and PPARγ, and a synergistic effect at simultaneous treatment with both (335). Rexinoid and TZD treatment differs in that rexinoids decrease body weight in association with insulin sensitizing action, and also show a wider range of tissue action than do TZD, having effects primarily on adipose tissue (321). The widespread effects of rexinoids are probably due to activation of RXR when this receptor hererodimerizes with nuclear receptors other than PPARγ.

PRESENT INVESTIGATION

AIMS

The primary aim of this thesis has been to broaden our knowledge of HSL by investigating a mouse model with a targeted disruption of the HSL gene. In view of the central role of HSL, highly involved in lipid mobilization, and the reported dysregulation of HSL in common metabolic disorders such as obesity-related insulin resistance and T2D, increased knowledge about the function of this lipase would be of great importance in studies aimed at exploring HSL as a new target for antobesity and antidiabetic drugs.

The functional consequences of an ablated HSL expression was initially studied at the whole body level (paper I), and subsequently narrowed down to focus on the white adipose tissue aspects (papers II-IV), a tissue that was shown to be greatly affected by the loss of HSL. The unexpected lean phenotype seen in paper I was further challenged with a HFD in subsequent studies (papers II-IV). A global expression analysis at the protein level was made (paper II), that was used in a hypothesis-generating manner. An early hypothesis based on initial findings was subsequently tested by more functional studies to confirm and strengthen the theory. An attempt to investigate the mechanisms underlying the observed, but unexpected lean phenotype, was made in paper III, and in paper IV a major hypothesis that could help explain previous findings from papers I-III was tested, and a rescue study was performed.

PAPER I

Given the potential pathogenic role of both circulating and stored lipids, HSL may play an important role in the development of insulin resistance and T2DM. A decreased function of HSL could lead to ectopic accumulation of lipids, subsequently impairing insulin sensitivity in these tissues. To address the role of HSL in these events, we generated a mouse with a targeted disruption of the HSL gene.

In agreement with previous reports on other models of HSL null mice (272) (273), the mice were lean, and male mice were sterile due to oligo- and azospermia. The consequences of lack of HSL were studied using lipolysis studies performed on isolated adipocytes showing that catecholamine-induced glycerol release is almost completely blunted and catecholamine-induced FFA release is significantly reduced in HSL null compared to WT adipocytes, in agreement with results from another line (251). These results and the fact that basal lipolysis is not lower in HSL null mice, suggested the presence of another TG lipase in adipose tissue capable of at least partly compensating for the lack of HSL. A confirmation of a previously reported accumulation of DG (251), and a severely impaired DG lipase activity in white adipocytes from HSL null mice, suggests that HSL is the sole lipase capable of hydrolyzing DGs in the adipocytes. Further, *in vitro* data suggest that whereas HSL is crucial for DG hydrolysis in WAT, in liver and muscle other lipases might be responsible for this activity, since no difference in DG lipase activity in these tissues was shown. A high neutral cholesterol ester hydrolase activity in HSL is a unique property among mammalian lipases (245). The importance of HSL in this action as a cholesterol ester hydrolase is reflected by a severely reduced cholesterol ester hydrolase activity in white adipocytes, muscle and liver from HSL null mice.

In the fasted state, HSL null mice displayed hyperinsulinemia and slightly, but significantly elevated levels of glucose, suggesting that lack of HSL leads to impaired insulin sensitivity, as previously suggested (283). Decreased peripheral insulin sensitivity was further suggested by an increased secretion of insulin during both oral and intravenous glucose tolerance test and by the slower rates of glucose elimination during an insulin tolerance test.

To further characterize the impaired insulin sensitivity in the HSL null mice, at the level of the liver, a hyperinsulinemic and euglycemic clamp was performed. After an initial injection of a high dose of insulin to block endogenous insulin production, insulin was clamped at 5 nM and glucose was infused to uphold a glucose concentration of 6 mM. A pronounced increase in baseline insulin levels and a trend towards decreased whole body insulin sensitivity was seen in HSL null mice. The endogenous glucose output (EGO) which mainly reflects hepatic glucose production, was slightly elevated already prior to the clamp, and was several fold increased at the end of the clamp in HSL null mice compared to WT. Insulin-stimulated glucose uptake into soleus muscle was impaired, as was insulin-stimulated lipogenesis in parametrial adipocytes from mice lacking HSL. Thus, insulin resistance was observed in multiple tissues including adipose tissue (**Fig 6**), skeletal muscle and liver, and was almost fully compensated by increased insulin secretion, as reflected in hyperinsulinemia and only slight hyperglycemia.

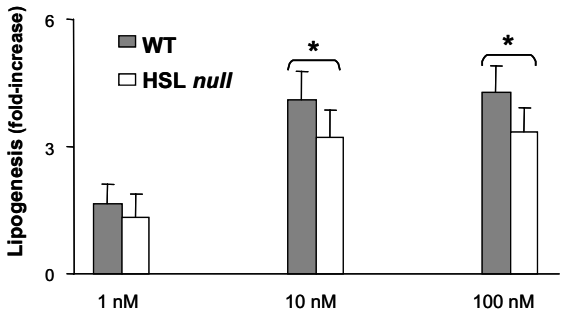

Figure 6 Insulin stimulated lipogenesis is decreased in isolated adipocytes from WAT of HSL null mice.

The capacity of the pancreatic β-cells to secrete insulin was investigated both *in vivo* by an arginine test and in vitro in perifused islets, on the basis of a previously reported secretory defect of the pancreatic β-cells (283). No decreased ability to secrete insulin was found in pancreatic β-cells from HSL null mice in response to either arginine, glucose, FFA or KCI. The absence of a secretory defect in β-cells from HSL null mice has been confirmed in a later extensive study (287). However, in perifusion experiments on isolated pancreatic islets there is an exaggerated response to glucose, suggesting an adaptation to the insulin resistant state. This is further supported by immunohistochemistry, showing a hypertrophy of the HSL ablated islets. An increase in both β-cell mass and insulin content in isolated islets from HSL null mice is likely the result of adaptations to insulin resistance.

The insulin resistance in this model is unusual in the sense that it occurs in the absence of increased adiposity or raised plasma NEFA levels. On the contrary, in the fasted state plasma NEFA and TG are reported to be decreased (251), which is normally associated with increased insulin sensitivity. The underlying mechanism of the insulin resistance seen in various tissues is not clear. However, the accumulation of DGs seen here in WAT, but also reported in muscle (251) could be a contributing factor since DGs are known to function as intracellular signaling molecules involved in the activation of atypical PKC (38) and/or IKKβ (336) which inhibits insulin signaling. It is also possible that accumulation of these lipids affects transcriptional activity and changes in gene expression in target tissues of insulin.

In conclusion, the present study demonstrates that an abrogation of regulated mobilization of lipids from cellular TG stores via disruption of HSL, results in impaired insulin sensitivity. A mild insulin resistance, seen in multiple tissues including WAT, muscle and liver is likely a confounding factor to the increased basal levels of circulating insulin levels. However, due to a compensatory increase in insulin secretion by the pancreatic β-cells, plasma glucose levels are almost fully restored. Also, whereas HSL appears to be the sole lipase capable of hydrolyzing DGs in the adipocyte, the presence of an alternative TG lipase in adipose tissue is suggested.

PAPER II AND III

A major finding of the HSL null mice is that they remain lean despite the fact that they lack a substantial capacity for TG hydrolysis. In order to further challenge this phenotype the mice were put on a long-term HFD, and a focus was made on the WAT since this is a tissue where HSL is normally highly expressed. Strikingly, HSL null mice were resistant also to HFD-induced obesity, in agreement with a previous study (279) (paper III). Using computer tomography, it was obvious that the massive increase of both subcutaneous and visceral WAT seen in high fat-fed WT mice was absent in mice lacking HSL (**Fig 7**). An even more pronounced heterogeneity in adipocyte cell size was seen by light microscopy. Also, HFD feeding provoked the appearance of an increased number of eosinophilic-like cells, reflecting an inflammatory state of the tissue.

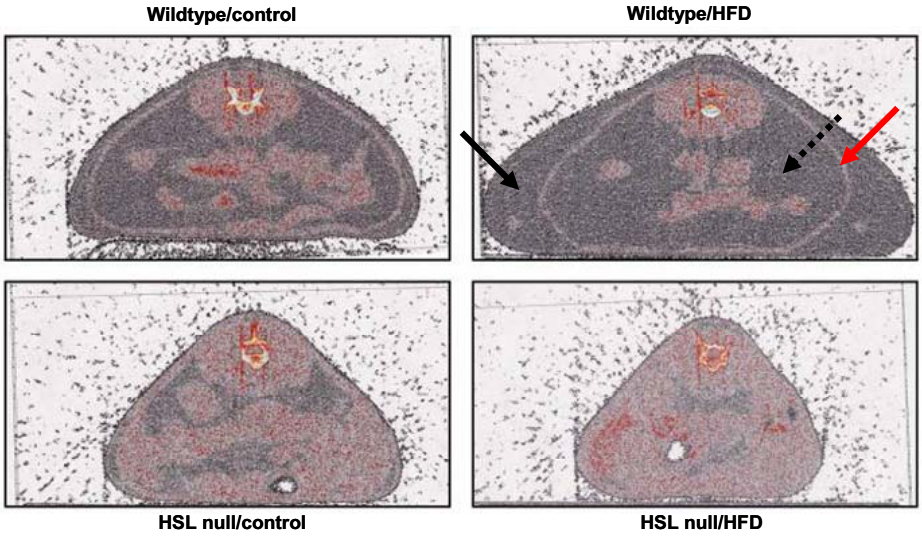

Figure 7 Subcutaneous and visceral adipose tissue stores are decreased in HSL null mice. Computer tomography scan images showing fat distribution in WT and HSL null mice fed either a control diet or a HFD for 6 months. The filled black arrow and the dotted black arrow represent the subcutaneous and visceral adipose, respectively. The red arrow indicates the location of the muscle layer separating the two adipose tissue depots.

Paper II is based on a proteomic analysis using both one-dimensional polyacrylamide gel electrophoresis (1D-PAGE) and 2D-PAGE, and was performed with the intention to generate a hypothesis for the observed phenotype. Methodological aspects of the analysis of data generated from 2D-PAGE were also investigated, with a focus on the handling of missing data points. A conclusion was made that setting missing data points to zero results in a large number of false positives. Also, a way to evaluate the many steps involved in analyzing data generated using 2D-PAGE, e.g. background extraction and normalization, is presented in this paper.

The expression data indicated an increased expression of inflammation-related genes including ferritin heavy chain, galectin-3 (GAL-3) and mast cell protease-4. These data, together with indications of inflammation from light microscopy data, and observations of an increased TNFα expression in WAT from HSL null mice (279), made us focus on a further characterization of the inflammatory reaction in this tissue. TNFα is an important cytokine and mediator of inflammation, often seen increased in insulin resistant states, and has further been shown to induce expression of ferritin heavy chain. The protein levels of both the membrane bound (26 kDa) and the secreted (17 kDa) isoform of TNFα were several fold increased in WAT from HSL null mice (**Fig 8A**). NFκB is a nuclear transcription factor regulating the expression of a majority of proinflammatory genes, including TNFα. In the inactivated state, NFκB is bound to IκB which keeps NFκB in the cytosol. Upon phosphorylation of IκB on Ser-32 by IKKβ, IκB is targeted for proteosomal degradation and releases from NFκB, which enters the nucleus and initiates transcription of several inflammatory mediators implicated in insulin resistance. An indirect measurement of NFκB activity using western blot technique and a phosphospecific antibody was made, and a several fold increase in phorphorylation of Ser-32 of IκB was observed in HSL null WAT. Increased protein level of the macrophage marker GAL-3 was confirmed using western blot analysis. Since TNFα is suggested to be secreted mainly by macrophages in WAT, and since an increased infiltration of macrophages into WAT has been associated with obesity and insulin resistance (15) (290) (159), we proceeded to measure macrophage infiltration in WAT. Using immunohistochemistry, an increase in the macrophage surface marker F4/80 was seen in HSL null mice under both normal and HFD feeding, indicating increased macrophage infiltration regardless of diet. The increase in F4/80 was confirmed on the mRNA level using real time quantitative PCR (rtPCR) on the SVF fraction from WAT (**Fig 8B**). Increased mRNA levels of F4/80 were observed in WT mice after a HFD compared to WT mice fed a normal chow diet. The inflammatory mediators TNFα of IL-6 could not be detected in plasma of HSL null mice, indicating that the inflammatory response is not systemic.

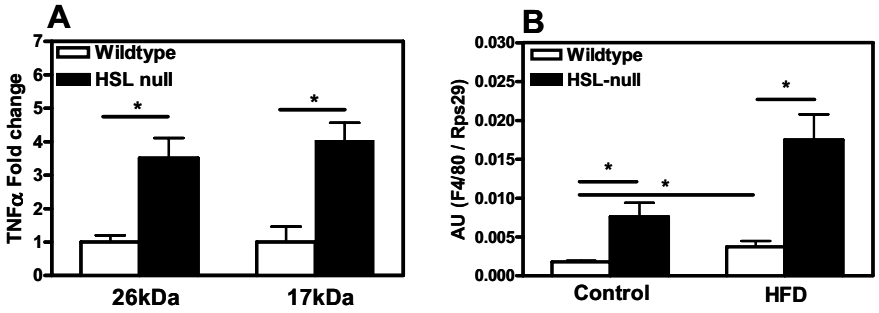

Figure 8 Increased inflammation and infiltration of macrophages in WAT of HSL null mice. (A) Protein levels of the membrane bound and soluble form of TNFα in WAT from HFD-fed mice. (B) mRNA levels of the macrophage marker F4/80 in SVF fraction of WAT from control or HFD-fed mice.

A connection between the inflammatory state of the WAT and the insulin resistance observed in HSL null mice is highly probable. However, the triggering factor behind obesity-induced inflammation is not known. The adipose tissue is an important endocrine organ and releases adipokines that can alter the metabolic behavior of peripheral tissues. An important adipokine involved in the development of insulin resistance is adiponectin. Adiponectin has insulin sensitizing and anti-inflammatory abilities and is often seen decreased in obese and insulin resistant states. In paper III, decreased plasma levels of adiponectin are seen already at the beginning of the HFD study, and the decrease is even more pronounced after 22 weeks of HFD. Decrease in local and circulating adiponectin could be a contributing factor to the observed inflammation and insulin resistance. The specific role of HSL for the generation of this inflammatory WAT phenotype is a key question.

The link between HSL expression and the regulation of target genes could be that HSL provides ligands for transcription factors, such as PPARγ or any of the retinoid nuclear receptors. Keeping in mind a broad substrate specificity of HSL, this ligand could be any of a number of candidates. It is possible that HSL generates a lipid ligand for PPARγ itself, or for its heterodimerization partner RXR, such as the long-chain polyunsaturated fatty acid DHA shown to be a specific activator of RXR (337). An appealing candidate could be a derivative/metabolite of retinol, i.e isomers of RA. HSL has previously been shown to possess retinyl ester (RE) hydrolase activity (249), and it is possible that HSL is important for the liberation of retinol, and secondary for the delivery of retinoic acid (RA) in the adipocyte, affecting various processes in the cell.
To test the hypothesis that the inflammatory response in WAT of HSL null mice might be due to a reduced ability to generate retinol and retinoic acid from RE stores, the RE hydrolase activity in WAT infranatants was investigated. A significant decrease in RE hydrolase activity was seen in HSL null WAT compared to WT, suggesting that HSL is responsible for mobilizing the retinyl esters stored in this tissue.
Retinoic acid has been shown to possess several anti-inflammatory abilities (306). In humans, retinol supplementation lowers plasma levels of TNFα. Mice fed a retinol deficient diet display an elevated NFκB activity that was repressed by an oral high dose of RA (307). Also, RA decreases TNFα levels in a macrophage cell line (338), and GAL-3 in a F9 cell line (339).

A working model can thus be formed suggesting that in the absence of HSL in adipocytes, and possibly also in other types of cells normally residing in WAT e.g. preadipocytes and macrophages, an RA ligand is not generated to suppress NFκB activity. The increased activity of NFκB then leads to increased transcription of proinflammatory cytokines such as TNFα in WAT. A decreased RA signaling in macrophages could lead to increased production of several proinflammatory mediators including TNFα, nitric oxide and IL-1β. Also, GAL-3 is known to be involved in chemotaxis, the attraction of inflammatory cells to the site of inflammation, which could contribute to the recruitment of macrophages and other inflammatory cells to WAT.

In paper III, the resistance to diet-induced obesity in HSL null mice could not be explained by decreased food intake, reduced intestinal absorption or increased physical activity. A block in adipogenesis has previously been suggested to be involved in the observed resistance

to diet-induced obesity in another HSL model (279). As previously shown, the mRNA levels of the key adipogenic transcription factors PPARγ and C/EBPα were decreased in WAT from HSL null mice. A decreased level of PPARγ was confirmed also on the protein level in our study (**Fig 9A**). Also, the protein level of stearoyl-CoA desaturase-1 (SCD-1), known to be expressed only in mature adipocytes, was almost absent in WAT from HSL null mice (**Fig 9B**).

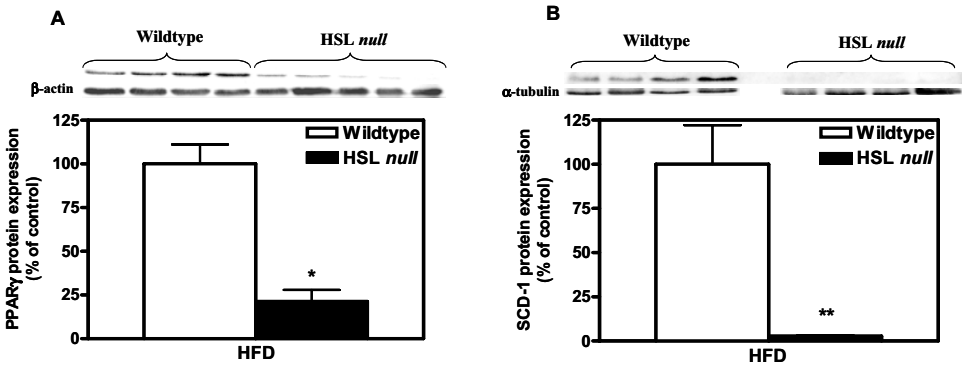

Figure 9 Decreased protein levels of adipogenic markers in WAT from HSL null mice. Protein levels of PPARγ (A) and SCD-1 (B) in WAT.

Using indirect calorimetry, an increased metabolic rate in HSL null mice was observed. Transmission electron microscopy on WAT revealed a 50% increased size of mitochondria from HSL null mice. A brownish appearance of WAT together with previous results led us to hypothesize that WAT of HSL null mice had attained BAT characteristics. When analyzing the protein level of the brown adipocyte marker UCP-1 in mitochondrial fractions from WAT we found a several fold increase in UCP-1 in HSL null mice. A confirmation on the mRNA level of increased UCP-1 expression in HSL null mice was performed on isolated adipocytes, to exclude any contribution from other cell types present in WAT (**Fig 10A**). The levels of carnitine palmitoyl transferase 1 (CPT1), the rate limiting enzyme for long-chain acyl-CoA transport into mitochondria, was also increased in adipocytes from HSL null mice (**Fig 10B**). CPT1 is usually expressed at higher levels in brown versus white adipocytes. A functional reflection of this could be the observed increase in palmitate oxidation in isolated HSL null adipocytes.

Transcriptional coregulators seem to play a major role in determination of differentiation into white versus brown adipocyte lineage. Two proteins that have been associated with white versus brown adipocyte transformation are pRb and RIP140. Although pRb is not a coregulator, it has been shown to function as a molecular switch determining white versus brown differentiation (194). RIP140 is a transcriptional corepressor that promotes differentiation into the white adipocyte lineage (195). The mRNA levels of both pRb and

RIP140 were decreased in white adipocytes from HSL null mice. The expression levels of the coactivator PGC-1α however, was not significantly changed between the two genotypes. To study the functional consequences of increased UCP-1 expression, oxygen consumption on isolated WAT was investigated. Basal oxygen consumption was markedly increased in WAT from HSL null mice (**Fig 10C**). Also, the level of electron transport chain uncoupling was markedly increased in WAT from mice lacking HSL (**Fig 10D**).

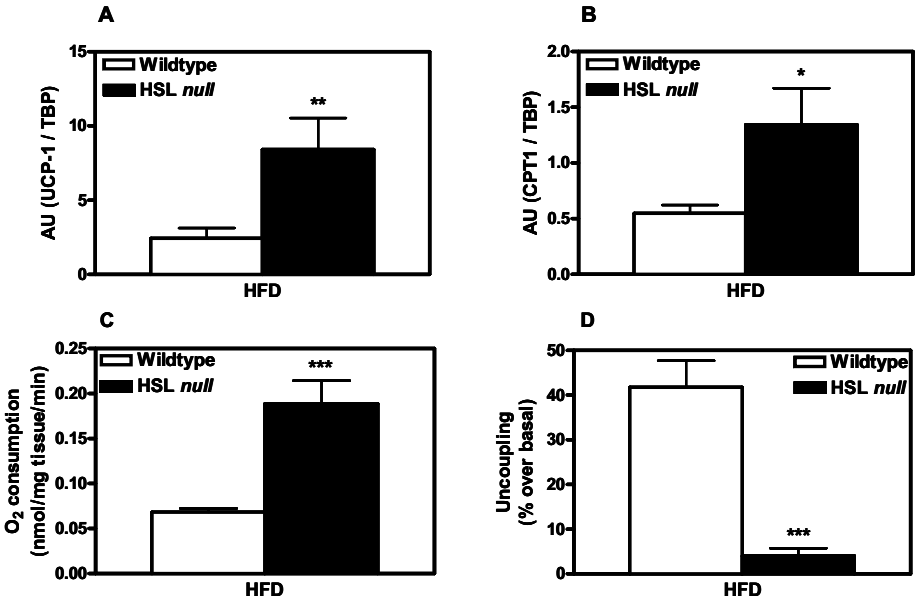

Figure 10 Acquirement of brown adipocyte features in WAT of HSL null mice. The mRNA levels of UCP-1 (A) and CPT1 (B) in isolated white adipocytes. Basal oxygen consumption (C) and the ability to increase uncoupling activity after administration to a chemical uncoupler (D) were measured on WAT tissue pieces.

In conclusion these data suggest that WAT of HSL null mice attains BAT characteristics that are functionally manifested as increased oxygen consumption and uncoupling activity. However, the increased oxygen consumption in WAT is likely to contribute little to the increase in total energy expenditure considering the small contribution of WAT to whole body energy consumption compared to that of a metabolically active tissue such as skeletal muscle. Although the underlying mechanisms and the physiological relevance remain to be resolved, these data recognize a role of HSL in the determination of cell fate during adipocyte differentiation. A parallel between HSL null mice and RIP140 null mice is the acquirement of BAT characteristics in WAT without increased PGC-1α expression. A recruitment of RIP140 to a key enhancer element in the UCP-1 promoter, demonstrates a direct role for RIP140 in repressing gene expression (216). It could thus be that in white adipocytes of HSL null mice, the decreased level of RIP140 allows the low levels of PGC-1α (and/or possibly PGC-1β) coactivator to promote the appearance of brown fat features.

The mechanism whereby HSL is involved in the determination of cell fate during adipocyte differentiation remains to be explored. However, the fact that both pRb and RIP140 are transcriptionally controlled by RA (331) (214), together with a dramatically reduced retinyl ester hydrolase activity in WAT from HSL null mice (291), makes future studies on this activity of HSL highly justified.

PAPER IV

In paper IV, an attempt to investigate the importance of HSL for retinol metabolism in the white adipocyte was made.

It has previously been demonstrated that HSL exhibits RE hydrolase activity (249). However, since the activity of HSL against RE has never been compared to the activity against other known substrates using purified homogenous preparations of HSL, this was done. The activity of rat HSL against retinyl palmitate (RP) was the highest activity of the tested substrates (**Fig 11A**). Compared to rat, human HSL showed a lower activity for all tested substrates (**Fig 11B**). Similar to rat HSL, human HSL exhibited high activity against RP, although diacylglycerols were the preferred substrate for the human enzyme. A dramatically reduced RE hydrolase activity was seen in WAT from HSL null mice compared to WT littermates (**Fig 11C**). In this study these analyses were extended to include both visceral and subcutaneous WAT as well as BAT. RE hydrolase activity was dramatically decreased in all these depots of HSL null mice, suggesting that HSL is the major RE hydrolase in adipose tissue. The largest decrease in activity was seen in visceral WAT from HSL null mice, exhibiting only 1% of remaining activity against RP compared to WT littermates.

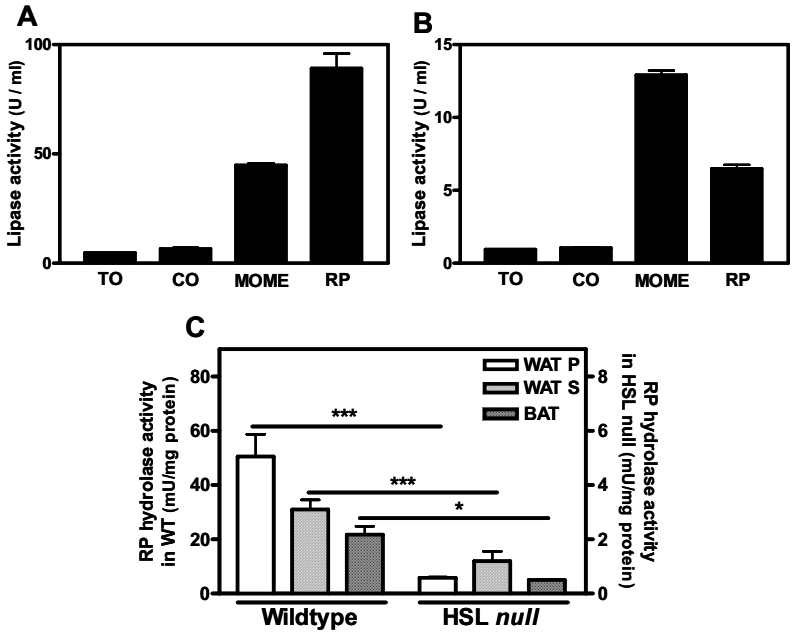

Figure 11 HSL has a prominent RE hydrolase activity seen from homogenous preparations of recombinant rat (A) and human (B) HSL assayed against triolein (TO), a diolein analogue (MOME), cholesteryl oleate (CO) and retinyl palmitate (RP). The RP hydrolase activity is severely decreased in subcutaneous and visceral WAT as well, as BAT from HSL null mice (C).

It is clearly shown that HSL has a prominent RE hydrolase activity and that this activity is severely impaired in WAT from HSL null mice. To further investigate retinol (ROH) metabolism in HSL null mice, retinoid metabolites were measured. In line with the reduced RE hydrolase activity, a marked increase in RE content and a decrease in ROH, retinaldehyde (RALD) and all-trans RA (atRA) levels were observed in perigonadal WAT from HSL null mice fed a HFD. These data suggest that a decreased ability of HSL to hydrolyze RE to generate ROH has an impact on the retinoid metabolism also downstream of retinol. The observation that the plasma levels of ROH did not differ between HSL null mice and WT littermates could mean that WAT plays a minor role in maintenance of normal plasma levels of ROH, i.e. ROH generated within WAT is mainly for local use. Also, plasma ROH cannot compensate for failure to generate RA within WAT. This is in agreement with the observation that chylomicron RE is an important source of adipocyte retinoids (326) and furthermore suggests that ROH taken up by adipocytes is rapidly reesterified to RE. Thus, the adipocyte appears to be critically dependent on a functional cytosolic RE hydrolase and our studies strongly suggest that HSL is serving this role.

Different enzymes involved in the conversion of ROH to RA were measured. Alcohol dehydrogenase 3 (Adh3), catalyzing the oxidation of ROH to RALD, and Raldh1, catalyzing the oxidation of RALD to RA are both positively regulated by RA and were found to be downregulated in HSL null mice. Raldh2, on the other hand, has been shown to be negatively regulated by RA, and its expression was elevated in HSL null mice. Raldh2 has been shown to be functionally more important and also more efficient and selective for RALD than Raldh1. Recently, it was reported that RALD repress adipogenesis and diet-induced obesity (314). As RALD levels are decreased in WAT of HSL null mice, this mechanism clearly does not contribute to the impairment of adipogenesis and resistance to diet-induced obesity in this model.

Next we investigated the mRNA levels of genes known to be transcriptionally regulated by RA. In agreement with the lowered RA level in WAT from HSL null mice, decreased mRNA levels of RIP140, RBP4, SPOT14, SREPB1c and RARα, were found in HSL null mice compared to WT littermates. A reduction of RARα protein in WAT of HSL null mice was confirmed using western blot analysis. Also, the mRNA level of RXRα was decreased in WAT of HSL null mice compared to WT littermates.

The severely reduced capacity to mobilize ROH, and thus RA, in WAT of HSL null mice, together with the inability of plasma ROH to compensate for this defect, was the rationale for using RA in the dietary intervention studies. Upon supplementation of the HFD with RA, the mass of WAT in HSL null mice increased in a dose-dependent manner with a maximal increase of 1.6-fold at the highest RA dose administered, whereas in WT mice RA supplementation caused a dose-dependent reduction in the size of the periovarial WAT compared to WT mice fed regular HFD. These opposite effects of RA supplementation reflect that while added RA in the HSL null mice compensates for the inability to generate RA within WAT, added RA in the WT mice may exert other effects. It is possible that in WT mice, with a retained capacity to mobilize endogenous RA, the effect of RA administration in the diet precipitates the previously reported more acute effect of RA, to induce a more oxidative phenotype in WAT, accompanied by UCP-1 induction and decreased adipocyte

mass (229). This is supported by the findings that WT mice fed the RA enriched diet, have a decreased pRb expression in white adipocytes compared to WT mice on a regular HFD. Also, a dual role of RA in adipogenesis has been described. Whereas low doses of RA have been shown to promote preadipocyte differentiation (329), other studies have recognized RA as a potent inhibitor of adipogenesis (106). The negative effect of RA on adipogenesis is reflected in reduced PPARγ levels in WT mice. In HSL null mice, no difference in PPARγ levels could be seen after RA supplementation, but interestingly, a significant increase of the late differentiation marker aP2 was noticed. A recently reported ability of RXR to form active homodimers with the capacity to bind PPRE recognition sites and enhance transcription of target genes independently of PPARγ, could be a possible mechanism (323).

RA supplementation partially or fully restored the expression of RA-regulated genes in white adipocytes of HSL null mice. Among these were pRb and RIP140, key factors in the determination of differentiation into the white versus the brown lineage. The downregulation of the expression of these factors could account for the described attainment of brown adipocyte features of WAT of HSL null mice (paper III). Consequently, normalized expression of these factors upon RA administration presumably accounts for the expansion of WAT mass in HSL null mice as normally expected during a HFD feeding. Diet intervention with RA, to a large extent reversed the phenotypic changes also of BAT of HSL null mice, i.e. BAT mass was significantly reduced and the mRNA levels of UCP1, PPARα and glycerol kinase, all markers of differentiated brown adipocytes, were restored to normal levels. RA administration appeared to have no effect on the defect in spermatogenesis seen in male HSL null mice, since no viable sperm could be detected in the testis at any time point during the rescue study. This could be explained by previous observations that RA in plasma is not absorbed through the testis barrier (311).

The exact mechanisms whereby perturbed retinoid metabolism in HSL null mice causes the observed disturbances in the differentiation program of adipocytes remain to be elucidated. We propose a working model (**Fig 12**) that HSL generates ROH from RE stores, which, following conversion to atRA, 9-cis-RA and possibly other RA species, act as ligands for RAR and RXR. This ensures a proper expression of RA-regulated genes, such as pRb and RIP140, with crucial roles in the determination of adipocyte cell fate. Provision of RA by HSL would also allow proper activation of the PPARγ:RXR heterodimer, the crucial transcription factor for adipogenesis and survival of mature adipocytes. This in turn allows the proper secretion of important adipokines such as adiponectin from WAT. Other effects of RA are to control inflammation by inhibiting NFκB activation and TNFα release, thereby blocking macrophage recruitment to WAT. Since inflammation in WAT is associated with insulin resistance, this effect of RA would prevent development of insulin resistance in WAT, and possibly also in other tissues. It should however be pointed out that HSL is induced late in the adipogenic program (62). The late induction of HSL in adipogenesis could suggest that the main role of HSL is to supply RA crucial for the survival of mature adipocytes.

In conclusion, HSL is the major RE hydrolase of white and brown adipocytes. The effects of its absence in WAT, is a perturbed retinoid metabolism, which could potentially result in failure to provide RA for signalling events that are crucial for determination of adipocyte fate. The importance of the internal RE stores for WAT function is underscored by the fact that plasma ROH is unable to compensate for failure to generate RA within WAT.
This study further supports the emerging view of HSL as a multifunctional enzyme capable of hydrolyzing a wide variety of substrates, with other functions in adipose tissue biology than fatty acid mobilization. Thus, in addition to its key role in energy homeostasis, HSL appears to play an important role by providing signals for transcriptional regulation. Detailed knowledge about these processes is necessary in order to explore HSL as a therapeutic target for treatment of metabolic diseases.

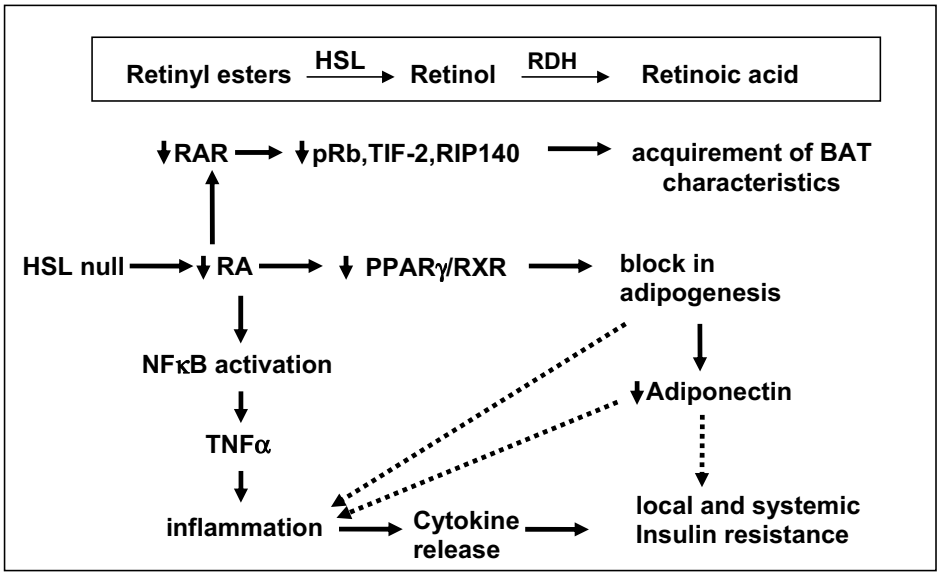

Figure 12 Working hypothesis describing the mechanisms underlying the observed phenotype of the HSL null mice. Via the generation of retinoid ligands HSL is important for adipocyte differentiation into the white adipocyte lineage, for adipogenesis and survival of the mature adipocyte, for the prevention of inflammation and cytokine release from the adipocyte and finally for the maintenance of insulin sensitivity.

CONCLUDING REMARKS

This thesis shows that in addition to its key role as an adipocyte acylglycerol lipase, HSL appears to play an important role in adipose tissue biology by providing retinoic acid for transcriptional regulation and various lipid signalling events. Through studies on mice where HSL has been deleted, several new functions of this lipase have been suggested. HSL seems to be important for maintaining insulin sensitivity in WAT but also on a systemic level. Further functions of HSL in WAT seem to include the regulation of inflammation, determination and differentiation and/or survival of mature adipocytes. An important effect of HSL seems to be the mobilization of stored RE. By regulating this process in the adipocyte, the enzyme ensures the delivery of important ligands involved in several processes. The absence of HSL leads to deceased levels of RA, and decreased signaling by this molecule. Inflammation and adipogenesis as well as the attainment of BAT characteristics of WAT could be explained by an inability of the adipocyte to generate RA in the absence of HSL. An increased inflammation could secondarily affect insulin sensitivity both locally and systemically through a decreased ability to release cytokines.

The major findings of the individual papers are listed below.

Paper I

This paper demonstrates that a disruption of HSL results in a lean mouse with impaired insulin sensitivity. A mild insulin resistance, seen in multiple tissues including WAT, muscle and liver is likely a confounding factor to the increased fasted levels of circulating insulin levels. However, due to a compensatory increase in insulin secretion by the pancreatic β-cells, plasma glucose levels are only slightly elevated. Also, whereas HSL appears to be the sole lipase capable of hydrolyzing DGs in the adipocyte, the presence of an alternative TG lipase in adipose tissue is suggested.

Paper II

This paper demonstrates a local inflammation in WAT of the non-obese HSL null mouse fed either a control diet or a HFD. An increased infiltration of macrophages into WAT is associated with the inflammation. New methodological aspects of analyzing data generated by 2D-PAGE are also presented.

Paper III

This paper describes a resistance to HFD-induced obesity of HSL null mice, possibly due to increased energy expenditure. A less differentiated WAT is also shown. This paper also reveals that WAT of HSL null mice attains BAT characteristics, functionally manifested as increased oxygen consumption and uncoupling activity. Although the underlying mechanisms and the physiological relevance remain to be resolved, these data recognize a role of HSL in the determination of cell fate during adipocyte differentiation.

Paper IV

This paper shows that HSL is the major RE hydrolase of white and brown adipocytes. In its absence in WAT, retinoid metabolism is perturbed, potentially leading to failure to provide RA for signaling events that are crucial for determination of adipocyte fate. The data also underscores the importance of the internal RE stores for WAT function and, furthermore, that

plasma retinol cannot compensate for failure to generate RA within WAT. The generation of RA is suggested as an important function of HSL in WAT.

Future studies

This new role of HSL in adipose tissue biology needs to be extensively verified. Although the rescue experiments where atRA was administered in the diet, strongly suggest that RA is an important ligand that HSL helps to generate, further studies need to be done. The concentration of RA administered could be further optimized. Measuring the concentration of RA in WAT after the RA intervention could help in the assessment of an optimal dose of RA administration. Further studies, using receptor selective ligands, such as rexinoids, could help distinguish between different pathways of HSL-mediated signaling.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Fetma och övervikt har nått epidemiska proportioner runt om i världen. Världshälsoorganisationen har i en mätning från 2005 uppskattat antalet överviktiga till 1.6 miljarder och antalet människor med kraftig övervikt (dvs. fetma) till 400 miljoner, ett antal som ökar i oroande hastighet. Med övervikt och fetma ökar risken för följdsjukdomar såsom diabetes, högt blodtryck, åderförkalkning och hjärt-kärlsjukdomar. Faktorer som starkt bidrar till ökningen av övervikt är försämrade kostvanor och en stillasittande livsstil. Evolutionärt sett har förmågan att lagra energi i form av fett varit absolut nödvändig för överlevnad under perioder av svält. I dagens samhälle råder dock ett överskott på fettintag och kroppens förmåga att lagra energi är inte längre enbart till gagn.

Mitt arbete har berört ett enzym som heter hormon-känsligt lipas (HKL). Detta enzym är viktigt för frigörandet av energi från fettväven. I kroppen lagras energi främst i form av triglycerider i fettväven. Vid fasta ökar aktiviteten av HKL, vilket medför en ökad förmåga att bryta ner triglycerider till fettsyror som kroppens övriga celler kan använda som energi. Till hjälp i mina studier har jag haft en musmodell där genen för HKL har slagits ut (HKL null mus), vilket innebär att dessa möss har en total avsaknad av detta enzym. Fokus har legat på fettväven, vilket är den vävnad som normalt sett innehåller den högsta HKL-koncentrationen.

Den ursprungliga och till synes logiska tanken var att om man tar bort HKL och sänker förmågan att bryta ner fett, borde mössen bli fetare. Detta visade sig vara fel eftersom ingen ökning i vare sig kroppsvikt eller fettmängd kunde ses i HKL null musen. Däremot kunde en mild insulinresistens påvisas i dessa möss (Arbete I). Insulin är ett livsnödvändigt hormon som frisätts från bukspottskörteln när nivåerna av blodsocker (glukos) stiger. Huvudsyftet för insulin är att sänka nivån av glukos i blodet genom att stimulera olika vävnader såsom muskler och fett att öka sitt upptag av glukos, samt att signalera till levern att stänga av sin glukosproduktion. Vid insulinresistens syns en minskad förmåga hos de olika vävnaderna att svara på insulin vilket innebär att högre doser av insulin är nödvändiga för att få samma effekt. Insulinresistens kunde påvisas i fettväv, muskel och lever. Blodnivåerna av glukos var dock enbart måttligt förhöjda pga. en kompensatorisk ökad utsöndring av insulin från bukspottskörteln.

Oförmågan att gå upp i vikt blev ännu mer påtaglig efter att mössen blivit utfodrade med en fettrik diet. Den kraftiga viktökning som observerades i normala möss och som var förknippad med en massivt ökad fettväv, kunde inte ses i HKL null möss. I arbete II användes s.k. proteomikteknik för att närmare undersöka förändringarna i fettväven hos HKL null mössen. Med hjälp av denna teknik kunde ökade nivåer av inflammatoriska proteiner påvisas i fettväv från möss utan HKL. Dessa initiala fynd bekräftades genom att mäta mängden av tumörnekrotisk faktor α vilket är ett protein som är förknippat med inflammation och som utsöndras från fettväven. Vidare kunde också ett ökat antal makrofager ses i fettväv från HKL null möss, vilket är associerat med den inflammation i fettväven som syns vid fetma och som kan leda till insulin resistens. Hypotesen som sattes upp föreslår att HKL är nödvändig för genererandet av en faktor som normalt sätt reglerar uppkomsten av inflammation i fettväven.

I arbete III gjordes en vidare utredning av oförmågan att gå upp i vikt hos HKL null musen. Det visade sig att faktorer som födointag, fettuptag i tarmen och fysisk aktivitet var oförändrade därför kunde avskrivas som tänkbara mekanismer. Däremot var den basala energiförbrukningen högre i HKL null musen. I möss finns förutom den vita fettväven även en speciell brun fettväv som har en temperaturreglerande funktion vid exempelvis dvala och kyla. Medan vit fettväv är specialiserad på att lagra fett, så har den bruna fettväven en hög förmåga till energiförbrukning via en process som kallas för termogenes. Det som skiljer termogenes från energiförbrukning i övriga celler är att det produceras värme istället för energi (ATP). Detta beror på uttrycket av ett unikt protein (UCP-1) som frikopplar ATP bildningen i cellernas mitokondrier, till förmån för värmeproduktion.
En kraftigt förhöjd mängd av UCP-1 protein kunde påvisas i vit fettväv hos HSL null möss. Sänkta nivåer av regulatoriska faktorer som är involverade i bildning av vita fettceller kunde även ses och tillsammans tyder detta på att en frånvaro av HKL leder till att de vita fettcellerna får brun fettcells-karaktär. En ökad energiförbrukning kunde ses i vit fettväv från HKL null musen, vilket stärker tidigare fynd.
Ett normalt sätt för fettväven att hantera ett ökat fettintag är att expandera i storlek. Detta sker genom att befintliga fettceller ökar sin fettinlagring och ökar i storlek, men även genom utmognad av nya fettceller från omogna celler i fettväven. HKL har visat sig viktig även för fettcellsutmognad och/eller överlevnad av mogna fettceller eftersom minskade nivåer av protein som är typiska för mogna fettceller kunde ses i fettväv från HKL null möss.

I arbete IV gjordes ett försök att utreda om HKLs förmåga att bryta ner lagringsformen av vitamin A, s.k retinylestrar (RE), kunde vara viktig för fettcellsutvecklingen, och således kunna vara en mekanism genom vilken HKL påverkar fettcellsomvandling. Den aktiva substansen av vitamin A, dvs. retinsyra, har tidigare visat sig vara viktig för utmognaden av vita fettceller, och fettväven är näst levern den största lagringsplatsen för vitamin A i kroppen. Förmågan att bryta ner RE visade sig vara kraftigt nedsatt i fettväv från HKL null möss. Vidare analyser visade att nivåerna av RE var förhöjda medan nivåerna av vitamin A och retinsyra var sänkta i fettväv från HKL null möss. Sänkt uttryck av gener som normalt sätt är positivt reglerade av retinsyra kunde också ses. Ett försök att återställa HKL musen gjordes genom att tillsätta retinsyra i kosten. Detta ledde till en 40%-ig ökning av den vita fettväven i HKL null musen vilket visar på att tillförsel av retinsyra delvis kunde återställa fettcellsutvecklingen i denna mus. Ökade nivåer av flera faktorer som är nödvändiga för fettcellsutmognad kunde ses efter retinsyratillskott i HKL null musen.

Sammanfattningsvis visar mina studier att HKL är viktig för utmognad av fettceller och/eller överlevnad av mogna fettceller genom att bilda retinsyra. Frånvaro av HKL leder till ofullständig fettcellsutmognad och även till inflammation i fettväven, vilket i sin tur leder till insulinresistens i vävnaden.

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Finally, I have come to the last part for me to write in this thesis. However, as I am almost out of words (and time) I will keep this short. There are nonetheless a couple of people that I would like to take the opportunity to thank.

I would like to start out by thanking my supervisor **Cecilia Holm** for giving me the opportunity to remain in the lab to do my PhD studies, after my initial 20 week "prao". I have really enjoyed my time as a PhD student in your group, not only because the project was very interesting and challenging, but also for the fantastic climate in the group. You have always allowed me to work very independently, but have still been very active in the project and have always been available when strange results needed debating or questions started to accumulate, which they did frequenly. It's also nice to see that there are more night owles out there. You are one of the few if not the only person I know who will respond to mails at 2 am in the morning, and still be up early in the morning.

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REFERENCES

1. O'Rahilly, S., and I. S. Farooqi. 2006. Genetics of obesity. *Philosophical transactions of the Royal Society of London* 361: 1095-1105.
2. Rosen, E. D., and B. M. Spiegelman. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 444: 847-853.
3. Cannon, B., and J. Nedergaard. 2004. Brown adipose tissue: function and physiological significance. *Physiological reviews* 84: 277-359.
4. Despres, J. P., and I. Lemieux. 2006. Abdominal obesity and metabolic syndrome. *Nature* 444: 881-887.
5. Carey, D. G., A. B. Jenkins, L. V. Campbell, J. Freund, and D. J. Chisholm. 1996. Abdominal fat and insulin resistance in normal and overweight women: Direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes* 45: 633-638.
6. Montague, C. T., and S. O'Rahilly. 2000. The perils of portliness: causes and consequences of visceral adiposity. *Diabetes* 49: 883-888.
7. Kahn, S. E., R. L. Hull, and K. M. Utzschneider. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444: 840-846.
8. Wajchenberg, B. L. 2000. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocrine reviews* 21: 697-738.
9. Reaven, G. M. 1995. The fourth musketeer--from Alexandre Dumas to Claude Bernard. *Diabetologia* 38: 3-13.
10. Schwartz, M. W., S. C. Woods, D. Porte, Jr., R. J. Seeley, and D. G. Baskin. 2000. Central nervous system control of food intake. *Nature* 404: 661-671.
11. Bouchard, C., A. Tremblay, J. P. Despres, A. Nadeau, P. J. Lupien, G. Theriault, J. Dussault, S. Moorjani, S. Pinault, and G. Fournier. 1990. The response to long-term overfeeding in identical twins. *The New England journal of medicine* 322: 1477-1482.
12. Levine, J. A., N. L. Eberhardt, and M. D. Jensen. 1999. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 283: 212-214.
13. Eckel, R. H., S. M. Grundy, and P. Z. Zimmet. 2005. The metabolic syndrome. *Lancet* 365: 1415-1428.
14. Moller, D. E., and K. D. Kaufman. 2005. Metabolic syndrome: a clinical and molecular perspective. *Annual review of medicine* 56: 45-62.
15. Weisberg, S. P., D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante, Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112: 1796-1808.
16. Kissebah, A. H., G. E. Sonnenberg, J. Myklebust, M. Goldstein, K. Broman, R. G. James, J. A. Marks, G. R. Krakower, H. J. Jacob, J. Weber, L. Martin, J. Blangero, and A. G. Comuzzie. 2000. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci U S A* 97: 14478-14483.
17. Benjamin, S. M., R. Valdez, L. S. Geiss, D. B. Rolka, and K. M. Narayan. 2003. Estimated number of adults with prediabetes in the US in 2000: opportunities for prevention. *Diabetes care* 26: 645-649.
18. Schwartz, M. W., S. C. Woods, R. J. Seeley, G. S. Barsh, D. G. Baskin, and R. L. Leibel. 2003. Is the energy homeostasis system inherently biased toward weight gain? *Diabetes* 52: 232-238.
19. Woods, S. C., E. C. Lotter, L. D. McKay, and D. Porte, Jr. 1979. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* 282: 503-505.
20. Shepherd, P. R., and B. B. Kahn. 1999. Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. *The New England journal of medicine* 341: 248-257.

21. Bell, G. I., T. Kayano, J. B. Buse, C. F. Burant, J. Takeda, D. Lin, H. Fukumoto, and S. Seino. 1990. Molecular biology of mammalian glucose transporters. *Diabetes care* 13: 198-208.
22. Bruning, J. C., J. Winnay, S. Bonner-Weir, S. I. Taylor, D. Accili, and C. R. Kahn. 1997. Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. *Cell* 88: 561-572.
23. Roden, M., and G. I. Shulman. 1999. Applications of NMR spectroscopy to study muscle glycogen metabolism in man. *Annual review of medicine* 50: 277-290.
24. Petersen, K. F., and G. I. Shulman. 2006. Etiology of insulin resistance. *The American journal of medicine* 119: S10-16.
25. Saltiel, A. R., and C. R. Kahn. 2001. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414: 799-806.
26. Kim, J. K., A. Zisman, J. J. Fillmore, O. D. Peroni, K. Kotani, P. Perret, H. Zong, J. Dong, C. R. Kahn, B. B. Kahn, and G. I. Shulman. 2001. Glucose toxicity and the development of diabetes in mice with muscle-specific inactivation of GLUT4. *J Clin Invest* 108: 153-160.
27. Carvalho, E., K. Kotani, O. D. Peroni, and B. B. Kahn. 2005. Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. *Am J Physiol Endocrinol Metab* 289: E551-561.
28. Shepherd, P. R., L. Gnudi, E. Tozzo, H. Yang, F. Leach, and B. B. Kahn. 1993. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. *J Biol Chem* 268: 22243-22246.
29. Kotani, K., O. D. Peroni, Y. Minokoshi, O. Boss, and B. B. Kahn. 2004. GLUT4 glucose transporter deficiency increases hepatic lipid production and peripheral lipid utilization. *J Clin Invest* 114: 1666-1675.
30. Okada, T., C. W. Liew, J. Hu, C. Hinault, M. D. Michael, J. Krtzfeldt, C. Yin, M. Holzenberger, M. Stoffel, and R. N. Kulkarni. 2007. Insulin receptors in beta-cells are critical for islet compensatory growth response to insulin resistance. *Proc Natl Acad Sci U S A* 104: 8977-8982.
31. Boden, G., and M. Laakso. 2004. Lipids and glucose in type 2 diabetes: what is the cause and effect? *Diabetes care* 27: 2253-2259.
32. Savage, D. B., K. F. Petersen, and G. I. Shulman. 2007. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiological reviews* 87: 507-520.
33. Jacobsson, B., G. Holm, P. Bjorntorp, and U. Smith. 1976. Influence of cell size on the effects of insulin and noradrenaline on human adipose tissue. *Diabetologia* 12: 69-72.
34. Goldrick, R. B. 1967. Effects of insulin on glucose metabolism in isolated human fat cells. *J Lipid Res* 8: 581-588.
35. Bluher, M., M. D. Michael, O. D. Peroni, K. Ueki, N. Carter, B. B. Kahn, and C. R. Kahn. 2002. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev Cell* 3: 25-38.
36. Randle, P. J., P. B. Garland, C. N. Hales, and E. A. Newsholme. 1963. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1: 785-789.
37. Randle, P. J., D. A. Priestman, S. C. Mistry, and A. Halsall. 1994. Glucose fatty acid interactions and the regulation of glucose disposal. *Journal of cellular biochemistry* 55 Suppl: 1-11.
38. Griffin, M. E., M. J. Marcucci, G. W. Cline, K. Bell, N. Barucci, D. Lee, L. J. Goodyear, E. W. Kraegen, M. F. White, and G. I. Shulman. 1999. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* 48: 1270-1274.
39. Boden, G. 2001. Free fatty acids-the link between obesity and insulin resistance. *Endocr Pract* 7: 44-51.
40. Boden, G. 2002. Interaction between free fatty acids and glucose metabolism. *Current opinion in clinical nutrition and metabolic care* 5: 545-549.

41. Hwang, J. H., J. W. Pan, S. Heydari, H. P. Hetherington, and D. T. Stein. 2001. Regional differences in intramyocellular lipids in humans observed by in vivo 1H-MR spectroscopic imaging. *J Appl Physiol* 90: 1267-1274.
42. Shulman, G. I. 2000. Cellular mechanisms of insulin resistance. *J Clin Invest* 106: 171-176.
43. Boden, G. 2006. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. *Current diabetes reports* 6: 177-181.
44. Weyer, C., T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R. E. Pratley, and P. A. Tataranni. 2001. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *The Journal of clinical endocrinology and metabolism* 86: 1930-1935.
45. Shoelson, S. E., J. Lee, and A. B. Goldfine. 2006. Inflammation and insulin resistance. *J Clin Invest* 116: 1793-1801.
46. Moitra, J., M. M. Mason, M. Olive, D. Krylov, O. Gavrilova, B. Marcus-Samuels, L. Feigenbaum, E. Lee, T. Aoyama, M. Eckhaus, M. L. Reitman, and C. Vinson. 1998. Life without white fat: a transgenic mouse. *Genes Dev* 12: 3168-3181.
47. Garg, A. 2000. Lipodystrophies. *The American journal of medicine* 108: 143-152.
48. Gavrilova, O., B. Marcus-Samuels, D. Graham, J. K. Kim, G. I. Shulman, A. L. Castle, C. Vinson, M. Eckhaus, and M. L. Reitman. 2000. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* 105: 271-278.
49. Shimomura, I., R. E. Hammer, S. Ikemoto, M. S. Brown, and J. L. Goldstein. 1999. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 401: 73-76.
50. Unger, R. H. 1995. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 44: 863-870.
51. Terauchi, Y., I. Takamoto, N. Kubota, J. Matsui, R. Suzuki, K. Komeda, A. Hara, Y. Toyoda, I. Miwa, S. Aizawa, S. Tsutsumi, Y. Tsubamoto, S. Hashimoto, K. Eto, A. Nakamura, M. Noda, K. Tobe, H. Aburatani, R. Nagai, and T. Kadowaki. 2007. Glucokinase and IRS-2 are required for compensatory beta cell hyperplasia in response to high-fat diet-induced insulin resistance. *J Clin Invest* 117: 246-257.
52. Kulkarni, R. N., J. C. Bruning, J. N. Winnay, C. Postic, M. A. Magnuson, and C. R. Kahn. 1999. Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96: 329-339.
53. Prentki, M., E. Joly, W. El-Assaad, and R. Roduit. 2002. Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. *Diabetes* 51 Suppl 3: S405-413.
54. Lee, Y., H. Hirose, M. Ohneda, J. H. Johnson, J. D. McGarry, and R. H. Unger. 1994. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A* 91: 10878-10882.
55. Mering, J., and O. Minkowski. 1890. Diabetes mellitus nach Pankreasexstirpation. *Arch Exp Pathol Pharmakol* 26.
56. Banting, F. G., and C. H. Best. 1922. The internal secretion of the pancreas. 1922. *J Lab Clin Invest* 7: 251-266.
57. Wild, S., G. Roglic, A. Green, R. Sicree, and H. King. 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes care* 27: 1047-1053.
58. Roglic, G., N. Unwin, P. H. Bennett, C. Mathers, J. Tuomilehto, S. Nag, V. Connolly, and H. King. 2005. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. *Diabetes care* 28: 2130-2135.
59. Buchanan, T. A., and A. H. Xiang. 2005. Gestational diabetes mellitus. *J Clin Invest* 115: 485-491.
60. Fajans, S. S., G. I. Bell, and K. S. Polonsky. 2001. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *The New England journal of medicine* 345: 971-980.
61. Leung, N., T. Sakaue, A. Carpentier, K. Uffelman, A. Giacca, and G. F. Lewis. 2004. Prolonged increase of plasma non-esterified fatty acids fully abolishes the stimulatory effect

of 24 hours of moderate hyperglycaemia on insulin sensitivity and pancreatic beta-cell function in obese men. *Diabetologia* 47: 204-213.
62. Ailhaud, G., P. Grimaldi, and R. Negrel. 1992. Cellular and molecular aspects of adipose tissue development. *Annu Rev Nutr* 12: 207-233.
63. Cinti, S. 2005. The adipose organ. *Prostaglandins, leukotrienes, and essential fatty acids* 73: 9-15.
64. Arner, P. 2001. Free fatty acids--do they play a central role in type 2 diabetes? *Diabetes, obesity & metabolism* 3 Suppl 1: S11-19.
65. Trayhurn, P. 2005. The biology of obesity. *The Proceedings of the Nutrition Society* 64: 31-38.
66. Stralfors, P. 1990. Autolysis of isolated adipocytes by endogenously produced fatty acids. *FEBS Lett* 263: 153-154.
67. Frayn, K. N. 2002. Adipose tissue as a buffer for daily lipid flux. *Diabetologia* 45: 1201-1210.
68. Danforth, E., Jr. 2000. Failure of adipocyte differentiation causes type II diabetes mellitus? *Nat Genet* 26: 13.
69. Okuno, A., H. Tamemoto, K. Tobe, K. Ueki, Y. Mori, K. Iwamoto, K. Umesono, Y. Akanuma, T. Fujiwara, H. Horikoshi, Y. Yazaki, and T. Kadowaki. 1998. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J Clin Invest* 101: 1354-1361.
70. Frayn, K. N., F. Karpe, B. A. Fielding, I. A. Macdonald, and S. W. Coppack. 2003. Integrative physiology of human adipose tissue. *Int J Obes Relat Metab Disord* 27: 875-888.
71. Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-432.
72. Timmons, J. A., K. Wennmalm, O. Larsson, T. B. Walden, T. Lassmann, N. Petrovic, D. L. Hamilton, R. E. Gimeno, C. Wahlestedt, K. Baar, J. Nedergaard, and B. Cannon. 2007. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc Natl Acad Sci U S A* 104: 4401-4406.
73. Dani, C., A. G. Smith, S. Dessolin, P. Leroy, L. Staccini, P. Villageois, C. Darimont, and G. Ailhaud. 1997. Differentiation of embryonic stem cells into adipocytes in vitro. *Journal of cell science* 110 (Pt 11): 1279-1285.
74. Green, H., and O. Kehinde. 1975. An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. *Cell* 5: 19-27.
75. Green, H., and O. Kehinde. 1976. Spontaneous heritable changes leading to increased adipose conversion in 3T3 cells. *Cell* 7: 105-113.
76. Jiang, H. P., and G. Serrero. 1992. Isolation and characterization of a full-length cDNA coding for an adipose differentiation-related protein. *Proc Natl Acad Sci U S A* 89: 7856-7860.
77. Brasaemle, D. L., T. Barber, N. E. Wolins, G. Serrero, E. J. Blanchette-Mackie, and C. Londos. 1997. Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. *J Lipid Res* 38: 2249-2263.
78. Ross, S. E., N. Hemati, K. A. Longo, C. N. Bennett, P. C. Lucas, R. L. Erickson, and O. A. MacDougald. 2000. Inhibition of adipogenesis by Wnt signaling. *Science* 289: 950-953.
79. Ross, S. E., R. L. Erickson, I. Gerin, P. M. DeRose, L. Bajnok, K. A. Longo, D. E. Misek, R. Kuick, S. M. Hanash, K. B. Atkins, S. M. Andresen, H. I. Nebb, L. Madsen, K. Kristiansen, and O. A. MacDougald. 2002. Microarray analyses during adipogenesis: understanding the effects of Wnt signaling on adipogenesis and the roles of liver X receptor alpha in adipocyte metabolism. *Mol Cell Biol* 22: 5989-5999.
80. Cornelius, P., O. A. MacDougald, and M. D. Lane. 1994. Regulation of adipocyte development. *Annu Rev Nutr* 14: 99-129.
81. Spiegelman, B. M., and S. R. Farmer. 1982. Decreases in tubulin and actin gene expression prior to morphological differentiation of 3T3 adipocytes. *Cell* 29: 53-60.

82. Umek, R. M., A. D. Friedman, and S. L. McKnight. 1991. CCAAT-enhancer binding protein: a component of a differentiation switch. *Science* 251: 288-292.
83. Krotkiewski, M., P. Bjorntorp, L. Sjostrom, and U. Smith. 1983. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J Clin Invest* 72: 1150-1162.
84. Rosen, E. D., C. J. Walkey, P. Puigserver, and B. M. Spiegelman. 2000. Transcriptional regulation of adipogenesis. *Genes Dev* 14: 1293-1307.
85. Gottlicher, M., E. Widmark, Q. Li, and J. A. Gustafsson. 1992. Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc Natl Acad Sci U S A* 89: 4653-4657.
86. Brun, R. P., P. Tontonoz, B. M. Forman, R. Ellis, J. Chen, R. M. Evans, and B. M. Spiegelman. 1996. Differential activation of adipogenesis by multiple PPAR isoforms. *Genes Dev* 10: 974-984.
87. Amri, E. Z., F. Bonino, G. Ailhaud, N. A. Abumrad, and P. A. Grimaldi. 1995. Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors. *J Biol Chem* 270: 2367-2371.
88. Berger, J., M. D. Leibowitz, T. W. Doebber, A. Elbrecht, B. Zhang, G. Zhou, C. Biswas, C. A. Cullinan, N. S. Hayes, Y. Li, M. Tanen, J. Ventre, M. S. Wu, G. D. Berger, R. Mosley, R. Marquis, C. Santini, S. P. Sahoo, R. L. Tolman, R. G. Smith, and D. E. Moller. 1999. Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPARdelta ligands produce distinct biological effects. *J Biol Chem* 274: 6718-6725.
89. Chawla, A., E. J. Schwarz, D. D. Dimaculangan, and M. A. Lazar. 1994. Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology* 135: 798-800.
90. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev* 8: 1224-1234.
91. Zhu, Y., C. Qi, J. R. Korenberg, X. N. Chen, D. Noya, M. S. Rao, and J. K. Reddy. 1995. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. *Proc Natl Acad Sci U S A* 92: 7921-7925.
92. Fajas, L., D. Auboeuf, E. Raspe, K. Schoonjans, A. M. Lefebvre, R. Saladin, J. Najib, M. Laville, J. C. Fruchart, S. Deeb, A. Vidal-Puig, J. Flier, M. R. Briggs, B. Staels, H. Vidal, and J. Auwerx. 1997. The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem* 272: 18779-18789.
93. Kliewer, S. A., K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans. 1992. Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature* 358: 771-774.
94. Gearing, K. L., M. Gottlicher, M. Teboul, E. Widmark, and J. A. Gustafsson. 1993. Interaction of the peroxisome-proliferator-activated receptor and retinoid X receptor. *Proc Natl Acad Sci U S A* 90: 1440-1444.
95. Tontonoz, P., S. Singer, B. M. Forman, P. Sarraf, J. A. Fletcher, C. D. Fletcher, R. P. Brun, E. Mueller, S. Altiok, H. Oppenheim, R. M. Evans, and B. M. Spiegelman. 1997. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. *Proc Natl Acad Sci U S A* 94: 237-241.
96. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell* 79: 1147-1156.
97. Rosen, E. D., P. Sarraf, A. E. Troy, G. Bradwin, K. Moore, D. S. Milstone, B. M. Spiegelman, and R. M. Mortensen. 1999. PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell* 4: 611-617.
98. Barak, Y., M. C. Nelson, E. S. Ong, Y. Z. Jones, P. Ruiz-Lozano, K. R. Chien, A. Koder, and R. M. Evans. 1999. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell* 4: 585-595.
99. He, W., Y. Barak, A. Hevener, P. Olson, D. Liao, J. Le, M. Nelson, E. Ong, J. M. Olefsky, and R. M. Evans. 2003. Adipose-specific peroxisome proliferator-activated receptor gamma

knockout causes insulin resistance in fat and liver but not in muscle. *Proc Natl Acad Sci U S A* 100: 15712-15717.
100. Christy, R. J., K. H. Kaestner, D. E. Geiman, and M. D. Lane. 1991. CCAAT/enhancer binding protein gene promoter: binding of nuclear factors during differentiation of 3T3-L1 preadipocytes. *Proc Natl Acad Sci U S A* 88: 2593-2597.
101. Cao, Z., R. M. Umek, and S. L. McKnight. 1991. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. *Genes Dev* 5: 1538-1552.
102. Yeh, W. C., Z. Cao, M. Classon, and S. L. McKnight. 1995. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev* 9: 168-181.
103. Lin, F. T., O. A. MacDougald, A. M. Diehl, and M. D. Lane. 1993. A 30-kDa alternative translation product of the CCAAT/enhancer binding protein alpha message: transcriptional activator lacking antimitotic activity. *Proc Natl Acad Sci U S A* 90: 9606-9610.
104. Zuo, Y., L. Qiang, and S. R. Farmer. 2006. Activation of CCAAT/enhancer-binding protein (C/EBP) alpha expression by C/EBP beta during adipogenesis requires a peroxisome proliferator-activated receptor-gamma-associated repression of HDAC1 at the C/ebp alpha gene promoter. *J Biol Chem* 281: 7960-7967.
105. Tanaka, T., N. Yoshida, T. Kishimoto, and S. Akira. 1997. Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. *Embo J* 16: 7432-7443.
106. Schwarz, E. J., M. J. Reginato, D. Shao, S. L. Krakow, and M. A. Lazar. 1997. Retinoic acid blocks adipogenesis by inhibiting C/EBPbeta-mediated transcription. *Mol Cell Biol* 17: 1552-1561.
107. Freytag, S. O., D. L. Paielli, and J. D. Gilbert. 1994. Ectopic expression of the CCAAT/enhancer-binding protein alpha promotes the adipogenic program in a variety of mouse fibroblastic cells. *Genes Dev* 8: 1654-1663.
108. Wang, N. D., M. J. Finegold, A. Bradley, C. N. Ou, S. V. Abdelsayed, M. D. Wilde, L. R. Taylor, D. R. Wilson, and G. J. Darlington. 1995. Impaired energy homeostasis in C/EBP alpha knockout mice. *Science* 269: 1108-1112.
109. Wu, Z., E. D. Rosen, R. Brun, S. Hauser, G. Adelmant, A. E. Troy, C. McKeon, G. J. Darlington, and B. M. Spiegelman. 1999. Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. *Mol Cell* 3: 151-158.
110. Rosen, E. D., C. H. Hsu, X. Wang, S. Sakai, M. W. Freeman, F. J. Gonzalez, and B. M. Spiegelman. 2002. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. *Genes Dev* 16: 22-26.
111. Tontonoz, P., J. B. Kim, R. A. Graves, and B. M. Spiegelman. 1993. ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. *Mol Cell Biol* 13: 4753-4759.
112. Kim, J. B., and B. M. Spiegelman. 1996. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. *Genes Dev* 10: 1096-1107.
113. Kim, J. B., H. M. Wright, M. Wright, and B. M. Spiegelman. 1998. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. *Proc Natl Acad Sci U S A* 95: 4333-4337.
114. Imai, T., R. Takakuwa, S. Marchand, E. Dentz, J. M. Bornert, N. Messaddeq, O. Wendling, M. Mark, B. Desvergne, W. Wahli, P. Chambon, and D. Metzger. 2004. Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. *Proc Natl Acad Sci U S A* 101: 4543-4547.
115. Chen, Z., J. I. Torrens, A. Anand, B. M. Spiegelman, and J. M. Friedman. 2005. Krox20 stimulates adipogenesis via C/EBPbeta-dependent and -independent mechanisms. *Cell Metab* 1: 93-106.
116. Oishi, Y., I. Manabe, K. Tobe, K. Tsushima, T. Shindo, K. Fujiu, G. Nishimura, K. Maemura, T. Yamauchi, N. Kubota, R. Suzuki, T. Kitamura, S. Akira, T. Kadowaki, and R.

Nagai. 2005. Kruppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. *Cell Metab* 1: 27-39.
117. Tang, Q. Q., T. C. Otto, and M. D. Lane. 2003. Mitotic clonal expansion: a synchronous process required for adipogenesis. *Proc Natl Acad Sci U S A* 100: 44-49.
118. Chen, P. L., D. J. Riley, Y. Chen, and W. H. Lee. 1996. Retinoblastoma protein positively regulates terminal adipocyte differentiation through direct interaction with C/EBPs. *Genes Dev* 10: 2794-2804.
119. Rosen, E. D., and O. A. MacDougald. 2006. Adipocyte differentiation from the inside out. *Nature reviews* 7: 885-896.
120. Onate, S. A., S. Y. Tsai, M. J. Tsai, and B. W. O'Malley. 1995. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* 270: 1354-1357.
121. Ge, K., M. Guermah, C. X. Yuan, M. Ito, A. E. Wallberg, B. M. Spiegelman, and R. G. Roeder. 2002. Transcription coactivator TRAP220 is required for PPAR gamma 2-stimulated adipogenesis. *Nature* 417: 563-567.
122. Farmer, S. R. 2006. Transcriptional control of adipocyte formation. *Cell Metab* 4: 263-273.
123. Kershaw, E. E., and J. S. Flier. 2004. Adipose tissue as an endocrine organ. *The Journal of clinical endocrinology and metabolism* 89: 2548-2556.
124. Friedman, J. M., and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. *Nature* 395: 763-770.
125. Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley, and J. M. Friedman. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269: 543-546.
126. Collins, S., C. M. Kuhn, A. E. Petro, A. G. Swick, B. A. Chrunyk, and R. S. Surwit. 1996. Role of leptin in fat regulation. *Nature* 380: 677.
127. Shimabukuro, M., K. Koyama, G. Chen, M. Y. Wang, F. Trieu, Y. Lee, C. B. Newgard, and R. H. Unger. 1997. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. *Proc Natl Acad Sci U S A* 94: 4637-4641.
128. Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, and et al. 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1: 1155-1161.
129. Halaas, J. L., C. Boozer, J. Blair-West, N. Fidahusein, D. A. Denton, and J. M. Friedman. 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci U S A* 94: 8878-8883.
130. Petersen, K. F., E. A. Oral, S. Dufour, D. Befroy, C. Ariyan, C. Yu, G. W. Cline, A. M. DePaoli, S. I. Taylor, P. Gorden, and G. I. Shulman. 2002. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *J Clin Invest* 109: 1345-1350.
131. Scherer, P. E., S. Williams, M. Fogliano, G. Baldini, and H. F. Lodish. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270: 26746-26749.
132. Hu, E., P. Liang, and B. M. Spiegelman. 1996. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271: 10697-10703.
133. Arita, Y., S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi, and Y. Matsuzawa. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and biophysical research communications* 257: 79-83.
134. Yang, W. S., W. J. Lee, T. Funahashi, S. Tanaka, Y. Matsuzawa, C. L. Chao, C. L. Chen, T. Y. Tai, and L. M. Chuang. 2001. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *The Journal of clinical endocrinology and metabolism* 86: 3815-3819.
135. Yamauchi, T., J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M. L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura,

M. Tomita, P. Froguel, and T. Kadowaki. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7: 941-946.
136. Combs, T. P., A. H. Berg, S. Obici, P. E. Scherer, and L. Rossetti. 2001. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* 108: 1875-1881.
137. Zou, C., and J. Shao. 2007. Role of adipocytokines in obesity-associated insulin resistance. *J Nutr Biochem*.
138. Maeda, N., I. Shimomura, K. Kishida, H. Nishizawa, M. Matsuda, H. Nagaretani, N. Furuyama, H. Kondo, M. Takahashi, Y. Arita, R. Komuro, N. Ouchi, S. Kihara, Y. Tochino, K. Okutomi, M. Horie, S. Takeda, T. Aoyama, T. Funahashi, and Y. Matsuzawa. 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8: 731-737.
139. Ouchi, N., S. Kihara, Y. Arita, Y. Okamoto, K. Maeda, H. Kuriyama, K. Hotta, M. Nishida, M. Takahashi, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Funahashi, and Y. Matsuzawa. 2000. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation* 102: 1296-1301.
140. Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima, and M. A. Lazar. 2001. The hormone resistin links obesity to diabetes. *Nature* 409: 307-312.
141. Patel, L., A. C. Buckels, I. J. Kinghorn, P. R. Murdock, J. D. Holbrook, C. Plumpton, C. H. Macphee, and S. A. Smith. 2003. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochemical and biophysical research communications* 300: 472-476.
142. Fukuhara, A., M. Matsuda, M. Nishizawa, K. Segawa, M. Tanaka, K. Kishimoto, Y. Matsuki, M. Murakami, T. Ichisaka, H. Murakami, E. Watanabe, T. Takagi, M. Akiyoshi, T. Ohtsubo, S. Kihara, S. Yamashita, M. Makishima, T. Funahashi, S. Yamanaka, R. Hiramatsu, Y. Matsuzawa, and I. Shimomura. 2005. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307: 426-430.
143. Tanaka, M., M. Nozaki, A. Fukuhara, K. Segawa, N. Aoki, M. Matsuda, R. Komuro, and I. Shimomura. 2007. Visfatin is released from 3T3-L1 adipocytes via a non-classical pathway. *Biochemical and biophysical research communications* 359: 194-201.
144. Yang, Q., T. E. Graham, N. Mody, F. Preitner, O. D. Peroni, J. M. Zabolotny, K. Kotani, L. Quadro, and B. B. Kahn. 2005. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 436: 356-362.
145. Sethi, J. K., and G. S. Hotamisligil. 1999. The role of TNF alpha in adipocyte metabolism. *Seminars in cell & developmental biology* 10: 19-29.
146. Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259: 87-91.
147. Kern, P. A., M. Saghizadeh, J. M. Ong, R. J. Bosch, R. Deem, and R. B. Simsolo. 1995. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 95: 2111-2119.
148. Fain, J. N., S. W. Bahouth, and A. K. Madan. 2004. TNFalpha release by the nonfat cells of human adipose tissue. *Int J Obes Relat Metab Disord* 28: 616-622.
149. Kanety, H., R. Feinstein, M. Z. Papa, R. Hemi, and A. Karasik. 1995. Tumor necrosis factor alpha-induced phosphorylation of insulin receptor substrate-1 (IRS-1). Possible mechanism for suppression of insulin-stimulated tyrosine phosphorylation of IRS-1. *J Biol Chem* 270: 23780-23784.
150. Uysal, K. T., S. M. Wiesbrock, M. W. Marino, and G. S. Hotamisligil. 1997. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389: 610-614.
151. Schreyer, S. A., S. C. Chua, Jr., and R. C. LeBoeuf. 1998. Obesity and diabetes in TNF-alpha receptor- deficient mice. *J Clin Invest* 102: 402-411.

152. Ofei, F., S. Hurel, J. Newkirk, M. Sopwith, and R. Taylor. 1996. Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. *Diabetes* 45: 881-885.
153. Mohamed-Ali, V., S. Goodrick, A. Rawesh, D. R. Katz, J. M. Miles, J. S. Yudkin, S. Klein, and S. W. Coppack. 1997. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *The Journal of clinical endocrinology and metabolism* 82: 4196-4200.
154. Klover, P. J., T. A. Zimmers, L. G. Koniaris, and R. A. Mooney. 2003. Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes* 52: 2784-2789.
155. Pedersen, B. K., and M. A. Febbraio. 2007. Point: Interleukin-6 does have a beneficial role in insulin sensitivity and glucose homeostasis. *J Appl Physiol* 102: 814-816.
156. Tontonoz, P., L. Nagy, J. G. Alvarez, V. A. Thomazy, and R. M. Evans. 1998. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* 93: 241-252.
157. Cousin, B., O. Munoz, M. Andre, A. M. Fontanilles, C. Dani, J. L. Cousin, P. Laharrague, L. Casteilla, and L. Penicaud. 1999. A role for preadipocytes as macrophage-like cells. *Faseb J* 13: 305-312.
158. Charriere, G., B. Cousin, E. Arnaud, M. Andre, F. Bacou, L. Penicaud, and L. Casteilla. 2003. Preadipocyte conversion to macrophage. Evidence of plasticity. *J Biol Chem* 278: 9850-9855.
159. Xu, H., G. T. Barnes, Q. Yang, G. Tan, D. Yang, C. J. Chou, J. Sole, A. Nichols, J. S. Ross, L. A. Tartaglia, and H. Chen. 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112: 1821-1830.
160. Curat, C. A., A. Miranville, C. Sengenes, M. Diehl, C. Tonus, R. Busse, and A. Bouloumie. 2004. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. *Diabetes* 53: 1285-1292.
161. Harman-Boehm, I., M. Bluher, H. Redel, N. Sion-Vardy, S. Ovadia, E. Avinoach, I. Shai, N. Kloting, M. Stumvoll, N. Bashan, and A. Rudich. 2007. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *The Journal of clinical endocrinology and metabolism* 92: 2240-2247.
162. Sartipy, P., and D. J. Loskutoff. 2003. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A* 100: 7265-7270.
163. Gerhardt, C. C., I. A. Romero, R. Cancello, L. Camoin, and A. D. Strosberg. 2001. Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. *Mol Cell Endocrinol* 175: 81-92.
164. Kim, C. S., H. S. Park, T. Kawada, J. H. Kim, D. Lim, N. E. Hubbard, B. S. Kwon, K. L. Erickson, and R. Yu. 2006. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *International journal of obesity (2005)* 30: 1347-1355.
165. Weisberg, S. P., D. Hunter, R. Huber, J. Lemieux, S. Slaymaker, K. Vaddi, I. Charo, R. L. Leibel, and A. W. Ferrante, Jr. 2006. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* 116: 115-124.
166. Kanda, H., S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, S. Kitazawa, H. Miyachi, S. Maeda, K. Egashira, and M. Kasuga. 2006. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* 116: 1494-1505.
167. Hirosumi, J., G. Tuncman, L. Chang, C. Z. Gorgun, K. T. Uysal, K. Maeda, M. Karin, and G. S. Hotamisligil. 2002. A central role for JNK in obesity and insulin resistance. *Nature* 420: 333-336.
168. Hotamisligil, G. S. 2006. Inflammation and metabolic disorders. *Nature* 444: 860-867.
169. Nguyen, M. T., H. Satoh, S. Favelyukis, J. L. Babendure, T. Imamura, J. I. Sbodio, J. Zalevsky, B. I. Dahiyat, N. W. Chi, and J. M. Olefsky. 2005. JNK and tumor necrosis factor-alpha mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. *J Biol Chem* 280: 35361-35371.

170. Yuan, M., N. Konstantopoulos, J. Lee, L. Hansen, Z. W. Li, M. Karin, and S. E. Shoelson. 2001. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293: 1673-1677.
171. Hundal, R. S., K. F. Petersen, A. B. Mayerson, P. S. Randhawa, S. Inzucchi, S. E. Shoelson, and G. I. Shulman. 2002. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J Clin Invest* 109: 1321-1326.
172. Arkan, M. C., A. L. Hevener, F. R. Greten, S. Maeda, Z. W. Li, J. M. Long, A. Wynshaw-Boris, G. Poli, J. Olefsky, and M. Karin. 2005. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 11: 191-198.
173. Ozcan, U., Q. Cao, E. Yilmaz, A. H. Lee, N. N. Iwakoshi, E. Ozdelen, G. Tuncman, C. Gorgun, L. H. Glimcher, and G. S. Hotamisligil. 2004. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* 306: 457-461.
174. Foster, D. O., and M. L. Frydman. 1978. Brown adipose tissue: the dominant site of nonshivering thermogenesis in the rat. *Experientia* 32: 147-151.
175. Lowell, B. B., S. S. V, A. Hamann, J. A. Lawitts, J. Himms-Hagen, B. B. Boyer, L. P. Kozak, and J. S. Flier. 1993. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 366: 740-742.
176. Cao, W., K. W. Daniel, J. Robidoux, P. Puigserver, A. V. Medvedev, X. Bai, L. M. Floering, B. M. Spiegelman, and S. Collins. 2004. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. *Mol Cell Biol* 24: 3057-3067.
177. Golozoubova, V., E. Hohtola, A. Matthias, A. Jacobsson, B. Cannon, and J. Nedergaard. 2001. Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *Faseb J* 15: 2048-2050.
178. Matthias, A., K. B. Ohlson, J. M. Fredriksson, A. Jacobsson, J. Nedergaard, and B. Cannon. 2000. 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* 275: 25073-25081.
179. Enerback, S., A. Jacobsson, E. M. Simpson, C. Guerra, H. Yamashita, M. E. Harper, and L. P. Kozak. 1997. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387: 90-94.
180. Puigserver, P., and B. M. Spiegelman. 2003. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocrine reviews* 24: 78-90.
181. Linhart, H. G., K. Ishimura-Oka, F. DeMayo, T. Kibe, D. Repka, B. Poindexter, R. J. Bick, and G. J. Darlington. 2001. C/EBPalpha is required for differentiation of white, but not brown, adipose tissue. *Proc Natl Acad Sci U S A* 98: 12532-12537.
182. Valmaseda, A., M. C. Carmona, M. J. Barbera, O. Vinas, T. Mampel, R. Iglesias, F. Villarroya, and M. Giralt. 1999. Opposite regulation of PPAR-alpha and -gamma gene expression by both their ligands and retinoic acid in brown adipocytes. *Mol Cell Endocrinol* 154: 101-109.
183. Nedergaard, J., N. Petrovic, E. M. Lindgren, A. Jacobsson, and B. Cannon. 2005. PPARgamma in the control of brown adipocyte differentiation. *Biochim Biophys Acta* 1740: 293-304.
184. Sears, I. B., M. A. MacGinnitie, L. G. Kovacs, and R. A. Graves. 1996. Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. *Mol Cell Biol* 16: 3410-3419.
185. Puigserver, P., Z. Wu, C. W. Park, R. Graves, M. Wright, and B. M. Spiegelman. 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92: 829-839.
186. Puigserver, P., J. Rhee, J. Lin, Z. Wu, J. C. Yoon, C. Y. Zhang, S. Krauss, V. K. Mootha, B. B. Lowell, and B. M. Spiegelman. 2001. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARgamma coactivator-1. *Mol Cell* 8: 971-982.
187. Lin, J., P. H. Wu, P. T. Tarr, K. S. Lindenberg, J. St-Pierre, C. Y. Zhang, V. K. Mootha, S. Jager, C. R. Vianna, R. M. Reznick, L. Cui, M. Manieri, M. X. Donovan, Z. Wu, M. P.

Cooper, M. C. Fan, L. M. Rohas, A. M. Zavacki, S. Cinti, G. I. Shulman, B. B. Lowell, D. Krainc, and B. M. Spiegelman. 2004. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell* 119: 121-135.

188. Leone, T. C., J. J. Lehman, B. N. Finck, P. J. Schaeffer, A. R. Wende, S. Boudina, M. Courtois, D. F. Wozniak, N. Sambandam, C. Bernal-Mizrachi, Z. Chen, J. O. Holloszy, D. M. Medeiros, R. E. Schmidt, J. E. Saffitz, E. D. Abel, C. F. Semenkovich, and D. P. Kelly. 2005. PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS biology* 3: e101.
189. Lin, J., C. Handschin, and B. M. Spiegelman. 2005. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab* 1: 361-370.
190. Barbera, M. J., A. Schluter, N. Pedraza, R. Iglesias, F. Villarroya, and M. Giralt. 2001. Peroxisome proliferator-activated receptor alpha activates transcription of the brown fat uncoupling protein-1 gene. A link between regulation of the thermogenic and lipid oxidation pathways in the brown fat cell. *J Biol Chem* 276: 1486-1493.
191. Lin, J., P. T. Tarr, R. Yang, J. Rhee, P. Puigserver, C. B. Newgard, and B. M. Spiegelman. 2003. PGC-1beta in the regulation of hepatic glucose and energy metabolism. *J Biol Chem* 278: 30843-30848.
192. Uldry, M., W. Yang, J. St-Pierre, J. Lin, P. Seale, and B. M. Spiegelman. 2006. Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab* 3: 333-341.
193. Picard, F., M. Gehin, J. Annicotte, S. Rocchi, M. F. Champy, B. W. O'Malley, P. Chambon, and J. Auwerx. 2002. SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell* 111: 931-941.
194. Hansen, J. B., C. Jorgensen, R. K. Petersen, P. Hallenborg, R. De Matteis, H. A. Boye, N. Petrovic, S. Enerback, J. Nedergaard, S. Cinti, H. te Riele, and K. Kristiansen. 2004. Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc Natl Acad Sci U S A* 101: 4112-4117.
195. Leonardsson, G., J. H. Steel, M. Christian, V. Pocock, S. Milligan, J. Bell, P. W. So, G. Medina-Gomez, A. Vidal-Puig, R. White, and M. G. Parker. 2004. Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc Natl Acad Sci U S A* 101: 8437-8442.
196. Cederberg, A., L. M. Gronning, B. Ahren, K. Tasken, P. Carlsson, and S. Enerback. 2001. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 106: 563-573.
197. Wu, Z., P. Puigserver, U. Andersson, C. Zhang, G. Adelmant, V. Mootha, A. Troy, S. Cinti, B. Lowell, R. C. Scarpulla, and B. M. Spiegelman. 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115-124.
198. Puigserver, P., G. Adelmant, Z. Wu, M. Fan, J. Xu, B. O'Malley, and B. M. Spiegelman. 1999. Activation of PPARgamma coactivator-1 through transcription factor docking. *Science* 286: 1368-1371.
199. Tiraby, C., G. Tavernier, C. Lefort, D. Larrouy, F. Bouillaud, D. Ricquier, and D. Langin. 2003. Acquirement of brown fat cell features by human white adipocytes. *J Biol Chem* 278: 33370-33376.
200. Spiegelman, B. M., and R. Heinrich. 2004. Biological control through regulated transcriptional coactivators. *Cell* 119: 157-167.
201. McKenna, N. J., and B. W. O'Malley. 2002. Minireview: nuclear receptor coactivators--an update. *Endocrinology* 143: 2461-2465.
202. Xu, J., Y. Qiu, F. J. DeMayo, S. Y. Tsai, M. J. Tsai, and B. W. O'Malley. 1998. Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. *Science* 279: 1922-1925.
203. Rocchi, S., F. Picard, J. Vamecq, L. Gelman, N. Potier, D. Zeyer, L. Dubuquoy, P. Bac, M. F. Champy, K. D. Plunket, L. M. Leesnitzer, S. G. Blanchard, P. Desreumaux, D. Moras, J. P. Renaud, and J. Auwerx. 2001. A unique PPARgamma ligand with potent insulin-sensitizing yet weak adipogenic activity. *Mol Cell* 8: 737-747.

204. Hoang, T., I. S. Fenne, C. Cook, B. Borud, M. Bakke, E. A. Lien, and G. Mellgren. 2004. cAMP-dependent protein kinase regulates ubiquitin-proteasome-mediated degradation and subcellular localization of the nuclear receptor coactivator GRIP1. *J Biol Chem* 279: 49120-49130.
205. Wang, Z., C. Qi, A. Krones, P. Woodring, X. Zhu, J. K. Reddy, R. M. Evans, M. G. Rosenfeld, and T. Hunter. 2006. Critical roles of the p160 transcriptional coactivators p/CIP and SRC-1 in energy balance. *Cell Metab* 3: 111-122.
206. Lipinski, M. M., and T. Jacks. 1999. The retinoblastoma gene family in differentiation and development. *Oncogene* 18: 7873-7882.
207. Hansen, J. B., R. K. Petersen, B. M. Larsen, J. Bartkova, J. Alsner, and K. Kristiansen. 1999. Activation of peroxisome proliferator-activated receptor gamma bypasses the function of the retinoblastoma protein in adipocyte differentiation. *J Biol Chem* 274: 2386-2393.
208. Hansen, J. B., R. K. Petersen, C. Jorgensen, and K. Kristiansen. 2002. Deregulated MAPK activity prevents adipocyte differentiation of fibroblasts lacking the retinoblastoma protein. *J Biol Chem* 277: 26335-26339.
209. Herzig, S., F. Long, U. S. Jhala, S. Hedrick, R. Quinn, A. Bauer, D. Rudolph, G. Schutz, C. Yoon, P. Puigserver, B. Spiegelman, and M. Montminy. 2001. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 413: 179-183.
210. Scime, A., G. Grenier, M. S. Huh, M. A. Gillespie, L. Bevilacqua, M. E. Harper, and M. A. Rudnicki. 2005. Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1alpha. *Cell Metab* 2: 283-295.
211. Dali-Youcef, N., C. Mataki, A. Coste, N. Messaddeq, S. Giroud, S. Blanc, C. Koehl, M. F. Champy, P. Chambon, L. Fajas, D. Metzger, K. Schoonjans, and J. Auwerx. 2007. Adipose tissue-specific inactivation of the retinoblastoma protein protects against diabesity because of increased energy expenditure. *Proc Natl Acad Sci U S A* 104: 10703-10708.
212. Cavailles, V., S. Dauvois, F. L'Horset, G. Lopez, S. Hoare, P. J. Kushner, and M. G. Parker. 1995. Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *Embo J* 14: 3741-3751.
213. White, R., D. Morganstein, M. Christian, A. Seth, B. Herzog, and M. G. Parker. 2008. Role of RIP140 in metabolic tissues: Connections to disease. *FEBS Lett* 582: 39-45.
214. Kerley, J. S., S. L. Olsen, S. J. Freemantle, and M. J. Spinella. 2001. Transcriptional activation of the nuclear receptor corepressor RIP140 by retinoic acid: a potential negative-feedback regulatory mechanism. *Biochemical and biophysical research communications* 285: 969-975.
215. White, R., G. Leonardsson, I. Rosewell, M. Ann Jacobs, S. Milligan, and M. Parker. 2000. The nuclear receptor co-repressor nrip1 (RIP140) is essential for female fertility. *Nat Med* 6: 1368-1374.
216. Christian, M., E. Kiskinis, D. Debevec, G. Leonardsson, R. White, and M. G. Parker. 2005. RIP140-targeted repression of gene expression in adipocytes. *Mol Cell Biol* 25: 9383-9391.
217. Powelka, A. M., A. Seth, J. V. Virbasius, E. Kiskinis, S. M. Nicoloro, A. Guilherme, X. Tang, J. Straubhaar, A. D. Cherniack, M. G. Parker, and M. P. Czech. 2006. Suppression of oxidative metabolism and mitochondrial biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. *J Clin Invest* 116: 125-136.
218. Hansen, J. B., and K. Kristiansen. 2006. Regulatory circuits controlling white versus brown adipocyte differentiation. *Biochem J* 398: 153-168.
219. Garruti, G., and D. Ricquier. 1992. Analysis of uncoupling protein and its mRNA in adipose tissue deposits of adult humans. *Int J Obes Relat Metab Disord* 16: 383-390.
220. Oberkofler, H., G. Dallinger, Y. M. Liu, E. Hell, F. Krempler, and W. Patsch. 1997. Uncoupling protein gene: quantification of expression levels in adipose tissues of obese and non-obese humans. *J Lipid Res* 38: 2125-2133.
221. Moulin, K., N. Truel, M. Andre, E. Arnauld, M. Nibbelink, B. Cousin, C. Dani, L. Penicaud, and L. Casteilla. 2001. Emergence during development of the white-adipocyte cell phenotype is independent of the brown-adipocyte cell phenotype. *Biochem J* 356: 659-664.

222. Klaus, S. 1997. Functional differentiation of white and brown adipocytes. *Bioessays* 19: 215-223.
223. Tsai, Y. S., H. J. Kim, N. Takahashi, H. S. Kim, J. R. Hagaman, J. K. Kim, and N. Maeda. 2004. Hypertension and abnormal fat distribution but not insulin resistance in mice with P465L PPARgamma. *J Clin Invest* 114: 240-249.
224. Fukui, Y., S. Masui, S. Osada, K. Umesono, and K. Motojima. 2000. A new thiazolidinedione, NC-2100, which is a weak PPAR-gamma activator, exhibits potent antidiabetic effects and induces uncoupling protein 1 in white adipose tissue of KKAy obese mice. *Diabetes* 49: 759-767.
225. Guerra, C., R. A. Koza, H. Yamashita, K. Walsh, and L. P. Kozak. 1998. Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J Clin Invest* 102: 412-420.
226. Sell, H., J. P. Berger, P. Samson, G. Castriota, J. Lalonde, Y. Deshaies, and D. Richard. 2004. Peroxisome proliferator-activated receptor gamma agonism increases the capacity for sympathetically mediated thermogenesis in lean and ob/ob mice. *Endocrinology* 145: 3925-3934.
227. Himms-Hagen, J., A. Melnyk, M. C. Zingaretti, E. Ceresi, G. Barbatelli, and S. Cinti. 2000. Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *American journal of physiology* 279: C670-681.
228. Granneman, J. G., M. Burnazi, Z. Zhu, and L. A. Schwamb. 2003. White adipose tissue contributes to UCP1-independent thermogenesis. *Am J Physiol Endocrinol Metab* 285: E1230-1236.
229. Mercader, J., L. Madsen, F. Felipe, A. Palou, K. Kristiansen, and M. L. Bonet. 2007. All-trans retinoic acid increases oxidative metabolism in mature adipocytes. *Cell Physiol Biochem* 20: 1061-1072.
230. Mercader, J., J. Ribot, I. Murano, F. Felipe, S. Cinti, M. L. Bonet, and A. Palou. 2006. Remodeling of white adipose tissue after retinoic acid administration in mice. *Endocrinology* 147: 5325-5332.
231. Zhou, Y. T., Z. W. Wang, M. Higa, C. B. Newgard, and R. H. Unger. 1999. Reversing adipocyte differentiation: implications for treatment of obesity. *Proc Natl Acad Sci U S A* 96: 2391-2395.
232. Orci, L., W. S. Cook, M. Ravazzola, M. Y. Wang, B. H. Park, R. Montesano, and R. H. Unger. 2004. Rapid transformation of white adipocytes into fat-oxidizing machines. *Proc Natl Acad Sci U S A* 101: 2058-2063.
233. Holm, C., P. Belfrage, and G. Fredrikson. 1987. Immunological evidence for the presence of hormone-sensitive lipase in rat tissues other than adipose tissue. *Biochemical and biophysical research communications* 148: 99-105.
234. Holm, C., G. Fredrikson, B. Cannon, and P. Belfrage. 1987. Hormone-sensitive lipase in brown adipose tissue: identification and effect of cold exposure. *Bioscience reports* 7: 897-904.
235. Small, C. A., A. J. Garton, and S. J. Yeaman. 1989. The presence and role of hormone-sensitive lipase in heart muscle. *Biochem J* 258: 67-72.
236. Langfort, J., T. Ploug, J. Ihlemann, M. Saldo, C. Holm, and H. Galbo. 1999. Expression of hormone-sensitive lipase and its regulation by adrenaline in skeletal muscle. *Biochem J* 340 (Pt 2): 459-465.
237. Small, C. A., J. A. Goodacre, and S. J. Yeaman. 1989. Hormone-sensitive lipase is responsible for the neutral cholesterol ester hydrolase activity in macrophages. *FEBS Lett* 247: 205-208.
238. Khoo, J. C., K. Reue, D. Steinberg, and M. C. Schotz. 1993. Expression of hormone-sensitive lipase mRNA in macrophages. *J Lipid Res* 34: 1969-1974.
239. Contreras, J. A., C. Holm, A. Martin, M. L. Gaspar, and M. A. Lasuncion. 1994. Presence of hormone-sensitive lipase mRNA in J774 macrophages. *Israel journal of medical sciences* 30: 778-781.

240. Grober, J., S. Lucas, M. Sorhede-Winzell, I. Zaghini, A. Mairal, J. A. Contreras, P. Besnard, C. Holm, and D. Langin. 2003. Hormone-sensitive lipase is a cholesterol esterase of the intestinal mucosa. *J Biol Chem* 278: 6510-6515.
241. Holst, L. S., D. Langin, H. Mulder, H. Laurell, J. Grober, A. Bergh, H. W. Mohrenweiser, G. Edgren, and C. Holm. 1996. Molecular cloning, genomic organization, and expression of a testicular isoform of hormone-sensitive lipase. *Genomics* 35: 441-447.
242. Mulder, H., L. S. Holst, H. Svensson, E. Degerman, F. Sundler, B. Ahren, P. Rorsman, and C. Holm. 1999. Hormone-sensitive lipase, the rate-limiting enzyme in triglyceride hydrolysis, is expressed and active in beta-cells. *Diabetes* 48: 228-232.
243. Holm, C., T. G. Kirchgessner, K. L. Svenson, G. Fredrikson, S. Nilsson, C. G. Miller, J. E. Shively, C. Heinzmann, R. S. Sparkes, T. Mohandas, and et al. 1988. Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-q13.3. *Science* 241: 1503-1506.
244. Lindvall, H., P. Nevsten, K. Strom, R. Wallenberg, F. Sundler, D. Langin, M. S. Winzell, and C. Holm. 2004. A novel hormone-sensitive lipase isoform expressed in pancreatic beta-cells. *J Biol Chem* 279: 3828-3836.
245. Fredrikson, G., P. Stralfors, N. O. Nilsson, and P. Belfrage. 1981. Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J Biol Chem* 256: 6311-6320.
246. Cook, K. G., S. J. Yeaman, P. Stralfors, G. Fredrikson, and P. Belfrage. 1982. Direct evidence that cholesterol ester hydrolase from adrenal cortex is the same enzyme as hormone-sensitive lipase from adipose tissue. *European journal of biochemistry / FEBS* 125: 245-249.
247. Lee, F. T., J. B. Adams, A. J. Garton, and S. J. Yeaman. 1988. Hormone-sensitive lipase is involved in the hydrolysis of lipoidal derivatives of estrogens and other steroid hormones. *Biochim Biophys Acta* 963: 258-264.
248. Tsujita, T., H. Ninomiya, and H. Okuda. 1989. p-nitrophenyl butyrate hydrolyzing activity of hormone-sensitive lipase from bovine adipose tissue. *J Lipid Res* 30: 997-1004.
249. Wei, S., K. Lai, S. Patel, R. Piantedosi, H. Shen, V. Colantuoni, F. B. Kraemer, and W. S. Blaner. 1997. Retinyl ester hydrolysis and retinol efflux from BFC-1beta adipocytes. *J Biol Chem* 272: 14159-14165.
250. Fredrikson, G., H. Tornqvist, and P. Belfrage. 1986. Hormone-sensitive lipase and monoacylglycerol lipase are both required for complete degradation of adipocyte triacylglycerol. *Biochim Biophys Acta* 876: 288-293.
251. Haemmerle, G., R. Zimmermann, M. Hayn, C. Theussl, G. Waeg, E. Wagner, W. Sattler, T. M. Magin, E. F. Wagner, and R. Zechner. 2002. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. *J Biol Chem* 277: 4806-4815.
252. Holm, C. 2003. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Biochemical Society transactions* 31: 1120-1124.
253. Lafontan, M., P. Barbe, J. Galitzky, G. Tavernier, D. Langin, C. Carpene, A. Bousquet-Melou, and M. Berlan. 1997. Adrenergic regulation of adipocyte metabolism. *Human reproduction (Oxford, England)* 12 Suppl 1: 6-20.
254. Egan, J. J., A. S. Greenberg, M. K. Chang, S. A. Wek, M. C. Moos, Jr., and C. Londos. 1992. Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. *Proc Natl Acad Sci U S A* 89: 8537-8541.
255. Tansey, J. T., C. Sztalryd, J. Gruia-Gray, D. L. Roush, J. V. Zee, O. Gavrilova, M. L. Reitman, C. X. Deng, C. Li, A. R. Kimmel, and C. Londos. 2001. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc Natl Acad Sci U S A* 98: 6494-6499.
256. Sztalryd, C., G. Xu, H. Dorward, J. T. Tansey, J. A. Contreras, A. R. Kimmel, and C. Londos. 2003. Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. *The Journal of cell biology* 161: 1093-1103.
257. Degerman, E., P. Belfrage, and V. C. Manganiello. 1997. Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). *J Biol Chem* 272: 6823-6826.

258. Greenberg, A. S., W. J. Shen, K. Muliro, S. Patel, S. C. Souza, R. A. Roth, and F. B. Kraemer. 2001. Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J Biol Chem* 276: 45456-45461.
259. Garton, A. J., D. G. Campbell, D. Carling, D. G. Hardie, R. J. Colbran, and S. J. Yeaman. 1989. Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. *European journal of biochemistry / FEBS* 179: 249-254.
260. Smih, F., P. Rouet, S. Lucas, A. Mairal, C. Sengenes, M. Lafontan, S. Vaulont, M. Casado, and D. Langin. 2002. Transcriptional regulation of adipocyte hormone-sensitive lipase by glucose. *Diabetes* 51: 293-300.
261. Raclot, T., M. Dauzats, and D. Langin. 1998. Regulation of hormone-sensitive lipase expression by glucose in 3T3-F442A adipocytes. *Biochemical and biophysical research communications* 245: 510-513.
262. Botion, L. M., and A. Green. 1999. Long-term regulation of lipolysis and hormone-sensitive lipase by insulin and glucose. *Diabetes* 48: 1691-1697.
263. Winzell, M. S., H. Svensson, P. Arner, B. Ahren, and C. Holm. 2001. The expression of hormone-sensitive lipase in clonal beta-cells and rat islets is induced by long-term exposure to high glucose. *Diabetes* 50: 2225-2230.
264. Deng, T., S. Shan, P. P. Li, Z. F. Shen, X. P. Lu, J. Cheng, and Z. Q. Ning. 2006. Peroxisome proliferator-activated receptor-gamma transcriptionally up-regulates hormone-sensitive lipase via the involvement of specificity protein-1. *Endocrinology* 147: 875-884.
265. Yajima, H., Y. Kobayashi, T. Kanaya, and Y. Horino. 2007. Identification of peroxisome-proliferator responsive element in the mouse HSL gene. *Biochemical and biophysical research communications* 352: 526-531.
266. Slavin, B. G., J. M. Ong, and P. A. Kern. 1994. Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. *J Lipid Res* 35: 1535-1541.
267. Sztalryd, C., and F. B. Kraemer. 1994. Regulation of hormone-sensitive lipase during fasting. *The American journal of physiology* 266: E179-185.
268. Winzell, M. S., C. Holm, and B. Ahren. 2003. Downregulation of islet hormone-sensitive lipase during long-term high-fat feeding. *Biochemical and biophysical research communications* 304: 273-278.
269. Watt, M. J., A. L. Carey, E. Wolsk-Petersen, F. B. Kraemer, B. K. Pedersen, and M. A. Febbraio. 2005. Hormone-sensitive lipase is reduced in the adipose tissue of patients with type 2 diabetes mellitus: influence of IL-6 infusion. *Diabetologia* 48: 105-112.
270. Large, V., S. Reynisdottir, D. Langin, K. Fredby, M. Klannemark, C. Holm, and P. Arner. 1999. Decreased expression and function of adipocyte hormone-sensitive lipase in subcutaneous fat cells of obese subjects. *J Lipid Res* 40: 2059-2066.
271. Langin, D., A. Dicker, G. Tavernier, J. Hoffstedt, A. Mairal, M. Ryden, E. Arner, A. Sicard, C. M. Jenkins, N. Viguerie, V. van Harmelen, R. W. Gross, C. Holm, and P. Arner. 2005. Adipocyte lipases and defect of lipolysis in human obesity. *Diabetes* 54: 3190-3197.
272. Osuga, J., S. Ishibashi, T. Oka, H. Yagyu, R. Tozawa, A. Fujimoto, F. Shionoiri, N. Yahagi, F. B. Kraemer, O. Tsutsumi, and N. Yamada. 2000. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. *Proc Natl Acad Sci U S A* 97: 787-792.
273. Wang, S. P., N. Laurin, J. Himms-Hagen, M. A. Rudnicki, E. Levy, M. F. Robert, L. Pan, L. Oligny, and G. A. Mitchell. 2001. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. *Obes Res* 9: 119-128.
274. Mulder, H., M. Sorhede-Winzell, J. A. Contreras, M. Fex, K. Strom, T. Ploug, H. Galbo, P. Arner, C. Lundberg, F. Sundler, B. Ahren, and C. Holm. 2003. Hormone-sensitive lipase null mice exhibit signs of impaired insulin sensitivity whereas insulin secretion is intact. *J Biol Chem* 278: 36380-36388.
275. Suzuki, J., W. J. Shen, B. D. Nelson, S. Patel, J. H. Veerkamp, S. P. Selwood, G. M. Murphy, Jr., E. Reaven, and F. B. Kraemer. 2001. Absence of cardiac lipid accumulation in transgenic mice with heart-specific HSL overexpression. *Am J Physiol Endocrinol Metab* 281: E857-866.

276. Lucas, S., G. Tavernier, C. Tiraby, A. Mairal, and D. Langin. 2003. Expression of human hormone-sensitive lipase in white adipose tissue of transgenic mice increases lipase activity but does not enhance in vitro lipolysis. *J Lipid Res* 44: 154-163.
277. Winzell, M. S., H. Svensson, S. Enerback, K. Ravnskjaer, S. Mandrup, V. Esser, P. Arner, M. C. Alves-Guerra, B. Miroux, F. Sundler, B. Ahren, and C. Holm. 2003. Pancreatic beta-cell lipotoxicity induced by overexpression of hormone-sensitive lipase. *Diabetes* 52: 2057-2065.
278. Reid, B. N., G. P. Ables, O. A. Otlivanchik, G. Schoiswohl, R. Zechner, W. S. Blaner, I. J. Goldberg, R. Schwabe, S. C. Chua, Jr., and L. S. Huang. 2008. Hepatic overexpression of hormone-sensitive lipase and adipose triglyceride lipase promotes fatty acid oxidation, stimulates direct release of free fatty acids and ameliorates steatosis. *J Biol Chem*.
279. Harada, K., W. J. Shen, S. Patel, V. Natu, J. Wang, J. Osuga, S. Ishibashi, and F. B. Kraemer. 2003. Resistance to high-fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice. *Am J Physiol Endocrinol Metab* 285: E1182-1195.
280. Fortier, M., S. P. Wang, P. Mauriege, M. Semache, L. Mfuma, H. Li, E. Levy, D. Richard, and G. A. Mitchell. 2004. Hormone-sensitive lipase-independent adipocyte lipolysis during beta-adrenergic stimulation, fasting, and dietary fat loading. *Am J Physiol Endocrinol Metab* 287: E282-288.
281. Ström, K., O. Hansson, S. Lucas, P. Nevsten, F. C., C. Klint, S. Movérare-Skrtic, F. Sundler, C. Ohlsson, and C. Holm. 2008. Attainment of Brown Adipocyte Features in White Adipocytes of Hormone-Sensitive Lipase Null Mice. *PLoS ONE*.
282. Okazaki, H., J. Osuga, Y. Tamura, N. Yahagi, S. Tomita, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, S. Kimura, T. Gotoda, H. Shimano, N. Yamada, and S. Ishibashi. 2002. Lipolysis in the absence of hormone-sensitive lipase: evidence for a common mechanism regulating distinct lipases. *Diabetes* 51: 3368-3375.
283. Roduit, R., P. Masiello, S. P. Wang, H. Li, G. A. Mitchell, and M. Prentki. 2001. A role for hormone-sensitive lipase in glucose-stimulated insulin secretion: a study in hormone-sensitive lipase-deficient mice. *Diabetes* 50: 1970-1975.
284. Voshol, P. J., G. Haemmerle, D. M. Ouwens, R. Zimmermann, R. Zechner, B. Teusink, J. A. Maassen, L. M. Havekes, and J. A. Romijn. 2003. Increased hepatic insulin sensitivity together with decreased hepatic triglyceride stores in hormone-sensitive lipase-deficient mice. *Endocrinology* 144: 3456-3462.
285. Park, S. Y., H. J. Kim, S. Wang, T. Higashimori, J. Dong, Y. J. Kim, G. Cline, H. Li, M. Prentki, G. I. Shulman, G. A. Mitchell, and J. K. Kim. 2005. Hormone-sensitive lipase knockout mice have increased hepatic insulin sensitivity and are protected from short-term diet-induced insulin resistance in skeletal muscle and heart. *Am J Physiol Endocrinol Metab* 289: E30-39.
286. Backhed, F., H. Ding, T. Wang, L. V. Hooper, G. Y. Koh, A. Nagy, C. F. Semenkovich, and J. I. Gordon. 2004. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 101: 15718-15723.
287. Fex, M., C. S. Olofsson, U. Fransson, K. Bacos, H. Lindvall, M. Sorhede-Winzell, P. Rorsman, C. Holm, and H. Mulder. 2004. Hormone-sensitive lipase deficiency in mouse islets abolishes neutral cholesterol ester hydrolase activity but leaves lipolysis, acylglycerides, fat oxidation, and insulin secretion intact. *Endocrinology* 145: 3746-3753.
288. Haemmerle, G., R. Zimmermann, J. G. Strauss, D. Kratky, M. Riederer, G. Knipping, and R. Zechner. 2002. Hormone-sensitive lipase deficiency in mice changes the plasma lipid profile by affecting the tissue-specific expression pattern of lipoprotein lipase in adipose tissue and muscle. *J Biol Chem* 277: 12946-12952.
289. Zimmermann, R., G. Haemmerle, E. M. Wagner, J. G. Strauss, D. Kratky, and R. Zechner. 2003. Decreased fatty acid esterification compensates for the reduced lipolytic activity in hormone-sensitive lipase-deficient white adipose tissue. *J Lipid Res* 44: 2089-2099.
290. Cinti, S., G. Mitchell, G. Barbatelli, I. Murano, E. Ceresi, E. Faloia, S. Wang, M. Fortier, A. S. Greenberg, and M. S. Obin. 2005. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res* 46: 2347-2355.

291. Hansson, O., K. Strom, N. Guner, N. Wierup, F. Sundler, P. Hoglund, and C. Holm. 2006. Inflammatory response in white adipose tissue in the non-obese hormone-sensitive lipase null mouse model. *Journal of proteome research* 5: 1701-1710.
292. Wang, S. P., S. Chung, K. Soni, H. Bourdages, L. Hermo, J. Trasler, and G. A. Mitchell. 2004. Expression of human hormone-sensitive lipase (HSL) in postmeiotic germ cells confers normal fertility to HSL-deficient mice. *Endocrinology* 145: 5688-5693.
293. Vallet-Erdtmann, V., G. Tavernier, J. A. Contreras, A. Mairal, C. Rieu, A. M. Touzalin, C. Holm, B. Jegou, and D. Langin. 2004. The testicular form of hormone-sensitive lipase HSLtes confers rescue of male infertility in HSL-deficient mice. *J Biol Chem* 279: 42875-42880.
294. Fortier, M., K. Soni, N. Laurin, S. P. Wang, P. Mauriege, F. R. Jirik, and G. A. Mitchell. 2005. Human hormone-sensitive lipase (HSL): expression in white fat corrects the white adipose phenotype of HSL-deficient mice. *J Lipid Res* 46: 1860-1867.
295. Zimmermann, R., J. G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger, M. Riederer, A. Lass, G. Neuberger, F. Eisenhaber, A. Hermetter, and R. Zechner. 2004. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 306: 1383-1386.
296. Villena, J. A., S. Roy, E. Sarkadi-Nagy, K. H. Kim, and H. S. Sul. 2004. Desnutrin, an adipocyte gene encoding a novel patatin domain-containing protein, is induced by fasting and glucocorticoids: ectopic expression of desnutrin increases triglyceride hydrolysis. *J Biol Chem* 279: 47066-47075.
297. Jenkins, C. M., D. J. Mancuso, W. Yan, H. F. Sims, B. Gibson, and R. W. Gross. 2004. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J Biol Chem* 279: 48968-48975.
298. Haemmerle, G., A. Lass, R. Zimmermann, G. Gorkiewicz, C. Meyer, J. Rozman, G. Heldmaier, R. Maier, C. Theussl, S. Eder, D. Kratky, E. F. Wagner, M. Klingenspor, G. Hoefler, and R. Zechner. 2006. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science* 312: 734-737.
299. Lass, A., R. Zimmermann, G. Haemmerle, M. Riederer, G. Schoiswohl, M. Schweiger, P. Kienesberger, J. G. Strauss, G. Gorkiewicz, and R. Zechner. 2006. Adipose triglyceride lipase-mediated lipolysis of cellular fat stores is activated by CGI-58 and defective in Chanarin-Dorfman Syndrome. *Cell Metab* 3: 309-319.
300. Subramanian, V., A. Rothenberg, C. Gomez, A. W. Cohen, A. Garcia, S. Bhattacharyya, L. Shapiro, G. Dolios, R. Wang, M. P. Lisanti, and D. L. Brasaemle. 2004. Perilipin A mediates the reversible binding of CGI-58 to lipid droplets in 3T3-L1 adipocytes. *J Biol Chem* 279: 42062-42071.
301. Schweiger, M., R. Schreiber, G. Haemmerle, A. Lass, C. Fledelius, P. Jacobsen, H. Tornqvist, R. Zechner, and R. Zimmermann. 2006. Adipose triglyceride lipase and hormone-sensitive lipase are the major enzymes in adipose tissue triacylglycerol catabolism. *J Biol Chem* 281: 40236-40241.
302. Granneman, J. G., H. P. Moore, R. L. Granneman, A. S. Greenberg, M. S. Obin, and Z. Zhu. 2007. Analysis of lipolytic protein trafficking and interactions in adipocytes. *J Biol Chem* 282: 5726-5735.
303. Jocken, J. W., D. Langin, E. Smit, W. H. Saris, C. Valle, G. B. Hul, C. Holm, P. Arner, and E. E. Blaak. 2007. Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state. *The Journal of clinical endocrinology and metabolism* 92: 2292-2299.
304. Granneman, J. G., and H. P. Moore. 2008. Location, location: protein trafficking and lipolysis in adipocytes. *Trends in endocrinology and metabolism: TEM* 19: 3-9.
305. Chertow, B. S., W. S. Blaner, N. G. Baranetsky, W. I. Sivitz, M. B. Cordle, D. Thompson, and P. Meda. 1987. Effects of vitamin A deficiency and repletion on rat insulin secretion in vivo and in vitro from isolated islets. *J Clin Invest* 79: 163-169.
306. Stephensen, C. B. 2001. Vitamin A, infection, and immune function. *Annu Rev Nutr* 21: 167-192.

307. Austenaa, L. M., H. Carlsen, A. Ertesvag, G. Alexander, H. K. Blomhoff, and R. Blomhoff. 2004. Vitamin A status significantly alters nuclear factor-kappaB activity assessed by in vivo imaging. *Faseb J* 18: 1255-1257.
308. Blomhoff, R., M. H. Green, and K. R. Norum. 1992. Vitamin A: physiological and biochemical processing. *Annu Rev Nutr* 12: 37-57.
309. De Luca, L. M., R. L. Shores, E. F. Spangler, and M. L. Wenk. 1989. Inhibition of initiator-promoter-induced skin tumorigenesis in female SENCAR mice fed a vitamin A-deficient diet and reappearance of tumors in mice fed a diet adequate in retinoid or beta-carotene. *Cancer research* 49: 5400-5406.
310. O'Byrne, S. M., N. Wongsiriroj, J. Libien, S. Vogel, I. J. Goldberg, W. Baehr, K. Palczewski, and W. S. Blaner. 2005. Retinoid absorption and storage is impaired in mice lacking lecithin:retinol acyltransferase (LRAT). *J Biol Chem* 280: 35647-35657.
311. Kurlandsky, S. B., M. V. Gamble, R. Ramakrishnan, and W. S. Blaner. 1995. Plasma delivery of retinoic acid to tissues in the rat. *J Biol Chem* 270: 17850-17857.
312. Kawaguchi, R., J. Yu, J. Honda, J. Hu, J. Whitelegge, P. Ping, P. Wiita, D. Bok, and H. Sun. 2007. A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. *Science* 315: 820-825.
313. Duester, G., F. A. Mic, and A. Molotkov. 2003. Cytosolic retinoid dehydrogenases govern ubiquitous metabolism of retinol to retinaldehyde followed by tissue-specific metabolism to retinoic acid. *Chemico-biological interactions* 143-144: 201-210.
314. Ziouzenkova, O., G. Orasanu, M. Sharlach, T. E. Akiyama, J. P. Berger, J. Viereck, J. A. Hamilton, G. Tang, G. G. Dolnikowski, S. Vogel, G. Duester, and J. Plutzky. 2007. Retinaldehyde represses adipogenesis and diet-induced obesity. *Nat Med* 13: 695-702.
315. Gagnon, I., G. Duester, and P. V. Bhat. 2002. Kinetic analysis of mouse retinal dehydrogenase type-2 (RALDH2) for retinal substrates. *Biochim Biophys Acta* 1596: 156-162.
316. Kojima, R., T. Fujimori, N. Kiyota, Y. Toriya, T. Fukuda, T. Ohashi, T. Sato, Y. Yoshizawa, K. Takeyama, H. Mano, and et al. 1994. In vivo isomerization of retinoic acids. Rapid isomer exchange and gene expression. *J Biol Chem* 269: 32700-32707.
317. Chambon, P. 1994. The retinoid signaling pathway: molecular and genetic analyses. *Seminars in cell biology* 5: 115-125.
318. Mangelsdorf, D. J., and R. M. Evans. 1995. The RXR heterodimers and orphan receptors. *Cell* 83: 841-850.
319. Park, E. Y., A. Dillard, E. A. Williams, E. T. Wilder, M. R. Pepper, and M. A. Lane. 2005. Retinol inhibits the growth of all-trans-retinoic acid-sensitive and all-trans-retinoic acid-resistant colon cancer cells through a retinoic acid receptor-independent mechanism. *Cancer research* 65: 9923-9933.
320. Kliewer, S. A., K. Umesono, D. J. Mangelsdorf, and R. M. Evans. 1992. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. *Nature* 355: 446-449.
321. Villarroya, F., R. Iglesias, and M. Giralt. 2004. Retinoids and retinoid receptors in the control of energy balance: novel pharmacological strategies in obesity and diabetes. *Current medicinal chemistry* 11: 795-805.
322. Mukherjee, R., P. J. Davies, D. L. Crombie, E. D. Bischoff, R. M. Cesario, L. Jow, L. G. Hamann, M. F. Boehm, C. E. Mondon, A. M. Nadzan, J. R. Paterniti, Jr., and R. A. Heyman. 1997. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* 386: 407-410.
323. IJpenberg, A., N. S. Tan, L. Gelman, S. Kersten, J. Seydoux, J. Xu, D. Metzger, L. Canaple, P. Chambon, W. Wahli, and B. Desvergne. 2004. In vivo activation of PPAR target genes by RXR homodimers. *Embo J* 23: 2083-2091.
324. Tsutsumi, C., M. Okuno, L. Tannous, R. Piantedosi, M. Allan, D. S. Goodman, and W. S. Blaner. 1992. Retinoids and retinoid-binding protein expression in rat adipocytes. *J Biol Chem* 267: 1805-1810.

325. Zovich, D. C., A. Orologa, M. Okuno, L. W. Kong, D. A. Talmage, R. Piantedosi, D. S. Goodman, and W. S. Blaner. 1992. Differentiation-dependent expression of retinoid-binding proteins in BFC-1 beta adipocytes. *J Biol Chem* 267: 13884-13889.
326. Blaner, W. S., J. C. Obunike, S. B. Kurlandsky, M. al-Haideri, R. Piantedosi, R. J. Deckelbaum, and I. J. Goldberg. 1994. Lipoprotein lipase hydrolysis of retinyl ester. Possible implications for retinoid uptake by cells. *J Biol Chem* 269: 16559-16565.
327. Yen, C. L., M. Monetti, B. J. Burri, and R. V. Farese, Jr. 2005. The triacylglycerol synthesis enzyme DGAT1 also catalyzes the synthesis of diacylglycerols, waxes, and retinyl esters. *J Lipid Res* 46: 1502-1511.
328. Villarroya, F. 1998. Differential effects of retinoic acid on white and brown adipose tissues. An unexpected role for vitamin A derivatives on energy balance. *Annals of the New York Academy of Sciences* 839: 190-195.
329. Safonova, I., C. Darimont, E. Z. Amri, P. Grimaldi, G. Ailhaud, U. Reichert, and B. Shroot. 1994. Retinoids are positive effectors of adipose cell differentiation. *Mol Cell Endocrinol* 104: 201-211.
330. Villarroya, F., M. Giralt, and R. Iglesias. 1999. Retinoids and adipose tissues: metabolism, cell differentiation and gene expression. *Int J Obes Relat Metab Disord* 23: 1-6.
331. Chen, Q., and A. C. Ross. 2004. Retinoic acid regulates cell cycle progression and cell differentiation in human monocytic THP-1 cells. *Exp Cell Res* 297: 68-81.
332. Alvarez, R., J. de Andres, P. Yubero, O. Vinas, T. Mampel, R. Iglesias, M. Giralt, and F. Villarroya. 1995. A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene. *J Biol Chem* 270: 5666-5673.
333. Rial, E., M. Gonzalez-Barroso, C. Fleury, S. Iturrizaga, D. Sanchis, J. Jimenez-Jimenez, D. Ricquier, M. Goubern, and F. Bouillaud. 1999. Retinoids activate proton transport by the uncoupling proteins UCP1 and UCP2. *Embo J* 18: 5827-5833.
334. Bonet, M. L., J. Oliver, C. Pico, F. Felipe, J. Ribot, S. Cinti, and A. Palou. 2000. Opposite effects of feeding a vitamin A-deficient diet and retinoic acid treatment on brown adipose tissue uncoupling protein 1 (UCP1), UCP2 and leptin expression. *The Journal of endocrinology* 166: 511-517.
335. Schulman, I. G., G. Shao, and R. A. Heyman. 1998. Transactivation by retinoid X receptor-peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimers: intermolecular synergy requires only the PPARgamma hormone-dependent activation function. *Mol Cell Biol* 18: 3483-3494.
336. Kim, J. K., Y. J. Kim, J. J. Fillmore, Y. Chen, I. Moore, J. Lee, M. Yuan, Z. W. Li, M. Karin, P. Perret, S. E. Shoelson, and G. I. Shulman. 2001. Prevention of fat-induced insulin resistance by salicylate. *J Clin Invest* 108: 437-446.
337. de Urquiza, A. M., S. Liu, M. Sjoberg, R. H. Zetterstrom, W. Griffiths, J. Sjovall, and T. Perlmann. 2000. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science* 290: 2140-2144.
338. Mathew, J. S., and R. P. Sharma. 2000. Effect of all-trans-retinoic acid on cytokine production in a murine macrophage cell line. *International journal of immunopharmacology* 22: 693-706.
339. Lu, Y., B. Amos, E. Cruise, D. Lotan, and R. Lotan. 1998. A parallel association between differentiation and induction of galectin-1, and inhibition of galectin-3 by retinoic acid in mouse embryonal carcinoma F9 cells. *Biological chemistry* 379: 1323-1331.