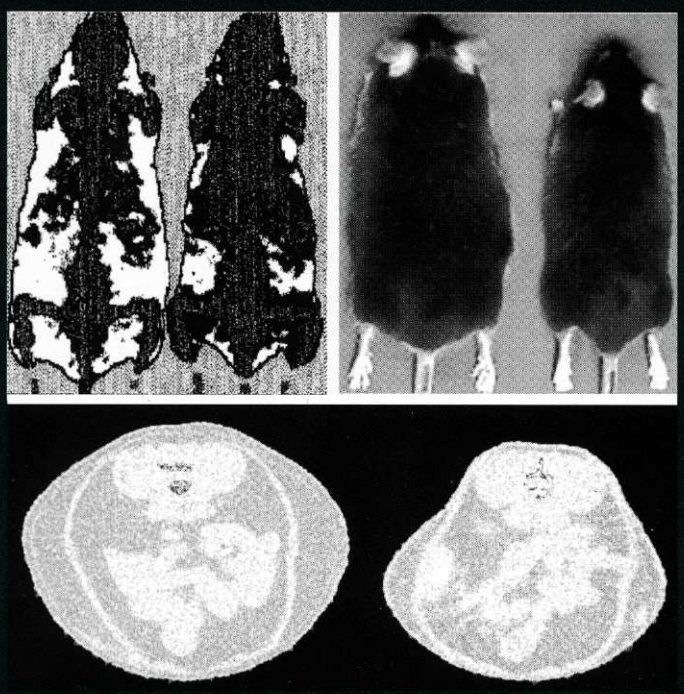
Insulin-like Growth Factor-I and Interleukin-6 Regulate Body Fat

Kristina Wallenius



Göteborg 2002

Insulin-like Growth Factor-I and Interleukin-6 Regulate Body Fat

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Göteborgs Universitet kommer att offentligen försvaras i Sahlgrenska Universitetssjukhusets aula i Göteborg, onsdagen den 29 maj 2002, kl 9.00

av

Kristina Wallenius
filosofie magister

Fakultetsopponent: Professor Peter Arner, Karolinska Institutet
Huddinge Universitets Sjukhus

Avhandlingen baseras på följande delarbeten:

I Wallenius K, Sjögren K, Peng X-D, Park S, Wallenius V, Liu J-L, Umaerus M, Wennbo H, Isaksson O, Frohman L, Kineman R, Ohlsson C and Jansson J-O.
Liver derived IGF-I regulates GH secretion at the pituitary level in mice
Endocrinology 2001, 142 (11): 4762-4770

II Sjögren K, Wallenius K, Liu J-L, Bohlooly-Y M, Pacini G, Svensson L, Törnell J, Isaksson O, Ahrén B, Jansson J-O and Ohlsson C.
Liver derived IGF-I is of importance for normal carbohydrate and lipid metabolism
Diabetes 2000, 50 (7): 1539-1545

III Wallenius V*, Wallenius K*, Ahrén B, Rudling M, Carlsten H, Dickson S, Ohlsson C and Jansson J-O. **Both authors have contributed equally*
Interleukin-6-deficient mice develop mature-onset obesity
Nature Medicine 2002, 8 (1): 75-79

IV Wallenius K, Wallenius V, Sunter D, Dickson S and Jansson J-O
Intracerebroventricular interleukin-6 treatment decreases body fat in rats
Biochemical Biophysical Research Communications 2002, 293 (1): 560-565

Insulin-like Growth Factor-I and Interleukin-6 Regulate Body Fat

Kristina Wallenius, Research Centre for Endocrinology and Metabolism, Department of Internal Medicine, The Sahlgrenska Academy, Göteborg University, Sweden

ABSTRACT

The aim of this thesis was to investigate the role of insulin-like growth factor-I (IGF-I) and interleukin-6 (IL-6) in the regulation of metabolism and body fat mass. Circulating IGF-I is mainly liver derived, while a large part of circulating IL-6 is produced by adipose tissue.

We have used a liver-specific and inducible IGF-I knockout (LI-IGF-I-/-) mouse model to study the role of liver derived IGF-I in the regulation of body fat. The LI-IGF-I-/- mice had decreased total body fat measured by dual-energy X-ray absorptiometry (DXA) and dissection of fat pads. Serum IGF-I levels were decreased by 85% in the LI-IGF-I-/- mice, while growth hormone (GH) levels were increased, due to lack of IGF-I feedback inhibition. Moreover, the LI-IGF-I-/- mice had increased numbers of pituitary GH-releasing factor (GHRF) receptors and GH-secretagogue (GHS) receptors and increased GH responsiveness to GHRF and GHS treatment. The LI-IGF-I-/- mice had elevated insulin levels both under basal conditions and after an intravenous glucose challenge. The elevated insulin levels may be caused by the lack of insulin-like effects of IGF-I, or by the diabetogenic effect of GH. GH may also be responsible for the decreased fat mass due to its lipolytic effects.

We found that IL-6 deficient (IL-6-/-) mice developed mature-onset obesity, insulin and leptin resistance and decreased glucose tolerance. Peripheral treatment with low doses of IL-6 partly reversed the obesity in IL-6-/- mice, but had no effect in control mice. To study the mechanism and site of action for the antiobesity effect of IL-6, we treated rats with a single intracerebroventricular (ICV) IL-6 injection and found that ICV IL-6 acutely increased energy expenditure, while the same dose peripherally had no effect. Moreover, chronic ICV treatment with IL-6 for two weeks decreased body weight, total fat pad weight and serum levels of the fat-derived hormone leptin, but did not change the weights of several non-fat organs.

The results from this thesis show that liver derived IGF-I and centrally acting IL-6 are important regulators of fat mass in rodents.

Keywords: Obesity, insulin-like growth factor I (IGF-I), interleukin-6 (IL-6), growth hormone (GH), GH releasing factor (GHRF), GH secretagogue (GHS), receptor, leptin, insulin, glucose, intracerebroventricular (ICV), dual-energy X-ray absorptiometry (DXA), indirect calorimetry.

ISBN 91-628-5245-0

Insulin-like Growth Factor-I and Interleukin-6 Regulate Body Fat

Kristina Wallenius

Research Centre for Endocrinology and Metabolism
Department of Internal Medicine
The Sahlgrenska Academy
Göteborg University

Göteborg 2002

Cover picture: Dual-energy X-ray Absorptiometry
and Computed Tomography scans of live IL-6 deficient and control mice
(Wallenius *et al.,* Nat Med 2002)

Printed by OFTA Grafiska, Göteborg, Sweden
ISBN 91-628-5245-0

Insulin-Like Growth Factor-I and Interleukin-6 Regulate Body Fat

ABSTRACT

Kristina Wallenius, Research Centre for Endocrinology and Metabolism, Department of Internal Medicine, The Sahlgrenska Academy, Göteborg University, Sweden.

The aim of this thesis was to investigate the role of insulin-like growth factor-I (IGF-I) and interleukin-6 (IL-6) in the regulation of metabolism and body fat mass. Circulating IGF-I is mainly liver derived, while a large part of circulating IL-6 is produced by adipose tissue.

We have used a liver-specific and inducible IGF-I knockout (LI-IGF-I-/-) mouse model to study the role of liver derived IGF-I in the regulation of body fat. The LI-IGF-I-/- mice had decreased total body fat measured by dual-energy X-ray absorptiometry (DXA) and dissection of fat pads. Serum IGF-I levels were decreased by 85% in the LI-IGF-I-/- mice, while growth hormone (GH) levels were increased, due to lack of IGF-I feedback inhibition. Moreover, the LI-IGF-I-/- mice had increased numbers of pituitary GH-releasing factor (GHRF) receptors and GH-secretagogue (GHS) receptors and increased GH responsiveness to GHRF and GHS treatment. The LI-IGF-I-/- mice had elevated insulin levels both under basal conditions and after an intravenous glucose challenge. The elevated insulin levels may be caused by the lack of insulin-like effects of IGF-I, or by the diabetogenic effect of GH. GH may also be responsible for the decreased fat mass due to its lipolytic effects.

We found that IL-6 deficient (IL-6-/-) mice developed mature-onset obesity, insulin and leptin resistance and decreased glucose tolerance. Peripheral treatment with low doses of IL-6 partly reversed the obesity in IL-6-/- mice, but had no effect in control mice. To study the mechanism and site of action for the antiobesity effect of IL-6, we treated rats with a single intracerebroventricular (ICV) IL-6 injection and found that ICV IL-6 acutely increased energy expenditure, while the same dose peripherally had no effect. Moreover, chronic ICV treatment with IL-6 for two weeks decreased body weight, total fat pad weight and serum levels of the fat-derived hormone leptin, but did not change the weights of several non-fat organs.

The results from this thesis show that liver derived IGF-I and centrally acting IL-6 are important regulators of fat mass in rodents.

Keywords: Obesity, insulin-like growth factor I (IGF-I), interleukin-6 (IL-6), growth hormone (GH), GH releasing factor (GHRF), GH secretagogue (GHS), receptor, leptin, insulin, glucose, intracerebroventricular (ICV), dual-energy X-ray absorptiometry (DXA), indirect calorimetry.

LIST OF PUBLICATIONS

This thesis is based on the following articles, which will be referred to by their roman numerals:

I Wallenius K, Sjögren K, Peng X-D, Park S, Wallenius V, Liu J-L, Umaerus M, Wennbo H, Isaksson O, Frohman L, Kineman R, Ohlsson C and Jansson J-O.
Liver derived IGF-I regulates GH secretion at the pituitary level in mice
Endocrinology 2001, 142 (11): 4762-4770

II Sjögren K, Wallenius K, Liu J-L, Bohlooly-Y M, Pacini G, Svensson L, Törnell J, Isaksson O, Ahrén B, Jansson J-O and Ohlsson C.
Liver derived IGF-I is of importance for normal carbohydrate and lipid metabolism
Diabetes 2000, 50 (7): 1539-1545

III Wallenius V*, Wallenius K*, Ahrén B, Rudling M, Carlsten H, Dickson S, Ohlsson C and Jansson J-O. **Both authors have contributed equally*
Interleukin-6-deficient mice develop mature-onset obesity
Nature Medicine 2002, 8 (1): 75-79

IV Wallenius K, Wallenius V, Sunter D, Dickson S and Jansson J-O
Intracerebroventricular interleukin-6 treatment decreases body fat in rats
Biochemical Biophysical Research Communications 2002, 293 (1): 560-565

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

αMSH	Melanocortin stimulating hormone
ACTH	Adrenocorticotropic hormone
AGRP	Agouti-related peptide
ALS	Acid labile subunit
Apo E	Apolipoprotein E
ARC	Arcuate nucleus
BAT	Brown adipose tissue
BBB	Blood brain barrier
BMI	Body mass index
CART	Cocaine and amphetamine regulated transcript
CNS	Central Nervous System
CNTF	Cilliary neurotrophic factor
Cre	Cyclization recombinase
CRH	Corticotrophin releasing hormone
CSF	Cerebrospinal fluid
CT	Computed tomography
CT-1	Cardiotrophin-1
Db/db	Leptin receptor deficient mice
DNA	Deoxyribonucleic acid
DXA	Dual energy x-ray absorptiometry
FFA	Free fatty acids
GH	Growth hormone
GHRF	GH-releasing factor (=GH releasing hormone, GHRH)
GHRF-R	GHRF-receptor
GHS	GH-secretagogue
GHS-R	GHS-receptor
gp	Glycoprotein
HDL	High density lipoprotein
HPA-axis	Hypothalamo-pituitary adrenal axis
ICAM-I	Intercellular adhesion molecule-1
ICV	Intracerebroventricular
IGF-I	Insulin-like growth factor I
IGF-BP	Insulin-like growth factor I binding protein
IL	Interleukin
JAK	Janus kinase
LDL	Low density lipoprotein
LIF	Leukemia inhibitory factor
LI-IGF-I-/-	Liver-specific inducible IGF-I knockout
Lit/lit	Little mice with mutation in GHRF-R
LPL	Lipoprotein lipase
LPS	Lipopolysacharide
Mac-1	leukocyte integrin $\alpha M\beta 2$
MC4-R	Melanocortin 4 receptor
MCH	Melanocyte concentration hormone
mRNA	Messenger ribonucleic acid

MUP	Major urinary protein
NPY	Neuropeptide Y
Ob/ob	Leptin deficient obese mice
ObRa	Short non-signaling leptin receptor isoform
ObRb	Long leptin receptor isoform
OSM	Oncostatin M
PBS	Phosphate buffered saline
POMC	Proopiomelanocortin
PPARγ	Peroxisome proliferating receptor γ
PRL-R	Prolactin receptor
PVN	Paraventricular nuclus
SAA	Serum amyloid A
SOCS	Suppressor of cytokine signaling
Sst1-5	Somatostatin receptors 1-5
STAT	Signal transducer and activator of transcription
TIMP	Tissue inhibitors of metalloproteinases
TNFα	Tumor Necrosis Factor α
VLDL	Very low density lipoprotein
WAT	White adipose tissue

INTRODUCTION

This thesis discusses insulin-like growth factor-I (IGF-I) and interleukin-6 (IL-6), in the regulation of metabolism and body fat mass. It is based on phenotypic examination of liver derived IGF-I-deficient mice and IL-6-deficient mice.

BACKGROUND IGF-I AND GROWTH HORMONE (GH)

As its name suggests, IGF-I has two main properties; 1) it mimics the metabolic effects of insulin and is structurally similar to insulin, 2) IGF-I is also an important growth factor, and it is produced in many tissues in response to both GH and insulin. Most of the circulating endocrine acting IGF-I is liver derived (1, 2). Local production of IGF-I in other tissues, such as muscle, adipose tissue, kidney and brain acts mainly via paracrine-autocrine mechanisms.

Insulin-like effects of IGF-I

IGF-I and its receptor are structurally and functionally related to insulin and the insulin receptor, respectively. However, the affinity of IGF-I to the insulin receptor is approximately 10 times lower than its affinity to the IGF-I receptor. The intracellular signaling cascades that are activated by binding of insulin and IGF-I to their respective receptors are similar, and both receptors are widely expressed in many tissues and cell types. The different roles of IGF-I and insulin may primarily be due to their biological availability because they have different secretion and serum profiles and are regulated by different factors, such as nutritional status and GH levels. IGF-I mimics the effects of insulin by stimulating glucose uptake, inhibiting gluconeogenesis and promoting lipogenesis (3).

IGF-I treatment in humans causes hypoglycemia indistinguishable from the response after an insulin injection (4). The hypoglycemic effect of IGF-I is exerted by free IGF-I, *i.e.* IGF-I that is not bound to IGF-I binding proteins (IGFBPs). Binding of IGF-I to IGFBP-1 neutralizes the insulin-like effects of IGF-I, *e.g.* during starvation (5). One difference between insulin and IGF-I, when given at doses that cause similar hypoglycemia, is that insulin inhibits lipolysis more potently (4).

Patients with type II diabetes have been treated with IGF-I in an attempt to improve glycemic control. IGF-I treatment improved glycemia in these patients and lead to decreased insulin levels and increased insulin sensitivity (6). During the 4-week treatment period body fat mass was decreased in these patients. IGF-I treatment also lead to markedly decreased levels of triglycerides and cholesterol in serum. IGFBP-1 and -2 levels were increased, which may have lead to increased transport of IGF-I through endothelium to help IGF-I access tissues were it can exert effects on glucose metabolism. IGF-I treatment is however, associated with side effects such as facial and peripheral edema and local pain at the injection site (6).

IGF-I binding proteins (IGFBP)

As mentioned above, IGF-I is usually bound to IGFBPs. Six different binding proteins have been characterized so far. IGFBP-3 is the most abundant one, and together with the so-called acid labile subunit (ALS) it binds most of the IGF-I that is present in blood during adulthood (3). The IGFBPs increase the half-life of IGF-I and alter its availability for binding to the IGF-I receptor. After birth serum levels of IGF-I and BP-3 increase and peak during puberty, where after they start to decrease. The opposite is true for BP-1 and BP-2 that are present at high levels at birth and decrease steadily until puberty when they start to rise again. Insulin can also regulate the activity of IGF-I by decreasing the levels of BP-1 (5). Insulin deficiency due to insulin-dependent diabetes mellitus or prolonged exercise leads to increased levels of circulating BP-1, while the opposite is true after an insulin injection or a meal.

BP-3 levels in serum are GH dependent and increase after a GH pulse. This is supported by the fact that patients with acromegaly have increased levels of BP-3 and GH-deficient patients have low levels of BP-3. GH also regulates the serum levels of ALS (3).

GH effects on postnatal body growth

GH is the major hormone that regulates postnatal body growth, including liver growth (7). Transgenic mice expressing human GH are almost twice as large as control littermates and have threefold larger livers (8). Serum IGF-I concentrations are 2-3 fold higher in these mice. Growth of GH transgenic mice accelerates at about three weeks of age, at the same time as IGF-I levels increase.

Regulation of GH secretion from the pituitary

Two hormones secreted from the hypothalamus, GH releasing factor (GHRF) and somatostatin, regulate GH secretion from the pituitary (Fig. 1). Somatostatin inhibits secretion but not production of GH (9). GH secretion can also be induced by treatment with synthetic peptides, so-called GH secretagogues (GHS), which act via the GHS-receptor (GHS-R) (10). The endogenous GHS-R ligand ghrelin was recently identified and its main site of production was shown to be the stomach (11). IGF-I has been shown to exert an inhibitory feedback action on GH secretion (12). However, it has been unclear how important endogenous liver derived IGF-I is for regulating GH secretion and if the effect of IGF-I is exerted at the hypothalamic or pituitary level (Fig. 1).

GH and sexual dimorphism

It is well known that there is a difference in the secretion pattern of GH between male and female rodents although the mean serum GH levels are similar in both sexes. Male rats have higher GH pulses and lower trough levels than female rats (13). In humans,

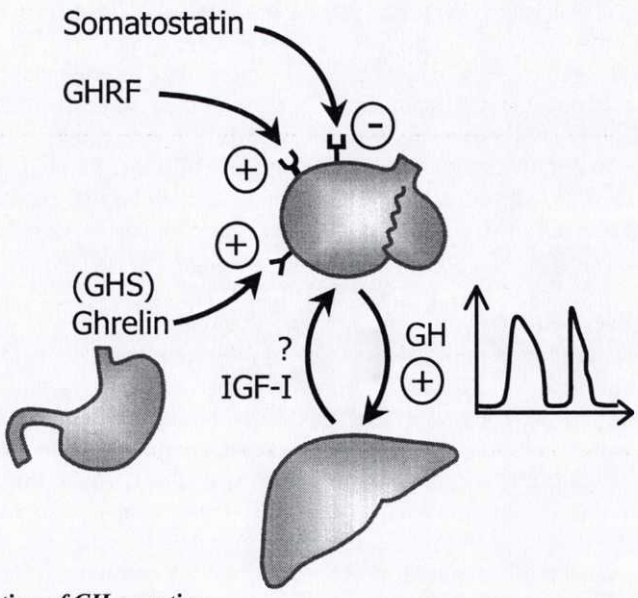


Figure 1. Regulation of GH secretion
GH secretion from the pituitary is pulsatile and is mainly regulated by the two hypothalamic hormones somatostatin and GH releasing factor (GHRF). Somatostatin inhibits GH secretion while GHRF induces GH secretion. GH secretion can also be induced by ghrelin, a peptide mainly produced in the stomach, although the physiological role of ghrelin in regulation of GH secretion is still unclear. Synthetic GH secretagogues (GHS) can also act via the ghrelin receptor. GH stimulates IGF-I release from the liver, and IGF-I may in turn inhibit GH release via negative feedback inhibition. It has been unclear to which extent this negative feedback effect is due to liver derived IGF-I and whether it is exerted at the pituitary level or at the hypothalamic level. These questions are addressed in article I.

men have a more pulsatile GH secretory pattern while women have less variable GH levels (14). Interestingly, men seem to have a more marked decrease in GH secretion in response to IGF-I than women (14). An indirect way of studying the GH secretory pattern in rodents is to measure the expression of liver specific and sexually dimorphic liver proteins, *e.g.* major urinary proteins (MUPs) and the prolactin receptor (PRL-R). Pulsatile GH secretion induces high levels of MUP protein in liver and urine, while constant levels of GH induce high expression of the PRL-R in liver (15). Liver growth is also influenced by the GH secretory pattern. Continuous GH treatment induces liver growth more than pulsatile treatment (16), while the opposite is true for body growth (17).

Roles of GH and IGF-I in postnatal growth

Many of the growth promoting effects of GH are mediated by IGF-I. GH enhances the levels of liver produced serum IGF-I as well as local production of IGF-I in other tissues (18). GH treatment to mice that lack GH is more potent than IGF-I in promoting growth, probably because GH promotes both endocrine and paracrine/autocine IGF-I

expression, while IGF-I treatment alone only restores the endocrine acting IGF-I. A patient lacking IGF-I was reported to have high levels of GH due to lack of negative feedback, and only responded with growth to IGF-I treatment (19). Mice lacking liver derived IGF-I also have increased levels of GH due to lack of feedback inhibition (1, 2). Insulin stimulates hepatic IGF-I production and young patients with insulin-dependent diabetes have growth impairment due to low IGF-I levels, despite high GH levels. Others and we have shown that liver specific deletion of IGF-I in mice, resulting in 85% decreased serum IGF-I levels, does not affect body growth up to 3 months of age (1, 2), suggesting that serum IGF-I has a minor role in regulating postnatal body growth.

GH-receptor deficiency

The Laron syndrome is a recessively inherited disease with GH resistance primarily due to a mutation in the GH-receptor (GHR) and GH-binding protein (GHBP) (20). These patients have high GH levels but low IGF-I levels and show similar growth retardation as GH deficient children. Treatment of children with the Laron syndrome with IGF-I leads to increased linear growth, showing that IGF-I promotes growth in GH insensitive patients (21, 22). It also shows that endocrine acting IGF-I can promote growth. IGF-I therapy seems to be well tolerated in these patients, the only side effect reported was hypoglycemia that could be avoided by food intake at the time of injection. At the start of treatment these patients also lost body fat. A GHR knockout mouse has been made which has the same characteristics as patients with the Laron syndrome (23). The GHR knockout mice show severe postnatal growth retardation, proportionate dwarfism, absence of GHBP, greatly decreased serum IGF-I and increased GH levels. These mice also develop truncal obesity, delayed puberty and recurrent hypoglycemia. The increase in GH secretion associated with GH receptor deficiency is probably due to decreased negative feedback by IGF-I and decreased short-loop feedback by GH itself.

IGF-I and IGF-I receptor knockout mice

Several studies have shown the importance of IGF-I and its receptor for normal body growth. The birth weights of IGF-I knockout mice are reduced to approximately 60% of those in normal littermates (24). The IGF-I knockout mice can not be used for studies of IGF-I effects during adult life as most mice die shortly after birth. IGF-I receptor knockout mice die at birth because of respiratory failure and exhibit an even more severe growth deficiency (45% of normal weight), probably reflecting the fact that both IGF-I and IGF-II act via the IGF-I receptor during fetal life (24).

IGF-II

IGF-II is normally not expressed in adult rodents while it is abundantly expressed during embryonic development (25). In contrast, in humans IGF-II is expressed in adults, but its role is unclear. IGF-II can bind to the IGF-I receptor and is able to mimic some of the effects of IGF-I (3). The birth weights of IGF-II knockout mice are reduced

to about 60% of those in normal littermates (26). However, IGF-II gene knockout mice grow normally after birth and are fertile.

BACKGROUND IL-6

IL-6 and IL-6 receptor expression and signaling

IL-6 expression
Several different cell types and tissues, including monocytes, fibroblasts, lymphocytes, glial cells, adipose and muscle tissue can produce IL-6 (27, 28). LPS and a variety of cytokines including IL-1β and TNFα, as well as β-adrenergic stimulation can induce IL-6 expression (29, 30), while glucocorticoids and estrogens can suppress production of IL-6 (31). Plasma levels of IL-6 are low under normal conditions *i.e.* in absence of infection, severe stress or exercise.

IL-6 receptor expression
The IL-6 receptor is mainly found on leukocyte subpopulations and hepatocytes (32). It has also been reported that there are IL-6 receptors in hypothalamic nuclei including the ventromedial and dorsomedial hypothalamus (Fig. 2) (33-35), and peripheral IL-6 infusion activates neurons in the paraventricular nucleus (PVN) of the hypothalamus (36). These hypothalamic nuclei are of importance for regulation of metabolic functions and body composition.

IL-6 receptor signaling
IL-6 acts via binding to a specific IL-6 receptor α (gp80) (37). This complex then dimerizes with a secondary transmembrane signal transducing receptor, glycoprotein (gp) 130, to eventually form a hexamer consisting of two of each of IL-6, IL-6 receptor α and gp130 (38). The signaling molecule gp130 is shared by a group of cytokines sometimes referred to as the IL-6 family of cytokines, with specific α-receptors and gp130 as the common secondary receptor (37). The IL-6 cytokine family includes oncostatin M (OSM), leukemia inhibitory factor (LIF), IL-11, cardiotrophin-1 (CT-1) and ciliary neurotrophic factor (CNTF). IL-6, LIF and OSM are secretory proteins, while CNTF and CT-1 are released after cell injury. The gp130 receptor lacks tyrosin kinase domains and is therefore dependent on activation of janus kinases (JAKs) for its down stream signaling (31). Activated JAK phosphorylates specific tyrosine residues on gp130, thereby creating docking sites recognized by the signal transducer and activator of transcription (STAT) proteins. STATs can be activated either by binding to the phoshorylated gp130 or to activated JAKs. Activated STATs form homo or heterodimers, which can enter the nucleus and activate transcription. There are several different JAKs and STATs that are involved in IL-6 signaling. JAK1, JAK2 and Tyk2 all bind to gp130. STAT3, 1 and 5 have all been shown to play a primary role in down stream signaling upon IL-6 activation (31). GH acts via a similar signaling pathway.

Soluble IL-6 receptor
Circulating IL-6 is bound to a soluble form of the IL-6 receptor α (gp80), that is produced either by shedding of the membrane bound IL-6 receptor, or by alternative

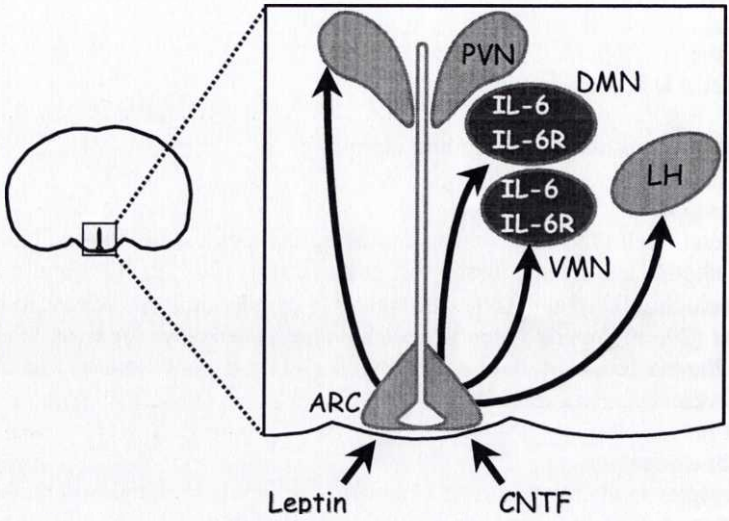


Figure 2. Expression of IL-6 and IL-6 receptors in the hypothalamus
According to several reports IL-6 and IL-6 receptors are expressed in the hypothalamus (33, 34, 35), and according to one report they are expressed in the ventromedial nucleus (VMN) and dorsomedial nucleus (DMN) of the hypothalamus (33). Leptin and ciliary neurotrophic factor (CNTF), two other cytokines, probably decrease body fat via effects on the arcuate nucleus (ARC) (141). Neuronal pathways from the ARC regulate other hypothalamic areas such as the paraventricular nucleus (PVN), the lateral hypothalamus (LH), VMN, and DMN.

splicing. This complex can then bind to membrane bound gp130 and activate IL-6 signaling pathways, even in cells that do not express the specific IL-6 α-receptor (39).

Immune-modulating properties of IL-6

Induction of acute-phase reaction
IL-6 is one of the major regulators of the acute-phase reaction that involves several different processes (27). The most obvious is fever, but it also involves leukocytosis, negative nitrogen balance, increased vascular permeability, alterations in plasma metal ion and steroid concentrations and an increase in hepatic acute-phase protein production (27). The hepatic acute-phase proteins that increase most are C-reactive protein and serum amyloid A (SAA), followed by fibrinogen, haptoglobin, α-antitrypsin and α-antichymotrypsin. The functions of these proteins and acute-phase reaction has not been entirely clarified, but the acute-phase proteins may be separated into three groups; 1) anti-microbial proteins, 2) proteins that regulate blood coagulation (both haematostatic and anti-thrombotic), and 3) anti-inflammatory proteins. Plasma levels of some liver derived plasma proteins, *e.g.* albumin and transferrin, are decreased during acute-phase response (40). Acute-phase reaction can be induced experimentally by treatment with bacterial wall components from gram-negative bacteria, *i.e.* lipopolysaccharides (LPS). IL-6 levels in serum rise prominently within 30 min after LPS treatment, and reach maximal levels at 2-3 hours, to decline back to baseline levels at 6-8 hours. During

inflammation and acute-phase reaction, IL-6 is often induced together with the pro-inflammatory cytokines TNFα and IL-1β (41).

Differentiation of immune cells
IL-6 has several other immunological functions besides induction of acute-phase reaction, such as induction of differentiation of B-cells into antibody-producing plasma cells, promotion of T-cell growth and differentiation (together with IL-1β) and development of antigen-specific cytotoxic T lymphocytes (29). It was also shown that IL-6 can induce differentiation of myeloid leukemic cell lines into macrophages (37).

Immunological effects of IL-6 deficiency
Studies in IL-6 deficient mice have shown that IL-6 is important for clearing infections with certain intracellular bacteria, *e.g. Listeria monocytogenes*, various parasites and some viral infections (42). However, IL-6 deficient mice develop normally and do not have substantially increased mortality rates or decreased longevity compared to their control littermates, unless challenged with specific pathogens. The acute-phase reaction is severely impaired after tissue damage or infection but is only moderately affected after LPS challenge, demonstrating the importance of other cytokines in regulation of acute-phase reaction. The number of thymocytes and peripheral T-cells are decreased in IL-6 deficient mice while lymphoid development is not seriously affected (31, 37).

Anti-inflammatory properties of IL-6
Experiments in IL-6 deficient mice have shown that IL-6 inhibits the production of several classical inflammatory mediators such as TNFα and INFγ (41). IL-6 also induces expression of acute-phase reactants with anti-inflammatory potential such as tissue inhibitor of metalloproteinases (TIMP) and it has been demonstrated that IL-6 can protect against both septic and toxic shock (43). IL-6 can induce activation of the hypothalamo-pituitary adrenal (HPA) axis, by inducing corticotropin releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) production, which in turn induce production of adrenal hormones including glucocorticoids (44). The latter are important stress hormones with potent anti-inflammatory properties. It was recently shown that deletion of IL-6 in apolipoprotein E knockout (ApoE-/- & IL-6-/-) mice causes an impairment of the cardiovascular phenotype compared to single ApoE-/- mice. These data suggest that IL-6 also has anti-inflammatory properties in atherosclerosis (45). Another aspect strengthening the role of IL-6 as an anti-inflammatory protein is that high doses of IL-6 are well tolerated in humans. Treatment with IL-6 does not induce hypotension in contrast to TNFα and IL-1β, which both cause increased vascular permeability, hypotension and circulatory shock at relatively low doses (46).

Other functions of IL-6

Besides its immune-modulating functions, IL-6 is important for normal liver growth (47) and liver regeneration (48, 49). After 70% partial hepatectomy the liver normally regenerates to its original size within approximately 10 days, while in IL-6 deficient mice this process is impaired.

BACKGROUND OBESITY

The obesity epidemic

Obesity is an increasing problem in society. In the USA, 60% of the population have a body mass index (BMI; kg/m^2) above the upper normal limit of 25 and approximately 40% are obese with a BMI of above 30. In Sweden and the rest of western societies, these figures are slightly lower, but are increasing rapidly (50). Obesity leads to several severe metabolic perturbations, such as hypertriglyceridemia, insulin resistance and diabetes, as well as cardiovascular disease that all significantly reduce the expected life span of patients. Nutritional education and physical activity are important approaches, but have statistically had limited efficiency (51, 52). This shows the need for the development of pharmacological treatments that together with behavioral approaches might improve chances of weight loss and weight maintenance. To find new pharmacological targets, it is important to increase our knowledge on the regulation of energy homeostasis, *i.e.* the balance between food intake and energy expenditure.

Regulation of fat mass by the hypothalamus

Early studies in the 1950´s showed that lesions of the ventromedial hypothalamus caused hyperphagia and obesity, while disruption of the lateral hypothalamus caused hypophagia and weight loss. Lesions of both the ventromedial hypothalamus and the lateral hypothalamus caused hypophagia, leading to the conclusion that there is a satiety center in medial hypothalamus, which inhibits the hunger center in the lateral hypothalamus. Later it has also become clear that the hypothalamus is important for regulation of energy expenditure (53).

Role of the medial hypothalamus in regulation of appetite
It is now well known that several peptides are found and act together to regulate energy homeostasis in the hypothalamus. These peptides are either anorexigenic, *i.e.* they suppress appetite, or orexigenic, *i.e.* they stimulate food intake (Fig. 3). Two anorectic peptides, α-melanocyte stimulating factor (α-MSH) and cocaine-amphetamine regulated transcript (CART) are found in the same neurons in the arcuate nucleus (ARC) of the medial hypothalamus, *i.e.* the satiety center mentioned above. The orexigenic or appetite stimulating neuropeptides, neuropeptide Y (NPY) and agouti-related peptide (AGRP) are found in another group of neurons in the arcuate nucleus. The latter neurons partly act by suppressing the effect of the anorectic α-MSH/CART neurons, in line with finding that loss of both by lesion of the satiety center results in hyperphagia and obesity (53).

Both α-MSH and AGRP act via the melanocortin-4 receptor (MC4-R). Activation of the MC4-R by α-MSH leads to suppression of food intake while inhibition of the MC4-R by AGRP induces food intake (Fig. 3). MC4-R mutations cause severe obesity in mice (54) and humans (55). Mice with ectopic expression of agouti, which similar to AGRP acts as an antagonist to the MC4-R, develop mature onset obesity, hyperleptinemia and hyperinsulinemia (56). As agouti in these mice also antagonises MC1-R in the skin, they

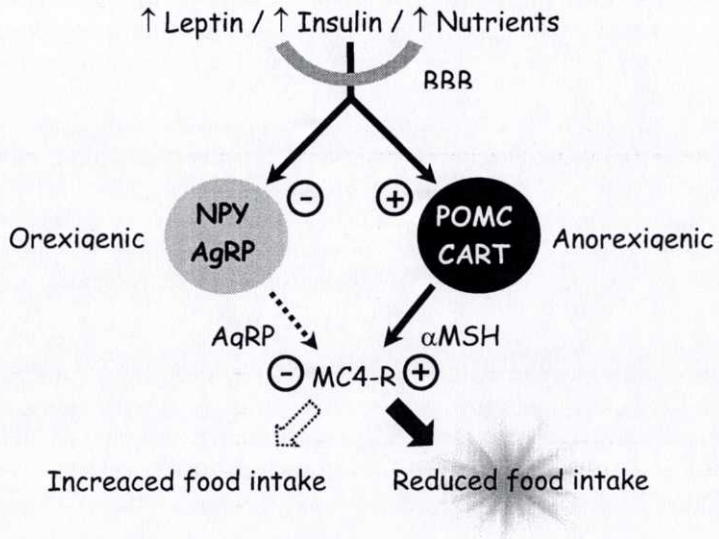


Figure 3. Hypothalamic regulation of food intake
A high nutritional status, i.e. high leptin levels and possibly also high insulin levels and abundance of nutrients, signal to the brain that energy stores are sufficient and therefore food intake can be decreased. The signaling cascades that are activated include the anorexigenic POMC pathway that leads to activation of the MC4-R by αMSH, which in turn, reduces food intake. The anorexigenic neurons also produce cocaine-amphetamine regulated transcript (CART). The orexigenic pathway, including the neurons that produce NPY and AGRP, is down regulated by surplus of nutrients. AGRP is an antagonist to αMSH and inhibits activation of the MC4-R. If nutrients are scarce on the other hand, the orexigenic pathway is activated and the anorexigenic pathway is inactivated, leading to inhibition of the MC4-R and increased food intake.

also get a reddish hair color (57, 58). A similar phenotype is also present in mice and humans with inactivating mutations of POMC, the precursor to α-MSH, but they also get hypofunction of the adrenal cortex due to lack of ACTH, which is another cleavage product of POMC (59). Mutations of the MC4-R are more common in humans and result in obesity without effects on pigmentation or adrenal function (60-62).

Role of the lateral hypothalamus in regulation of appetite
Two orexigenic peptides, melanocyte concentrating hormone (MCH) and orexin have been found in lateral hypothalamus (63, 64), which is well in line with the old findings that this is a hunger centre.

The fat-derived adipostatic hormone leptin
A large part of our current knowledge about hormones and metabolic pathways involved in energy balance has come from the analysis of extreme phenotypes in rodents such as the leptin deficient *ob/ob* mice, and the leptin receptor deficient *db/db* mice. These mice are markedly hyperphagic, have decreased energy expenditure, and develop severe obesity. By replacement therapy with leptin, the phenotype of *ob/ob* mice can be reversed (65, 66). The reduced food intake following leptin treatment is only seen until fat stores are depleted. After that, leptin treatment does not lead to a

further reduction of food intake or body weight. These data lead to high expectations for leptin as an antiobesity treatment for humans. It was later shown however, that obese people are generally leptin resistant and do not respond to leptin treatment (67).

Leptin is secreted from adipose tissue and regulates synthesis and release of several of the above-mentioned neuropeptides in the medial hypothalamus, which either increase or decrease food intake. Leptin inhibits expression of the orexigenic peptides NPY (68) and AGRP (69), and leptin stimulates expression of the anorexigenic peptides α-MSH and CART (Fig.3) (70). As far as known at present, leptin inhibits the hunger center of the lateral hypothalamus indirectly via effects on the satiety center in the medial hypothalamus.

Several pieces of information make it likely that the hypothalamus is the principle site of leptin´s actions: 1) Central treatment with leptin is much more potent in reducing body weight than peripheral treatment (65), 2) peripheral treatment with leptin activates neurons with an established role in regulation of food intake and energy expenditure (70) and 3) there are leptin receptors expressed in the activated areas (70).

Leptin levels in cerebrospinal fluid (CSF) are positively correlated with BMI. However, the efficiency of the uptake of leptin to CSF from plasma (the CSF:plasma leptin ratio) decreases with increasing obesity (71, 72). This indicates that leptin is transported from serum to CSF but that the mechanism is saturable. It has been suggested that this contributes to the leptin resistance in obese patients. The exact mechanism for how leptin is transported from serum to CSF is not known, one proposed mechanism is transport via the short non-signaling leptin receptor, ObRa, that is expressed *e.g.* in the choroid plexus (73). It has also been proposed that other, post-receptor mechanisms, such as upregulation of the suppressor of cytokine signalling (SOCS)-3 protein, that can inhibit signal transduction by the long leptin receptor, ObRb, could contribute to leptin resistance (74).

Genetic causes of obesity in humans

As mentioned above, several mutations discovered in mouse models have later been shown to be the underlying cause of obesity also in some humans. The most common mutations found so far are those in the MC4-R gene, which may account for up to 5% of all cases of severe human obesity (54, 55). These mutations, however, only explain obesity in a few of the total numbers of obese subjects. For instance, mutations of the leptin and the leptin receptor genes are extremely rare in humans (75, 76). However, the leptin deficient humans respond well to leptin treatment, and therefore this discovery has been beneficial for these individuals (77).

Most likely, several different contributing factors, genetical and behavioral, and combinations of these lead to obesity in humans. It is possible that heterozygous mutations of known genes such as the MC4-R gene contribute to obesity in larger groups of people. A precedent that heterozygous deletions cause less severe obesity has been shown for the leptin gene (78).

Adipose tissue

Adipose tissue can be divided into two different types, white adipose tissue (WAT) and brown adipose tissue (BAT). The main function of WAT is to store fat in the form of triglycerides, while BAT can participate in the regulation of body temperature by producing heat. This is achieved by so-called non-shivering thermogenesis via uncoupling of mitochondrial respiration (79). BAT is mainly found in neonates and during infancy in humans. There is evidence however, that brown adipocytes recide in white adipose tissue also in adults (80). Rodents maintain an interscapular BAT depot also after infancy.

White adipose tissue is a heterogeneous organ both in terms of differences between individual fat depots and in the range of cells that are present within the tissue. White adipose tissue consists of approximately 50% adipocytes and 50% stromal-vascular cells, which include endothelial cells, vascular smooth muscle cells, fibroblasts, immune cells (macrophages, B-cells and T-cells) and nerve fibers (81). Besides being important for the storage of energy, white adipose tissue secretes several proteins to the circulation, which have important functions in the regulation of metabolism. These include *e.g.* leptin, TNFα, IL-6, adipsin, resistin and adiponectin, which are described below.

Fat derived factors that affect insulin sensitivity

The two major effects of insulin are to stimulate glucose uptake into peripheral tissues, mainly muscle, and to stop hepatic glucose production. It has been assumed for many years that fat tissue regulates insulin sensitivity in other organs, *e.g.* skeletal muscle, probably via blood-borne factors. Obesity is often associated with type II diabetes, which is characterized by insulin resistance, *i.e.* hyperglycemia despite high insulin levels. Patients with severe lipodystrophy that have leptin deficiency are insulin resistant and have hypertriglyceridemia, as well as hepatic steatosis. These data show that both too much and too little fat is associated with insulin resistance. It is also known that drugs acting on peroxisome proliferator activated receptor (PPAR) γ, which is mainly expressed in fat tissue, can indirectly affect insulin sensitivity in skeletal muscle. Below are listed some fat-derived hormones that have been suggested to participate in the regulation of insulin sensitivity.

Leptin

Leptin levels in blood increase within hours after a meal and decrease within hours in response to fasting (82, 83). These effects are probably mediated by insulin. Insulin levels peak after a meal and are followed by a peak in leptin. A similar effect is seen after insulin treatment (84). Leptin is known to enhance insulin actions by improving its inhibition of hepatic glucose production (85). Recently, it has been reported that leptin treatment partly or completely can reverse the severe hypoglycemia seen in mice with experimentally induced lipoatrophy (86, 87). Also, in patients that lack adipose tissue, treatment with leptin has recently been shown to decrease fasting glucose, triglycerides and liver steatosis (88). When leptin levels were restored in these patients they were

able to discontinue, or greatly reduce, insulin therapy for diabetes. This shows that leptin deficiency contributes to insulin resistance, and that these two hormones act hand in hand.

TNFα

TNFα mRNA levels in adipose tissue increase with increasing obesity and cultured adipocytes secrete TNFα into the medium (89-91). Inhibition of TNFα in rodents increases insulin induced glucose uptake and therefore TNFα has been proposed to play an important role in insulin resistance in obesity (92). It was shown that TNFα inhibits insulin action by inducing serine phosphorylation of the insulin receptor substrate-1 (IRS-1). Serine-phosphorylated IRS-1 acts as an inhibitor of insulin receptor activity (93, 94). However, there are data suggesting that TNFα inhibition in obese diabetic patients does not affect insulin resistance (95). Moreover, TNFα concentrations were not higher in venous blood draining subcutaneous adipose tissue, compared to arterial blood in humans, implying an autocrine or paracrine, rather than endocrine, role for this cytokine within adipose tissue (96).

IL-6

IL-6 is secreted from adipose tissue to the circulation, and its expression is positively correlated to BMI and total body fat tissue mass (96, 97). Approximately 30% of all basal circulating IL-6, in absence of exercise and inflammation, is derived from adipose tissue (96). It has also been shown that intraperitoneal fat produces more IL-6 than subcutaneous fat (98). Serum IL-6 levels have been linked to metabolic disturbances, *e.g.* insulin resistance, and cardiovascular morbidity (99, 100), however, a cause-effect relationship has not yet been convincingly established. IL-6 treatment to humans has been shown to give a small, dose-dependent increase in blood glucose, probably via glycogen breakdown and secretion of glucose from the liver and/or by inducing peripheral resistance to insulin action (46). It has however also been shown that IL-6 infusion in humans leads to increased glucose clearance (101), and IL-6 induces increased glucose uptake in cultured adipocytes (102). It is possible that the increased production of IL-6 during inflammation or severe stress may contribute to the increased glucose levels and insulin resistance that accompanies these conditions. This could be an adaptive response for limited periods of time and might be a way to direct energy to the immune system rather than to energy storage.

Resistin

Adipocytes secrete resistin, and resistin levels in serum are increased in both genetic and diet-induced obesity (103). Resistin was found when screening for a factor that was up-regulated during adipocyte differentiation but down-regulated in mature adipocytes exposed to thiazolidinediones, a group of PPARγ agonistic anti-diabetic drugs (103). It was reported that insulin actions are blunted by resistin treatment while neutralizing antibodies against resistin enhance insulin-stimulated glucose uptake (103). However, another group has recently reported that resistin expression in fat is decreased by obesity and increased by thiazolidinedione treatment (104). Moreover, resistin may not be secreted from adipose tissue at all in humans (105). Therefore, more studies are needed to clarify the exact biological function of resistin.

Adiponectin

Adiponectin/adipocyte complement-related protein of 30 kDa (Acrp30) is an adipocyte-specific secreted protein (106). The plasma concentrations of adiponectin are decreased in obese subjects and patients with type II diabetes (107). Treatment with adiponectin has been reported to decrease hepatic glucose production and to prevent the increase in free fatty acids that occurs after meals (106). More studies are however needed to determine the therapeutic potential of this novel interesting hormone.

AIMS OF THE THESIS

The aims of this thesis were to characterize the roles of IGF-I and IL-6 in metabolism and regulation of body fat mass.

Specific aims of articles I-IV were:

I To investigate how liver derived IGF-I regulates the GH secretory pattern.

II To study the long-term effects of lack of liver derived IGF-I on body composition and metabolism.

III A) To characterize the mature-onset obesity and the obesity-related metabolic perturbations found in IL-6-deficient mice.

B) To identify the site and mechanism of action for the anti-obesity effect of endogenous IL-6.

IV To see if long-term central treatment with IL-6 could induce a specific decrease in adipose tissue mass in non-IL-6-deficient animals.

METHODOLOGICAL CONSIDERATIONS

Generation of liver-specific inducible IGF-I knockout (LI-IGF-I-/-) mice

To study the effect of liver derived IGF-I *in vivo* we have made a liver-specific inducible knock-out mouse (LI-IGF-I -/- mice) (1). We did this by using the Cre/loxP recombination system (108). This method makes it possible to generate time and tissue specific deletions of genes. This is especially important for the study of genes that are necessary for survival during embryonic development. The Cre-loxP system is based on the generation of two transgenic mouse strains. One mouse strain expresses the Cre enzyme only in the preferred tissue or cell type thanks to a tissue specific promoter. In the other mouse strain a crucial exon of the gene of interest is flanked by specific 35 nucleotide sequences (loxP sites), which are recognised by the Cre enzyme. The Cre enzyme specifically cuts out DNA sequences that are located between loxP sites. In the present model, loxP sites flank exon 4 of the IGF-I gene (109) and the MX-I-promoter is switched on mainly in hepatocytes (low expression also in the spleen) by treating the animals with interferon $\alpha 2/\alpha 1$ (108). By crossing these two strains of mice, we get mice in which the IGF-I gene can be inactivated specifically in the liver after treatment with interferon. This leads to a pronounced decrease in serum IGF-I levels enabling us to separate the effects of liver derived serum IGF-I from those of locally produced IGF-I in other tissues. In the present studies (articles I and II) the mice were treated with interferon at the time of weaning, at three weeks of age. They were treated with interferon every other day for a total of three days. The time for induction, 3 weeks of age, was chosen for several reasons: 1) GH dependent postnatal growth does not start until 3 weeks of age, 2) If pups are handled too early the dam can get stressed and reject or even kill the pups.

In the article where we first described these mice (1), three different control groups were included: 1) loxP homozygous mice treated with PBS, 2) loxP homozygous and MX-I-Cre transgenic mice treated with PBS, and 3) loxP homozygous mice treated with interferon. Because we could not detect any differences between the three control groups we only used littermates homozygous for loxP treated with interferon as controls in articles I and II. In the study where the mice were characterized (1) I measured mRNA levels of IGF-I by RNAse protection assay (RPA) in several different tissues to confirm that the IGF-I deletion was liver specific, and to study possible compensatory increases in IGF-I mRNA expression in other tissues. I found that IGF-I mRNA levels were decreased by more than 80% in the liver and by approximately 60% in the spleen but was unchanged in all other tissues examined. To test the degree of IGF-I deletion in hepatocytes, I isolated and cultured hepatocytes from control and LI-IGF-I-/- mice and measured IGF-I levels in the culture medium. IGF-I levels were measurable in medium from control hepatocytes, but not in medium from LI-IGF-I-/- hepatocytes. This shows that deletion of IGF-I production was complete in the hepatocytes from LI-IGF-I-/- mice.

Dual energy X-ray absorptiometry (DXA)

DXA is a non-invasive method that can be used for determining fat tissue mass. This technique enables us to study body composition in live animals. With the DXA technique, an emitted X-ray is divided into two different energy levels by a filter. The absorbance of X-ray energy is different in tissues with different densities, and the amount of radiation absorbed when each X-ray is passed through the body is measured. The DXA system used was the Norland pDEXA Sabre (Fort Atkinson, Wisconsin, USA) together with the Sabre Research software (3.9.2). To analyze the percentage of body fat the software % fat procedure was used together with a setting that made areas with more than 50% fat appear white on the image. The image was then printed, scanned and imported to the Scion Image software (Scion Corporation, Maryland, USA). The imported image was set to a threshold of 50 arbitrary units, making lean mass and bone appear black, while areas with more than 50% fat appeared white. Then the "analyze particle" procedure was performed, first with the white areas of the mice included (A1 = total area) and then with the white areas excluded (A2 = lean area + bone area). The % fat area was then calculated as [(A1-A2)/A1]*100. In all studies three mice were analyzed in each DXA measurement, one of which was included in all scans as an internal standard to avoid inter-scan variations. The % of fat measured by DXA has been shown to be correlated to the amount of dissected fat (110).

Peripheral quantitative computerized tomography (pQCT)

Figure 1*e* in article III shows the results of an abdominal CT scan of wild-type control mice and IL-6 deficient mice. The scan clearly shows the subcutaneous fat area between the skin and peritoneum. In the same article, figures 3*b* & *c*, we used the results of several abdominal CT scans to evaluate the effect of IL-6 treatment on the total abdominal and intraperitoneal areas. The scan was performed 5-mm proximal of the iliac crest, identified by a longitudinal pre-scan. The pQCT consists of a rotating X-ray force, which moves to fix even-distanced positions, 360° around the specimen. The local attenuation data from each position are processed in a computer that gives an image representing a section though the specimen. In this thesis measurements were performed with the Stratec pQCT (XCT) (Norland, software version 5.4B) operating at a resolution of 70 μm.

Indirect calorimetry

Measurements of oxygen consumption (Vo_2) and carbon dioxide production (Vco_2) were used to assess energy expenditure after ICV IL-6 or NaCl treatment in article III. The Oxymax system (Columbus Intruments, OH, USA) for indirect open-circuit calorimetry was used. The system monitors oxygen and carbon dioxide concentrations by volume at the inlet and outlet ports of a chamber through which a known flow of air is being forcibly ventilated. While passing through the chamber, the respiratory exchange of the animal reduces the oxygen content of the air and increases its carbon dioxide content. At the exit of the chamber, the air is dried, the airflow is measured and

samples of the cage air are distributed to gas analyzers that monitor its oxygen and carbon dioxide contents, relevant to the to the oxygen and carbon dioxide content of the air supplying the metabolic chamber. The difference in gas concentrations along with flow information is used in the calculations of oxygen consumption, carbon dioxide production and respiratory exchange ratio.

The system was calibrated daily with a gas of known composition (20.50% O_2, 0.50% CO_2 in N_2). Before start of all experiments, I checked that the airflow in each individual cage was higher than the air being withdrawn to the analyzers, to make sure that the system did not leak. In between every round of sampling, the room air (reference air) was analyzed to minimize eventual effects of changes in the environment. We did not measure activity during the experiments, however, all experiments were performed during light-phase and the animals fell asleep soon after being placed in the chambers. We did not take account for the metabolically active mass of the rats as they were their own controls, *i.e.* we compared Vo_2 and Vco_2 before and after treatments in each individual rat. The data collected during the first 1.5 hours was discarded in all experiments to allow for adequate equilibration of the chamber air to the animal.

RESULTS

I. Liver derived IGF-I regulates GH secretion at the pituitary level in mice

In an earlier article (1) we characterized a liver specific inducible IGF-I knockout (LI-IGF-I-/-) mouse model. This model made it possible for us to distinguish between the effects of liver derived IGF-I, that comprises 85% of serum IGF-I, and the effects of IGF-I produced locally in other tissues. The results from that study showed that post-natal growth was normal in LI-IGF-I-/- mice during the first 3 months, and that these mice had increased GH levels and enlarged livers. In article I, I studied the mechanism for how liver derived IGF-I regulated GH secretion and whether the GH secretory pattern was affected. Because it is very difficult to directly measure the GH secretory pattern in mice, an indirect way of assessing the GH secretory pattern was needed. Several different GH regulated functions are regulated in a sexually dimorphic way depending on the GH secretory pattern. Liver growth and PRL-R expression are stimulated by the feminine more continuous GH secretory pattern. A more pulsatile, male specific, GH secretory pattern stimulates expression of MUP and body growth. Expression of the PRL-R was increased and MUP expression was decreased in male LI-IGF-I-/- mice, *i.e.* the expression patterns of these two genes were feminized. These data, together with the increased liver size in the LI-IGF-I-/- mice, indicated that the basal GH levels were increased, *i.e.* a loss of GH troughs in-between pulses in the male LI-IGF-I-/- mice. To study the mechanism behind the increased GH levels in male LI-IGF-I-/- mice, we analyzed several hypothalamic hormones known to regulate GH secretion as well as their corresponding pituitary receptors. We found that the LI-IGF-I-/- mice had increased pituitary expression of receptors for GHRF and GHS. Treatment with GHRF and a GHS, ipamorelin, showed increased GH responsiveness in the LI-IGF-I-/- mice, results that are in line with the increased GHRF-R and GHS-R expression. To summarize, liver derived IGF-I has an important role in regulating GH secretion, and indirectly several liver functions, and acts by inhibiting expression of receptors for GHRF and GHS at the pituitary level. This is in line with the existence of a liver-pituitary axis.

II. Liver derived IGF-I is of importance for normal carbohydrate and lipid metabolism

In this article, we followed the LI-IGF-I-/- mice for more than a year and studied body growth and body composition. We also measured blood lipids, basal insulin levels and performed glucose tolerance tests. IGF-I levels were continuously low throughout the study in LI-IGF-I-/- mice. Although postnatal growth was not affected during the first 3 months, at 13 months of age, body weights were decreased by 20% in LI-IGF-I-/- mice. DXA analysis of body fat in these mice showed that the LI-IGF-I-/- mice had 25% decreased body fat content compared to the control mice. Fat dissection confirmed these results and showed that the retroperitoneal and gonadal fat depots were significantly smaller in the LI-IGF-I-/- mice. Surprisingly, leptin levels were also increased in young LI-IGF-I-/- mice. Basal insulin levels were increased and the mice responded with hypersecretion of insulin after a glucose charge. However, the hypersecretion of insulin

adequately lowered glucose levels after the glucose tolerance test. Cholesterol levels were increased in young LI-IGF-I-/- mice and lipoprotein profiles showed an increase in LDL and HDL cholesterol fractions.

III. Interleukin-6-deficient mice develop mature-onset obesity

We found that the body weights of IL-6 knockout (IL-6-/-) mice started to deviate from their wild-type controls at 6-7 months of age, and at 9 months of age they were 20% heavier than control mice. In this article, we characterized the IL-6-/- mice by measuring different metabolic functions and body fat content in the mice at different ages. Both DXA and CT analysis of body fat content, as well as fat dissections, showed that the mice had increased adipose tissue mass, mainly consisting of an increase in subcutaneous fat. Leptin levels were significantly increased and the IL-6 deficient mice did not respond to leptin treatment. We substituted the IL-6-/- mice with low doses of IL-6 to see if we could reverse the phenotype. The IL-6 treatment decreased body weight in IL-6-/- mice, but did not affect body weight in control mice at the doses used. Leptin measurements and abdominal CT scans were performed before and after IL-6 treatment. In IL-6-/- mice, but not in control mice, leptin levels and the total abdominal and intraperitoneal areas were decreased after IL-6 treatment, indicating that the IL-6 substitution in part reversed the obesity in these mice. A single intracerebroventricular (ICV) injection of IL-6 to rats acutely increased energy expenditure, measured by oxygen consumption and carbon dioxide production. The same dose of IL-6 intraperitoneally had no effect on energy expenditure. These data suggest that IL-6 acts centrally to activate neurons involved in the regulation of metabolic function and body composition.

IV. Intracerebroventricular interleukin-6 treatment decreases body fat in rats

In article III, we found that a single injection with IL-6 ICV acutely increased energy expenditure. In this study I wanted to see if chronic IL-6 treatment ICV could decrease fat mass in rats. Rats fed a high fat diet for 4 weeks before, and during the study, were treated with either IL-6 or saline ICV for 2 weeks. Body weight was significantly decreased in the rats treated with IL-6. Food intake was not decreased on a daily basis, but was decreased by 15% on average over the whole study. Fat dissections showed that the retroperitoneal and mesenteric fat pads, as well as total fat mass, were significantly decreased in the IL-6 treated rats. The weights of several non-adipose organs were not changed. Levels of the acute-phase protein haptoglobin were not changed in the livers of the IL-6 treated compared to the saline treated rats. This study shows that central IL-6 can regulate adipose tissue mass, and that IL-6 treatment can decrease adipose tissue mass in animals that have normal endogenous levels of IL-6 without causing acute-phase reaction.

DISCUSSION

WHAT IS THE FUNCTION OF LIVER DERIVED SERUM IGF-I?

Liver IGF-I deficiency causes decreased fat mass

At about one year of age, the LI-IFGF-I -/- mice had 20% decreased body weight, and an even more pronounced decrease in total fat content. The reason for this adipostatic effect of decreased liver IGF-I is unknown, but some possible mechanisms are shown in figure 4. Insulin is important for the storage of nutrients, but also for signaling the amount of available nutrients to the brain. It has been shown that neuron specific insulin receptor knockout results in increased obesity (111). Therefore, the decreased fat mass may be due to the high insulin levels that send an afferent adipostatic signal to insulin receptors in the brain, that adipose stores have high fat levels. Peripheral insulin treatment causes obesity, probably because of increased food intake due to the hypoglycemic effect of insulin (112). In our study, the central effects of high insulin levels may simply override the peripheral effects, possibly because the diabetogenic effect of lack of liver IGF-I (see below) may prevent hypoglycemia. Young LI-IGF-I-/- mice also had increased leptin levels despite similar or somewhat decreased adipose tissue mass. Insulin is known to stimulate leptin secretion (84) and it is possible that the increase is merely due to the increased insulin levels.

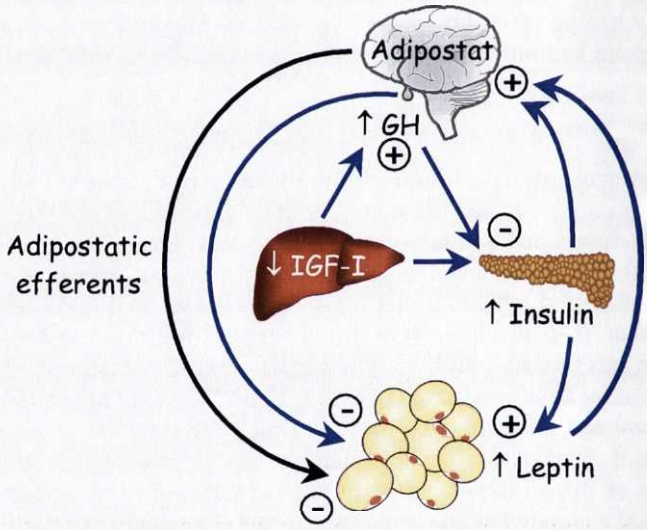


Figure 4. Possible mechanisms for regulation of fat mass by liver derived IGF-I
Deletion of liver derived IGF-I leads to 85% decreased serum levels of IGF-I. The observed increase in insulin levels could be a mechanism to compensate for the lack of insulin-like effects of IGF-I. The high insulin levels could in turn stimulate leptin secretion from adipose tissue. Leptin and insulin both signal to the adipostat in the brain that the body has sufficient energy stores (67, 111), which could decrease adipose tissue mass in the LI-IGF-I-/- mice via adipostatic efferents. Another possibility is that the increased GH levels, due to lack of negative feedback by IGF-I, cause or contribute to both the insulin resistance and decrease in adipose tissue mass.

Liver IGF-I deficiency impairs insulin sensitivity

Results from IGF-I treatment to insulin resistant patients show that IGF-I can mimic the glucose lowering effects of insulin, and can act as a back-up system for insulin (21). The importance of endogenous IGF-I for insulin action is supported by the identification of a patient lacking IGF-I, who had signs of insulin resistance, which was normalized after IGF-I treatment (113). This study, however, does not provide any information about the site of production of the IGF-I that exerts this effect. In article II we show that liver derived IGF-I is essential to facilitate the effect of insulin. In our mouse model serum IGF-I levels were decreased by 85% and it seems possible that the increased insulin levels may be a compensatory mechanism for the low IGF-I levels. Yakar *et al.* have published data supporting our data on the metabolic effects of IGF-I (114). Their liver specific IGF-I-/- mouse model is also insulin resistant and they found that insulin-induced autophosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1) was completely abolished in skeletal muscle, but normal in liver and fat. Replacement therapy with IGF-I decreased basal insulin secretion and enhanced insulin sensitivity in muscle.

Lipolytic and diabetogenic effects of GH

GH is well known to exert lipolytic and diabetogenic (insulin antagonizing) effects. Therefore, the high GH levels in LI-IGF-I-/- mice (Article I) may be a potential cause of both the decreased adipose tissue mass and the insulin resistance in LI-IGF-I-/- mice. In fact, it has been shown in another model of liver specific IGF-I knockout that lowering of the high GH levels by treatment with a GHRF-antagonist improves insulin sensitivity (114). Low GH levels or GH receptor defects are associated with abdominal obesity. Substitution treatment with GH to adult men with GH deficiency has been shown to decrease abdominal obesity (115). Fasting in humans is followed by a drop in serum IGF-I and an increase in GH secretion. The increase in GH secretion is reversed by treatment with IGF-I in a dose that does not suppress blood glucose. Therefore, the starvation-induced increase in GH, which in turn causes lipolysis and decreased glucose utilization, is probably mediated by decreased IGF-I production by the liver (116).

Does liver IGF-I affect appetite or energy expenditure?

The decrease in adipose tissue mass in the LI-IGF-I-/- mice could be due to changes in food intake and/or energy expenditure (increased basal metabolic rate and/or activity). We have so far not seen any differences in food intake between LI-IGF-I-/- mice and wild-type siblings. This might argue that the effects of decreased liver IGF-I are not mediated by changes in leptin or insulin, which are known to affect food intake. However, more studies are needed to clarify this issue, *e.g.* to examine oxygen consumption and motor activity in mice with liver specific IGF-I knockout.

Effects of liver IGF-I on the liver size

Liver weight in relation to body weight was increased in the LI-IGF-I-/- mice. We believe that this is due to and reflects the increased basal levels of GH because it is well-known that GH treatment can increase liver weight, and continuous GH may be more effective than pulsatile GH treatment (117). Furthermore, GH transgenic mice have increased liver weight in relation to body weight, while the opposite is true for mice that lack GH (8, 118).

Negative feedback loop

We have shown that mice which lack liver derived IGF-I have increased basal GH levels due to decreased IGF-I feedback. We were not able to measure whether low serum levels of IGF-I also caused increased GH pulse height. It is known that inhibition of GH action increases both pulse amplitude and basal GH levels in humans treated with a GH-receptor antagonist. This treatment lowers serum IGF-I levels efficiently but it is not known whether the enhanced GH levels are due to decreased short-loop feedback by GH itself or due to decreased IGF-I mediated feedback (119). Studies on a patient with IGF-I gene deletion have shown that complete lack of IGF-I causes enhanced basal GH secretion as well as increased pulse height (120). This study did not elucidate, however, whether the increase in GH secretion was caused by lack of circulating IGF-I or IGF-I produced locally in the hypothalamo-pituitary system.

It has been speculated a long time if IGF-I feedback inhibition is due to increased somatostatin secretion or decreased GHRF secretion from the hypothalamus. Very few reports have addressed the possibility that IGF-I at the pituitary level might regulate the expression of receptors that are important for GH secretion. A report by Kamegai *et al.* shows that IGF-I treatment to a spontaneous dwarf rat, that lacks endogenous GH due to a point mutation in the GH gene, did not affect expression of hypothalamic peptides, but decreased GHRF-R expression in the pituitary (121). This study supports our conclusion that circulating IGF-I acts on the pituitary level to regulate GH secretion, by inhibiting expression of GHRF-R.

REGULATION OF ADIPOSE TISSUE MASS BY IL-6

Central effects of IL-6

In articles III and IV we have shown that by increasing energy expenditure and possibly also by decreasing food intake, centrally acting IL-6 can decrease adipose tissue mass. We have also studied IL-6 levels in CSF from humans with varying BMI (Stenlöf K *et al.*, manuscript in preparation) and found that IL-6 levels in CSF were negatively correlated to both total fat mass, and subcutaneous fat mass. In some patients the levels of IL-6 in CSF were several-fold higher than in serum. It is well known that IL-6 levels in serum are positively correlated to BMI (96, 97), and we found a similar correlation in our study. In line with these results, there was no correlation between serum and CSF IL-6 levels. Finally, we found in this human study that IL-6 levels in serum, but not in

CSF, decreased during fasting. Taken together, these results suggest that local production of IL-6 within the CNS is the primary source of CSF IL-6. Moreover, CSF IL-6 levels could reflect the levels of IL-6 that affect brain centers regulating energy expenditure, appetite and adipose tissue mass, as indicated in figure 5. Hence, it may be speculated that obese patients have insufficient IL-6 production within the CNS. IL-6 is produced by several different cell types in the CNS such as astrocytes and microglia (122), but it has also been reported that IL-6 and its receptor are expressed in neurons in hypothalamic nuclei, *e.g.* VMN, that are involved in the regulation of body composition (33-35).

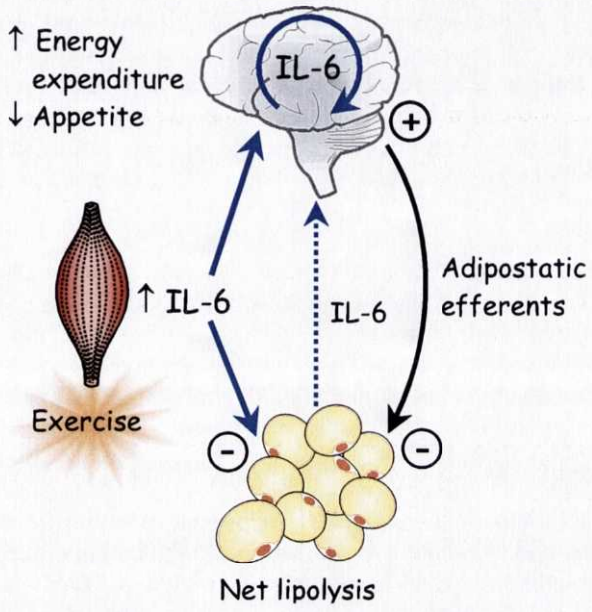


Figure 5. Regulation of fat mass by IL-6
We have shown that central IL-6 treatment reduces adipose tissue mass in rats by increasing energy expenditure and decreasing appetite (Articles III and IV). We do not know how central levels of IL-6 are regulated during physiological conditions. During exercise, when IL-6 levels increase 100-fold (123), it is possible that peripheral IL-6 has central effects. The IL-6 that is produced in skeletal muscle during exercise may also have direct effects on adipose tissue inducing lipolysis. Serum IL-6 is to a large extent derived from adipose tissue (96) during normal non-inflammatory conditions, and in the absence of exercise. We have shown that CSF levels of IL-6 do not correlate to the comparatively low serum levels of IL-6 during these conditions (Stenlöf K et al., manuscript in preparation). Therefore, under normal conditions it seems likely that the major proportion of CSF IL-6 is not derived from the periphery by active transport of IL-6 to the CNS. These data in conjunction with reports that IL-6 can be produced in the hypothalamus (33, 34, 35), raise the possibility of actions of locally produced IL-6 within the brain. The adipostatic efferent signals from the CNS to the periphery to decrease adipose tissue mass probably includes the sympathetic nervous system in addition to appetite regulation.

Regulation of IL-6 in the circulation

IL-6 levels in serum increase prominently after physical exercise, *e.g.* it was shown that IL-6 levels increase up to 100-fold after a marathon race (123). IL-6 is released from muscle and it increases already after moderate-intensity exercise. This effect is dependent on muscle glycogen content (124). It has been shown that the release of IL-6 is not caused by muscle damage (28, 125, 126). Exercise has many beneficial effects on the immune system, body weight regulation and in particular adipose tissue mass, as well as on insulin sensitivity (127). It is an intriguing possibility that IL-6 is one of the regulators of some of these effects. IL-6 produced from muscle tissue may be a signal for increased substrate release from fat, by lipolysis, and from liver by glucose production by glycogenolysis and gluconeogenesis. These effects may be direct on peripheral target tissues such as fat and liver, or indirect via central effects in the hypothalamus that affect peripheral tissues via various afferents (these possibilities are indicated for fat tissue in Fig. 5). When IL-6 levels in serum increase almost 100-fold, there might be an increased transport across the blood brain barrier. There are also data that show that IL-6 both stimulates release of glucose from the liver and increases glucose clearance from the circulation in humans, *i.e.* IL-6 increases glucose turnover (101).

The IL-6 levels in the circulation are considerably lower in the absence of exercise or inflammation. During basal conditions IL-6 is released to a large extent from fat, and, as noted above, serum IL-6 is positively correlated with BMI (96, 97). It is unclear whether these low serum levels of IL-6 can pass the blood-brain barrier in substantial amounts to affect fat regulating centra (Fig. 5). However, food intake has been shown to regulate IL-6 levels in fat and serum, further supporting a role for IL-6 in regulation of nutritional functions (100, 128).

We have started setting up a system were we can combine treadmill running and indirect calorimetry and plan to use this setup to study the effects of running exercise in IL-6 deficient compared to wild-type mice, in regards to running endurance, energy expenditure and macro-nutrient utilization. We also plan to measure blood glucose, glucose clearance and uptake, insulin and FFA levels, lipolysis in adipose tissue and glycogen stores in different tissues. The studies on exercise are exiting because they represent a state with a prominent physiological increase in serum IL-6 that is not associated with illness.

High serum levels of IL-6 have often been associated with cachexia. However, during illness or acute-phase reaction several other strongly pro-inflammatory cytokines such as IL-1β and TNFα are also increased. IL-6-deficient mice have prolonged acute-phase response showing that IL-6 is not only pro-inflammatory, but also has important anti-inflammatory properties, *e.g.* down-regulation of TNFα and IL-1β after acute-phase reaction (41). Metzger and colleagues transplanted mice with tumors secreting high levels of IL-6 and compared them to non-secreting tumors and found a specific effect on decrease in adipose tissue in the IL-6 secreting tumor bearing mice, but no decrease in lean body mass (129, 130). This study supports our results that IL-6 can have specific effects on adipose tissue mass without causing illness and degradation of muscle tissue. In article IV I found that food intake was significantly decreased in the IL-6 treated rats,

although the animals showed no signs of illness, such as behavioral alterations with reluctance to move, reduced grooming or starred coat. Moreover, the rats did not stop to eat, but decreased food intake moderately, by 15%, arguing against cachexia and illness. This was also supported by the absence of changes in the acute-phase protein haptoglobin levels in the livers of IL-6 treated rats compared to saline treated controls.

Peripheral treatment with IL-6

We have also performed studies with measurements of indirect calorimetry in wild-type mice treated continuously with IL-6 or saline for 5 days subcutaneously via miniosmotic pumps. These mice also had telemetry probes intra-abdominally enabling us to follow core body temperature, heart rate and activity during the IL-6 or saline treatment. Because the mice were treated with IL-6 peripherally, we used higher doses than those used in the ICV experiments. Four days after implantation of the miniosmotic pumps, we started measurements of oxygen consumption and carbon dioxide production. Carbon dioxide production (Vco_2) and oxygen consumption (Vo_2) was increased during the light phase in the IL-6 treated compared to the saline treated mice (Vco_2 IL-6 vs. saline: 2534±184 vs. 2120±244 ml/kg/h, $P<0.001$; Vo_2 IL-6 vs. saline: 2791±190 vs. 2547±179, $P=0.02$). Respiratory exchange ratio (RER), that reflects macro-nutrient utilization, was also increased in the IL-6 treated mice (RER IL-6 vs. saline: 0.914±0.025 vs. 0.823±0.050, $P<0.001$). At the same time, we did not see any differences in activity, body temperature or heart rate in these mice, indicating that the increase in energy expenditure is not caused by thermogenesis and increased body temperature. We have also measured glucose, FFA and SAA levels in serum during these studies. SAA is a sensitive acute-phase protein whose expression is stimulated by IL-6 in mice. We did see a minor, but significant, increase in SAA levels. However, the levels were considerable lower than those seen during acute-phase reaction in association with illness.

To summarize, treatment with IL-6 either centrally or, at higher doses peripherally, can have beneficial effects on adipose tissue mass by increasing energy expenditure. The increase in energy expenditure does not seem to be associated with fever or any other sickness-associated symptoms or behavioral alterations. Moreover, changes in acute-phase reactants were small or absent in the present experiments. Others have treated humans with IL-6 (doses of 0.1-10 μg/kg bodyweight) and seen a dose-dependent increase in energy expenditure with few and slight side effects such as nausea and slight increases in core body temperature at the highest doses used (46, 131, 132). Furthermore, the fact that IL-6 levels can rise up to 100-fold after physical exercise argues against a risk of deleterious long-term effects of high IL-6 levels. Therefore, I believe that IL-6 is a promising future drug candidate for the treatment of human obesity, at least at doses that mimic the levels seen during physical exercise. Thereby, it is possible that IL-6 could be used as an adjuvant, together with life-style changes of diet and increased exercise, to enhance the positive effects of physical exercise in obese subjects.

IMMUNE FUNCTION IN RELATION TO BODY FAT MASS

Leptin and immune function

Both leptin deficient and leptin receptor deficient animals have altered immune and inflammatory responses including abnormal cytokine gene expression after LPS challenge and impaired phagocytosis (133). This is also seen during starvation and malnutrition, two conditions characterized by low leptin levels. The fall in leptin levels seems to be a signal to the brain to adapt to starvation and conserve energy by shutting off "luxury" functions like reproduction and the innate immune system. Leptin treatment has been shown to reverse the immunological effects of starvation, such as impaired T-cell immunity (134).

True to its cytokine nature, leptin can increase body temperature, probably acting via IL-1β (135), and this effect may be part of the mechanism by which leptin treatment can inhibit the decrease in energy expenditure that is usually associated with starvation in rodents (65). In line with this, leptin deficient *ob/ob* mice also have decreased core body temperature (66).

Ciliary neurotrophic factor (CNTF)

One of the most promising anti-obesity drug candidates that is being tested in clinical phase 3 studies at the moment is CNTF, a cytokine belonging to the IL-6 family. It has been reported that three months of treatment with CNTF causes a substantial (~10%) weight loss in obese subjects (www.regeneron.com). Even more strikingly, the patients treated with CNTF maintained the decreased body weight for a period of more than 9 months after cessation of treatment. The three dimensional structures of IL-6 and CNTF are closely related, and the ligand binding parts of the CNTF receptor and IL-6 receptor are also structurally related (136). After binding to their specific receptors, both compounds signal via the same secondary transmembrane signal transducing receptor, gp130. Also, given peripherally to experimental animals, they have similar effects on body temperature, acute-phase reaction and blood lipids, with the difference that Espat *et al* also showed that CNTF, but not IL-6, caused lean tissue wasting in mice (137-139). The main difference is that CNTF seems to act in the arcuate nucleus to mainly decrease feeding in a similar way as shown for leptin (140, 141), while IL-6 seems to affect mainly energy expenditure (article III) and may act on receptors in hypothalamic nuclei other than the arcuate nucleus (33). This may suggest that these two cytokines after all act via different mechanisms and sites to reduce body weight.

TNFα

TNFα has been reported to inhibit LPL activity and stimulate lipolysis in adipocytes *in vitro* (142, 143). However, gene knockout mice lacking TNFα, TNF receptor-1, TNF receptor-2, or both TNF receptors do not spontaneously develop obesity (ref 144, own observations), indicating that TNFα has no major effect on fat mass *in vivo*. There is some inconsistency in the reports whether disruption of TNF-signaling improves or

impairs glucose metabolism in obese mice. In diet-induced obese mice, disruption of TNF-signaling impaired glucose metabolism (144), whereas in *ob/ob* mice insulin sensitivity was improved by TNF disruption (145). Thus, the presence or absence of leptin may influence the effects of TNFα on insulin sensitivity.

ICAM-1

Intercellular adhesion molecule-1 (ICAM-1) is a cell-surface receptor on *e. g.* endothelial cells and hepatocytes that mediates leukocyte adhesion by docking to cellular counter receptors on leukocytes such as Mac-1. In 1997, it was reported that both ICAM-1 and Mac-1 deficient mice developed mature onset obesity, and were more prone to diet-induced obesity than the wild-type controls (146). There is some controversy around this finding because another group recently reported that ICAM-1 knockout mice only get a transient increase in fat mass compared to controls when fed high fat diet, and do not show any difference in body weight on ordinary chow (147). The reason for this discrepancy is unknown. One possibility is differences between the two knockout models.

Dong *et al* reported that the ICAM-1 knockout mice did not have increased food intake, but the exact mechanism causing the obesity in the absence of ICAM-1 is not known (146). In one large population study there was a clear correlation between soluble ICAM-1 levels and insulin resistance (148). In another study by Targher *et al.* no correlation was seen between insulin resistance and ICAM-1, but instead BMI was the only independent predictor of plasma ICAM-1 levels after multiple regression analyses (149). As mentioned above, circulating IL-6 levels have also been found to correlate with both BMI and insulin resistance in different studies (96, 100).

These findings indicate that several regulators of immune function also have an important role in the regulation of body weight and metabolism. More studies are needed to clarify whether these findings are details in a larger pattern of interactions between the immune system and metabolic function, and especially body fat regulation.

SUMMARY AND FUTURE PERSPECTIVES

I Liver derived IGF-I, *i.e.* the major proportion of serum IGF-I, suppresses GH secretion from the pituitary by decreasing the number of pituitary receptors for GHRF and GHS. In line with this, the liver specific IGF-I knockout mice (LI-IGF-I-/-) showed increased GH responsiveness to treatment with GHRF and GHS. The increased GH levels in these mice reflect increased basal GH levels, thereby causing an alteration of the male-specific GH secretory pattern and feminization of several GH-regulated liver functions.

II Mice that lack liver derived IGF-I become leaner than their littermate controls, probably due to increased circulating levels of two adipostatic hormones, GH and/or leptin. The mice are also insulin resistant, although they can decrease blood glucose levels adequately after a glucose tolerance test. The markedly elevated insulin levels may be a compensation for the decreased IGF-I levels, as insulin and IGF-I share metabolic properties. The diabetogenic effect of GH could also contribute to the increased insulin levels.

III IL-6 gene knockout mice develop mature-onset obesity, which is partly reversed by IL-6 replacement. The increased adipose tissue mass leads to increased leptin levels and leptin resistance. In addition, the IL-6 deficient mice develop insulin resistance and decreased glucose tolerance. Female IL-6 knockout mice also have increased plasma triglyceride levels.

IV Intracerebroventricular (ICV) IL-6 treatment to rats leads to an acute increase in energy expenditure, while the same dose had no effect when given peripherally. This suggests that IL-6 can act within the CNS to regulate energy expenditure and thereby affect adipose tissue mass.

V Chronic treatment with IL-6 ICV leads to a decrease in body weight and adipose tissue mass, without causing acute-phase reaction or affecting the weights of several non-fat organs. These results show that central IL-6 can regulate adipose tissue mass. Moreover, this effect was seen in rats with normal endogenous IL-6 production, indicating that IL-6 can have a therapeutic potential in non-IL-6 deficient individuals.

We do not know the exact mechanisms causing the decreased fat mass and insulin resistance in the LI-IGF-I-/- mice. As GH is both lipolytic and diabetogenic, the enhanced GH levels may contribute to these features in LI-IGF-I-/- mice. Therefore, it would be interesting to treat these mice with a GH antagonist to see if it thereby is possible to reverse the leanness and insulin resistance by inhibition of GH action.

I measured food intake in the LI-IGF-I-/- mice and found no differences between the LI-IGF-I-/- mice and controls. Therefore, it would be of interest to measure basal energy

expenditure to see if LI-IGF-I-/- mice have an increased basal metabolic rate. It would also be of interest to measure motor activity at the same time.

For IL-6 to have a future as an anti-obesity drug, it is necessary to show that *peripheral* IL-6 treatment can cause a decrease in adipose tissue mass also in individuals without a severe deficiency of endogenous IL-6 production. There are no known cases of complete IL-6 deficiency in humans, and it seems likely that this condition is very rare. It appears likely that there should be a therapeutic interval where the anti-obesity effect can be achieved without loss of lean body mass or other severe side effects.

It would also be of great interest to find out more about how central nervous system levels of IL-6 are regulated. We have initiated studies to analyze the effects of starvation and other stimuli on IL-6 and IL-6 receptor mRNA expression in the hypothalamus by *in situ* hybridization and real-time PCR techniques.

As discussed above, it is now well known that circulating IL-6 levels increase markedly during physical exercise. Therefore, exercise could be a natural regulator of endogenous IL-6 levels, and it would be of interest to examine whether also central levels of IL-6 increase during exercise. Due to the high circulating levels of IL-6 induced by exercise, it is possible that substantial amounts of IL-6 reach the CNS. We are now performing studies to investigate the effects of exercise in IL-6 knockout mice.

It seems likely that a hormone with the experimental properties described for IL-6 in this thesis indeed has important positive effects on metabolism. Treatment with IL-6 increases energy expenditure and decreases adipose tissue mass, and lack of IL-6 causes obesity. Furthermore, IL-6 is one of few known hormones to show prominent regulation during physical exercise. It remains to be investigated whether this hormone will also find a use for treatment of obese humans. It might be speculated that IL-6 treatment could be of value especially in individuals that have defective endogenous IL-6 production in the CNS, or an acquired deficiency of IL-6 *e.g.* due to a low level of physical activity. Alternatively, IL-6 could be used as an adjuvant treatment to exercise in obese patients with insufficient endogenous IL-6 release from working skeletal muscle.

ACKNOWLEDGEMENTS

I wish to express my gratitude to everyone who has in one way or another contributed to this thesis. Especially, I would like to thank:

John-Olov Jansson, my supervisor, for your encouragement and never-ending enthusiasm.

Olle Isaksson and Bengt-Åke Bengtsson for providing excellent research facilities at RCEM.

Claes Ohlsson and Klara Sjögren for excellent collaboration with the work on the liver specific IGF-I deficient mice.

Susanne Dicksson, for providing great research facilities in Cambridge and for making Ville and me feel very welcome.

Peter Murgatroyd, for all help and encouragement setting up indirect calorimetry.

Håkan Billig and Staffan Edén for your efforts making GGSB a great start for scientific research.

Bo Ahrén, Lena Kvist, Mia Umaerus, Håkan Wennbo, Jan Törnell and Hans Carlsten for great collaborations.

Rhonda Kineman and Larry Frohman for fruitful collaboration and for taking care of all ordeals associated with sending samples across the Atlantic.

Present and former members of JOJs group, especially Petra Henning for being a great friend, Ingrid Wernstedt, Ted Fjällman, Stanko Skrtic and Jenny Fäldt.

Sabrina & Tim, Loraine, Adrian and David for making our stay in Cambridge great fun!

All friends and colleagues at RCEM, special thanks to Kajsa Sjöholm for being a great room-mate, Jenny Johansson and Louise Olofsson for a nice time together in Keystone, Britt Gabrielsson, Margareta Jernås, Gunnel Hellgren, Per-Arne Svensson, Bob Ohlsson, Magnus Johnsson, Jenny Kindblom, Marie Lindberg, Sofia Moverare, Åsa Tivesten, Marion Walser, Anette Hansevi and Olle Vidal for nice discussions and making the working environment at RCEM so nice! Special thanks to our Japanese postdocs, Masanori Hisaoka and Yoshiaki Itoh and their families.

All the people working at the Dept. of Endocrine Physiology, especially Mohammad Bohlooly, Ola Brusehed, Jon Kindblom, Jan Oscarsson, Daniel Lindén, Fredrik Frick and Linda Carlsson for always having time to discuss science and sharing experiences.

Maud Petterson for all your help with serum analyses.

Ulla-Britt Liebera and Kajsa Torstensson for making sure that things run smooth and fixing all different kinds of practical things.

Per Martinsson for quick help with computer problems and for sharing your interest in cooking.

Mary Tofano, for your love and all the time you spent encouraging me to read books.

Maria, Jessica and Susanne for support and fun during the years since we started studying chemistry together at the University. Victoria and Johanna for your long friendship and for knowing me so well that I can discuss anything and everything with you.

My twin sister Anna, for being my best friend and sharing all life's ups and downs with me.

My brother Robert, and his family for your friendship.

My grandmother Alice, for always having open arms waiting to comfort me.

My mother and father, for your love and for always listening and supporting me.

Ville for being a great work partner but most of all for your love and being my life companion. MRSEEEEP!

REFERENCES

1. **Sjogren K, Liu JL, Blad K, et al.** 1999 Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci U S A 96:7088-92
2. **Yakar S, Liu JL, Stannard B, et al.** 1999 Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci U S A 96:7324-9
3. **Jones JI, Clemmons DR** 1995 Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3-34
4. **Guler HP, Zapf J, Froesch ER** 1987 Short-term metabolic effects of recombinant human insulin-like growth factor I in healthy adults. N Engl J Med 317:137-40.
5. **Phillips LS, Harp JB, Goldstein S, Klein J, Pao CI** 1990 Regulation and action of insulin-like growth factors at the cellular level. Proc Nutr Soc 49:451-8.
6. **Moses AC, Young SC, Morrow LA, O'Brien M, Clemmons DR** 1996 Recombinant human insulin-like growth factor I increases insulin sensitivity and improves glycemic control in type II diabetes. Diabetes 45:91-100.
7. **Holder AT, Spencer EM, Preece MA** 1981 Effect of bovine growth hormone and a partially pure preparation of somatomedin on various growth parameters in hypopituitary dwarf mice. J Endocrinol 89:275-82
8. **Shea BT, Hammer RE, Brinster RL** 1987 Growth allometry of the organs in giant transgenic mice. Endocrinology 121:1924-30
9. **Minami S, Kamegai J, Sugihara H, Suzuki N, Wakabayashi I** 1998 Growth hormone inhibits its own secretion by acting on the hypothalamus through its receptors on neuropeptide Y neurons in the arcuate nucleus and somatostatin neurons in the periventricular nucleus. Endocr J 45 Suppl:S19-26
10. **Bowers CY** 1998 Growth hormone-releasing peptide (GHRP). Cell Mol Life Sci 54:1316-29
11. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656-60
12. **Ghigo E, Gianotti L, Arvat E, et al.** 1999 Effects of recombinant human insulin-like growth factor I administration on growth hormone (GH) secretion, both spontaneous and stimulated by GH-releasing hormone or hexarelin, a peptidyl GH secretagogue, in humans. J Clin Endocrinol Metab 84:285-90
13. **Jansson JO, Eden S, Isaksson O** 1985 Sexual dimorphism in the control of growth hormone secretion. Endocr Rev 6:128-50
14. **Jaffe CA, Ocampo-Lim B, Guo W, et al.** 1998 Regulatory mechanisms of growth hormone secretion are sexually dimorphic. J Clin Invest 102:153-64
15. **Norstedt G, Palmiter R** 1984 Secretory rhythm of growth hormone regulates sexual differentiation of mouse liver. Cell 36:805-12
16. **Clark RG, Jansson JO, Isaksson O, Robinson IC** 1985 Intravenous growth hormone: growth responses to patterned infusions in hypophysectomized rats. J Endocrinol 104:53-61

17. **Jansson JO, Albertsson-Wikland K, Eden S, Thorngren KG, Isaksson O** 1982 Circumstantial evidence for a role of the secretory pattern of growth hormone in control of body growth. Acta Endocrinol (Copenh) 99:24-30
18. **D'Ercole AJ, Stiles AD, Underwood LE** 1984 Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. Proc Natl Acad Sci U S A 81:935-9
19. **Camacho-Hubner C, Woods KA, Miraki-Moud F, et al.** 1999 Effects of recombinant human insulin-like growth factor I (IGF-I) therapy on the growth hormone-IGF system of a patient with a partial IGF-I gene deletion. J Clin Endocrinol Metab 84:1611-6
20. **Laron Z** 1999 The essential role of IGF-I: lessons from the long-term study and treatment of children and adults with Laron syndrome. J Clin Endocrinol Metab 84:4397-404
21. **Bondy CA, Underwood LE, Clemmons DR, Guler HP, Bach MA, Skarulis M** 1994 Clinical uses of insulin-like growth factor I. Ann Intern Med 120:593-601.
22. **Backeljauw PF, Underwood LE** 1996 Prolonged treatment with recombinant insulin-like growth factor-I in children with growth hormone insensitivity syndrome--a clinical research center study. GHIS Collaborative Group. J Clin Endocrinol Metab 81:3312-7
23. **Zhou Y, Xu BC, Maheshwari HG, et al.** 1997 A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). Proc Natl Acad Sci U S A 94:13215-20
24. **Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A** 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75:59-72
25. **Stewart CE, Rotwein P** 1996 Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. Physiol Rev 76:1005-26
26. **DeChiara TM, Efstratiadis A, Robertson EJ** 1990 A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 345:78-80
27. **Van Snick J** 1990 Interleukin-6: an overview. Annu Rev Immunol 8:253-78
28. **Pedersen BK, Steensberg A, Schjerling P** 2001 Muscle-derived interleukin-6: possible biological effects. J Physiol 536:329-37.
29. **Kishimoto T** 1989 The biology of interleukin-6. Blood 74:1-10.
30. **Mohamed-Ali V, Flower L, Sethi J, et al.** 2001 beta-Adrenergic regulation of IL-6 release from adipose tissue: in vivo and in vitro studies. J Clin Endocrinol Metab 86:5864-9
31. **Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L** 1998 Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J 334 (Pt 2):297-314
32. **Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM** 2001 The soluble interleukin 6 receptor: mechanisms of production and implications in disease. Faseb J 15:43-58.
33. **Schobitz B, de Kloet ER, Sutanto W, Holsboer F** 1993 Cellular localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. Eur J Neurosci 5:1426-35

34. **Miyahara S, Komori T, Fujiwara R, et al.** 2000 Effects of repeated stress on expression of interleukin-6 (IL-6) and IL-6 receptor mRNAs in rat hypothalamus and midbrain. Life Sci 66:PL93-8
35. **Gao Y, Ng YK, Lin JY, Ling EA** 2000 Expression of immunoregulatory cytokines in neurons of the lateral hypothalamic area and amygdaloid nuclear complex of rats immunized against human IgG. Brain Res 859:364-8
36. **Niimi M, Wada Y, Sato M, Takahara J, Kawanishi K** 1997 Effect of continuous intravenous injection of interleukin-6 and pretreatment with cyclooxygenase inhibitor on brain c-fos expression in the rat. Neuroendocrinology 66:47-53
37. **Hirano T** 1998 Interleukin 6 and its receptor: ten years later. Int Rev Immunol 16:249-84
38. **Paonessa G, Graziani R, De Serio A, et al.** 1995 Two distinct and independent sites on IL-6 trigger gp 130 dimer formation and signalling. Embo J 14:1942-51
39. **Hurst SM, Wilkinson TS, McLoughlin RM, et al.** 2001 Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. Immunity 14:705-14.
40. **Moshage H** 1997 Cytokines and the hepatic acute phase response. J Pathol 181:257-66
41. **Xing Z, Gauldie J, Cox G, et al.** 1998 IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. J Clin Invest 101:311-20.
42. **Kopf M, Baumann H, Freer G, et al.** 1994 Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature 368:339-42
43. **Barton BE** 1997 IL-6: insights into novel biological activities. Clin Immunol Immunopathol 85:16-20
44. **Imura H, Fukata J, Mori T** 1991 Cytokines and endocrine function: an interaction between the immune and neuroendocrine systems. Clin Endocrinol (Oxf) 35:107-15
45. **Elhage R, Clamens S, Besnard S, et al.** 2001 Involvement of interleukin-6 in atherosclerosis but not in the prevention of fatty streak formation by 17beta-estradiol in apolipoprotein E-deficient mice. Atherosclerosis 156:315-20.
46. **Tsigos C, Papanicolaou DA, Kyrou I, Defensor R, Mitsiadis CS, Chrousos GP** 1997 Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. J Clin Endocrinol Metab 82:4167-70
47. **Wallenius V, Wallenius K, Hisaoka M, et al.** 2001 Retarded liver growth in interleukin-6-deficient and tumor necrosis factor receptor-1-deficient mice. Endocrinology 142:2953-60.
48. **Cressman DE, Greenbaum LE, DeAngelis RA, et al.** 1996 Liver failure and defective hepatocyte regeneration in interleukin-6- deficient mice. Science 274:1379-83
49. **Wallenius V, Wallenius K, Jansson JO** 2000 Normal pharmacologically-induced, but decreased regenerative liver growth in interleukin-6-deficient (IL-6(-/-)) mice. J Hepatol 33:967-74.
50. **WHO** 2000 Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization, Geneva.
51. **Wadden TA** 1993 Treatment of obesity by moderate and severe caloric restriction. Results of clinical research trials. Ann Intern Med 119:688-93

52. **Goodrick GK, Foreyt JP** 1991 Why treatments for obesity don't last. J Am Diet Assoc 91:1243-7
53. **Spiegelman BM, Flier JS** 2001 Obesity and the regulation of energy balance. Cell 104:531-43.
54. **Huszar D, Lynch CA, Fairchild-Huntress V, et al.** 1997 Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131-41
55. **Farooqi IS, Yeo GS, Keogh JM, et al.** 2000 Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. J Clin Invest 106:271-9.
56. **Moussa NM, Claycombe KJ** 1999 The yellow mouse obesity syndrome and mechanisms of agouti-induced obesity. Obes Res 7:506-14
57. **Manne J, Argeson AC, Siracusa LD** 1995 Mechanisms for the pleiotropic effects of the agouti gene. Proc Natl Acad Sci U S A 92:4721-4.
58. **Ollmann MM, Wilson BD, Yang YK, et al.** 1997 Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science 278:135-8.
59. **Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A** 1998 Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat Genet 19:155-7
60. **Vaisse C, Clement K, Guy-Grand B, Froguel P** 1998 A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat Genet 20:113-4.
61. **Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S** 1998 A frameshift mutation in MC4R associated with dominantly inherited human obesity [letter]. Nat Genet 20:111-2
62. **Hinney A, Schmidt A, Nottebom K, et al.** 1999 Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. J Clin Endocrinol Metab 84:1483-6.
63. **Tritos NA, Maratos-Flier E** 1999 Two important systems in energy homeostasis: melanocortins and melanin- concentrating hormone. Neuropeptides 33:339-349
64. **Willie JT, Chemelli RM, Sinton CM, Yanagisawa M** 2001 To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci 24:429-58
65. **Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM** 1997 Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci U S A 94:8878-83.
66. **Pelleymounter MA, Cullen MJ, Baker MB, et al.** 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540-3
67. **Friedman JM, Halaas JL** 1998 Leptin and the regulation of body weight in mammals. Nature 395:763-70
68. **Stephens TW, Basinski M, Bristow PK, et al.** 1995 The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 377:530-2
69. **Mizuno TM, Mobbs CV** 1999 Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. Endocrinology 140:814-7

70. **Elmquist JK, Maratos-Flier E, Saper CB, Flier JS** 1998 Unraveling the central nervous system pathways underlying responses to leptin. Nat Neurosci 1:445-50
71. **Caro JF, Kolaczynski JW, Nyce MR, et al.** 1996 Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet 348:159-61
72. **Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D, Jr.** 1996 Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. Nat Med 2:589-93
73. **Tartaglia LA, Dembski M, Weng X, et al.** 1995 Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263-71
74. **Bjorbaek C, Elmquist JK, El-Haschimi K, et al.** 1999 Activation of SOCS-3 messenger ribonucleic acid in the hypothalamus by ciliary neurotrophic factor. Endocrinology 140:2035-43.
75. **Montague CT, Farooqi IS, Whitehead JP, et al.** 1997 Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903-8
76. **Clement K, Vaisse C, Lahlou N, et al.** 1998 A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392:398-401
77. **Farooqi IS, Jebb SA, Langmack G, et al.** 1999 Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med 341:879-84.
78. **Farooqi IS, Keogh JM, Kamath S, et al.** 2001 Partial leptin deficiency and human adiposity. Nature 414:34-5.
79. **Cannon B, Houstek J, Nedergaard J** 1998 Brown adipose tissue. More than an effector of thermogenesis? Ann N Y Acad Sci 856:171-87
80. **Krief S, Lonnqvist F, Raimbault S, et al.** 1993 Tissue distribution of beta 3-adrenergic receptor mRNA in man. J Clin Invest 91:344-9
81. **Greenwood MR, Johnson P** 1977 The adipose tissue. In: Weiss L, Greep R (eds) Histology, 4th ed. McGraw-Hill Book Company, New York, pp 179-203
82. **Frederich RC, Lollmann B, Hamann A, et al.** 1995 Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J Clin Invest 96:1658-63.
83. **Boden G, Chen X, Mozzoli M, Ryan I** 1996 Effect of fasting on serum leptin in normal human subjects. J Clin Endocrinol Metab 81:3419-23.
84. **Saladin R, De Vos P, Guerre-Millo M, et al.** 1995 Transient increase in obese gene expression after food intake or insulin administration. Nature 377:527-9.
85. **Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L** 1997 Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest 100:3105-10.
86. **Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL** 1999 Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. Nature 401:73-6.
87. **Ebihara K, Ogawa Y, Masuzaki H, et al.** 2001 Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes. Diabetes 50:1440-8.
88. **Oral EA, Simha V, Ruiz E, et al.** 2002 Leptin-replacement therapy for lipodystrophy. N Engl J Med 346:570-8.

89. **Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM** 1995 Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 95:2409-15.
90. **Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB** 1995 The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95:2111-9
91. **Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G** 2001 Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 280:E745-51.
92. **Hotamisligil GS, Shargill NS, Spiegelman BM** 1993 Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87-91
93. **Kanety H, Feinstein R, Papa MZ, Hemi R, Karasik A** 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 270:23780-4.
94. **Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM** 1996 IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 271:665-8.
95. **Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R** 1996 Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. Diabetes 45:881-5
96. **Mohamed-Ali V, Goodrick S, Rawesh A, et al.** 1997 Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 82:4196-200
97. **Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP** 1997 Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 82:1313-6.
98. **Fried SK, Bunkin DA, Greenberg AS** 1998 Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab 83:847-50
99. **Ershler WB, Keller ET** 2000 Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 51:245-70
100. **Bastard JP, Jardel C, Bruckert E, et al.** 2000 Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 85:3338-42.
101. **Stouthard JM, Romijn JA, Van der Poll T, et al.** 1995 Endocrinologic and metabolic effects of interleukin-6 in humans. Am J Physiol 268:E813-9.
102. **Stouthard JM, Oude Elferink RP, Sauerwein HP** 1996 Interleukin-6 enhances glucose transport in 3T3-L1 adipocytes. Biochem Biophys Res Commun 220:241-5
103. **Steppan CM, Bailey ST, Bhat S, et al.** 2001 The hormone resistin links obesity to diabetes. Nature 409:307-12.
104. **Way JM, Gorgun CZ, Tong Q, et al.** 2001 Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. J Biol Chem 276:25651-3.

105. **Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM** 2002 Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 10:1-5.
106. **Berg AH, Combs TP, Scherer PE** 2002 ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13:84-9
107. **Yamauchi T, Kamon J, Waki H, et al.** 2001 The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7:941-6.
108. **Kuhn R, Schwenk F, Aguet M, Rajewsky K** 1995 Inducible gene targeting in mice. Science 269:1427-9
109. **Liu JL, Grinberg A, Westphal H, et al.** 1998 Insulin-like growth factor-I affects perinatal lethality and postnatal development in a gene dosage-dependent manner: manipulation using the Cre/loxP system in transgenic mice. Mol Endocrinol 12:1452-62
110. **Sjogren K, Hellberg N, Bohlooly YM, et al.** 2001 Body fat content can be predicted in vivo in mice using a modified dual- energy X-ray absorptiometry technique. J Nutr 131:2963-6.
111. **Bruning JC, Gautam D, Burks DJ, et al.** 2000 Role of brain insulin receptor in control of body weight and reproduction. Science 289:2122-5
112. **DeFronzo RA** 1999 Pharmacologic therapy for type 2 diabetes mellitus. Ann Intern Med 131:281-303
113. **Woods KA, Camacho-Hubner C, Bergman RN, Barter D, Clark AJ, Savage MO** 2000 Effects of insulin-like growth factor I (IGF-I) therapy on body composition and insulin resistance in IGF-I gene deletion. J Clin Endocrinol Metab 85:1407-11.
114. **Yakar S, Liu JL, Fernandez AM, et al.** 2001 Liver-specific igf-1 gene deletion leads to muscle insulin insensitivity. Diabetes 50:1110-8.
115. **Johannsson G, Marin P, Lonn L, et al.** 1997 Growth hormone treatment of abdominally obese men reduces abdominal fat mass, improves glucose and lipoprotein metabolism, and reduces diastolic blood pressure. J Clin Endocrinol Metab 82:727-34.
116. **Hartman ML, Clayton PE, Johnson ML, et al.** 1993 A low dose euglycemic infusion of recombinant human insulin-like growth factor I rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans. J Clin Invest 91:2453-62
117. **Clark R, Mortensen D, Carlsson L, Carmignac D, Robinson I** 1995 Growth responses to patterned GH delivery. Endocrine Journal 3:717-723
118. **Behringer RR, Lewin TM, Quaife CJ, Palmiter RD, Brinster RL, D'Ercole AJ** 1990 Expression of insulin-like growth factor I stimulates normal somatic growth in growth hormone-deficient transgenic mice. Endocrinology 127:1033-40
119. **Veldhuis JD, Bidlingmaier M, Anderson SM, Wu Z, Strasburger CJ** 2001 Lowering total plasma insulin-like growth factor I concentrations by way of a novel, potent, and selective growth hormone (GH) receptor antagonist, pegvisomant (B2036-peg), augments the amplitude of GH secretory bursts and elevates basal/nonpulsatile GH release in healthy women and men. J Clin Endocrinol Metab 86:3304-10.

120. **Woods KA, Camacho-Hubner C, Savage MO, Clark AJ** 1996 Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 335:1363-7
121. **Kamegai J, Wakabayashi I, Miyamoto K, Unterman TG, Kineman RD, Frohman LA** 1998 Growth hormone-dependent regulation of pituitary GH secretagogue receptor (GHS-R) mRNA levels in the spontaneous dwarf Rat. Neuroendocrinology 68:312-8
122. **Aschner M** 1998 Immune and inflammatory responses in the CNS: modulation by astrocytes. Toxicol Lett 102-103:283-7.
123. **Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK** 1999 Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 515:287-91.
124. **Keller C, Steensberg A, Pilegaard H, et al.** 2001 Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. Faseb J 15:2748-50
125. **Jonsdottir IH, Schjerling P, Ostrowski K, Asp S, Richter EA, Pedersen BK** 2000 Muscle contractions induce interleukin-6 mRNA production in rat skeletal muscles. J Physiol 528 Pt 1:157-63.
126. **Ostrowski K, Schjerling P, Pedersen BK** 2000 Physical activity and plasma interleukin-6 in humans-effect of intensity of exercise. Eur J Appl Physiol 83:512-5
127. **Wallberg-Henriksson H, Rincon J, Zierath JR** 1998 Exercise in the management of non-insulin-dependent diabetes mellitus. Sports Med 25:25-35
128. **Orban Z, Remaley AT, Sampson M, Trajanoski Z, Chrousos GP** 1999 The differential effect of food intake and beta-adrenergic stimulation on adipose-derived hormones and cytokines in man. J Clin Endocrinol Metab 84:2126-33.
129. **Metzger S, Hassin T, Barash V, Pappo O, Chajek-Shaul T** 2001 Reduced body fat and increased hepatic lipid synthesis in mice bearing interleukin-6-secreting tumor. Am J Physiol Endocrinol Metab 281:E957-65.
130. **Metzger S, Goldschmidt N, Barash V, et al.** 1997 Interleukin-6 secretion in mice is associated with reduced glucose-6- phosphatase and liver glycogen levels. Am J Physiol 273:E262-7.
131. **Tsigos C, Papanicolaou DA, Defensor R, Mitsiadis CS, Kyrou I, Chrousos GP** 1997 Dose effects of recombinant human interleukin-6 on pituitary hormone secretion and energy expenditure. Neuroendocrinology 66:54-62.
132. **Mastorakos G, Chrousos GP, Weber JS** 1993 Recombinant interleukin-6 activates the hypothalamic-pituitary-adrenal axis in humans. J Clin Endocrinol Metab 77:1690-4
133. **Loffreda S, Yang SQ, Lin HZ, et al.** 1998 Leptin regulates proinflammatory immune responses. Faseb J 12:57-65.
134. **Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI** 1998 Leptin modulates the T-cell immune response and reverses starvation- induced immunosuppression. Nature 394:897-901.
135. **Luheshi GN, Gardner JD, Rushforth DA, Loudon AS, Rothwell NJ** 1999 Leptin actions on food intake and body temperature are mediated by IL-1. Proc Natl Acad Sci U S A 96:7047-52

136. **Sleeman MW, Anderson KD, Lambert PD, Yancopoulos GD, Wiegand SJ** 2000 The ciliary neurotrophic factor and its receptor, CNTFR alpha. Pharm Acta Helv 74:265-72.
137. **Espat NJ, Auffenberg T, Rosenberg JJ, et al.** 1996 Ciliary neurotrophic factor is catabolic and shares with IL-6 the capacity to induce an acute phase response. Am J Physiol 271:R185-90.
138. **Nonogaki K, Pan XM, Moser AH, et al.** 1996 LIF and CNTF, which share the gp130 transduction system, stimulate hepatic lipid metabolism in rats. Am J Physiol 271:E521-8
139. **Nonogaki K, Fuller GM, Fuentes NL, et al.** 1995 Interleukin-6 stimulates hepatic triglyceride secretion in rats. Endocrinology 136:2143-9
140. **Gloaguen I, Costa P, Demartis A, et al.** 1997 Ciliary neurotrophic factor corrects obesity and diabetes associated with leptin deficiency and resistance. Proc Natl Acad Sci U S A 94:6456-61
141. **Lambert PD, Anderson KD, Sleeman MW, et al.** 2001 Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin- resistant obesity. Proc Natl Acad Sci U S A 98:4652-7.
142. **Patton JS, Shepard HM, Wilking H, et al.** 1986 Interferons and tumor necrosis factors have similar catabolic effects on 3T3 L1 cells. Proc Natl Acad Sci U S A 83:8313-7
143. **Kern PA** 1997 Potential role of TNFalpha and lipoprotein lipase as candidate genes for obesity. J Nutr 127:1917S-1922S
144. **Schreyer SA, Chua SC, Jr., LeBoeuf RC** 1998 Obesity and diabetes in TNF-alpha receptor- deficient mice. J Clin Invest 102:402-11
145. **Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS** 1997 Protection from obesity-induced insulin resistance in mice lacking TNF- alpha function. Nature 389:610-4
146. **Dong ZM, Gutierrez-Ramos JC, Coxon A, Mayadas TN, Wagner DD** 1997 A new class of obesity genes encodes leukocyte adhesion receptors. Proc Natl Acad Sci U S A 94:7526-30
147. **Gregoire FM, Zhang Q, Smith SJ, et al.** 2002 Diet-induced obesity and hepatic gene expression alterations in C57BL/6J and ICAM-1-deficient mice. Am J Physiol Endocrinol Metab 282:E703-13
148. **Hak AE, Pols HA, Stehouwer CD, et al.** 2001 Markers of inflammation and cellular adhesion molecules in relation to insulin resistance in nondiabetic elderly: the Rotterdam study. J Clin Endocrinol Metab 86:4398-405
149. **Targher G, Bonadonna RC, Alberiche M, Zenere MB, Muggeo M, Bonora E** 2001 Relation between soluble adhesion molecules and insulin sensitivity in type 2 diabetic individuals: role of adipose tissue. Diabetes Care 24:1961-6

På grund av upphovsrättsliga skäl kan vissa ingående delarbeten ej publiceras här.
För en fullständig lista av ingående delarbeten, se avhandlingens början.

Due to copyright law limitations, certain papers may not be published here.
For a complete list of papers, see the beginning of the dissertation.

GÖTEBORGS UNIVERSITET