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The secretome of brown adipose tissue

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“Det har jag aldrig provat förut, så det klarar jag säkert.”
-Pippi Långstrump

To everyone who ever
shared a coffee or laughed
with me

Abstract

Brown adipose tissue has long been known for its heat-producing capacity, but less is known about its possible effects as a secretory organ. This thesis summarizes information about presently known factors secreted from brown adipose tissue and about their actions. We were able to add factors to the list by the use of a signal-sequence trap method. Results from the signal-sequence trap generated a list of suggested brown adipocyte secreted proteins; gene expression of these proteins was then further studied with microarray technique.

One of the genes further analyzed was the adipokine chemerin. Gene expression of chemerin in brown adipose tissue was decreased in cold acclimation but increased with a high-caloric diet. This indicates that factors other than norepinephrine influence chemerin gene expression. The effects on chemerin gene expression were not be reflected in serum levels; therefore, chemerin secreted from brown adipose tissue is ascribed an autocrine/paracrine role.

Signal-sequence trap and microarray studies suggested adrenomedullin, collagen type 3 a1, lipocalin 2 and Niemann Pick type C2 to be highly secreted from brown adipocytes. Gene expression of these factors was examined in vivo and in vitro. Our studies showed that both cold acclimation and high-caloric diet have an effect on gene expression of these factors. However, there was no effect on gene expression of chemerin and collagen type 3 a1 in norepinephrine-treated brown adipocyte cell cultures. This suggests that effects on gene expression of the examined possible brown adipocyte secreted proteins are not solely controlled by norepinephrine.

This thesis is based on the following papers, referred in the text by their Roman numerals.

I. A partial secretome of brown adipose tissue.
Ida R. Hansen, Satoru Ohgiya, Barbara Cannon and Jan Nedergaard
Manuscript

II. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues.
Tomas B. Waldén, **Ida R. Hansen**, James A. Timmons, Barbara Cannon and Jan Nedergaard
Am J Physiol Endocrinol Metab. 2012 Jan 1;302(1):E19-31.

III. Contrasting effects of cold and high-energy diets on chemerin gene expression in brown and brite adipose tissues.
Ida R. Hansen*, Kim M. Jansson*, Barbara Cannon and Jan Nedergaard
Submitted

IV. Physiological effects on gene expression of some secreted factors from brown adipose tissue.
Ida R. Hansen, Kim M. Jansson, Barbara Cannon and Jan Nedergaard
Manuscript

V. Effects of differentiation on gene expression of certain brown adipocyte-secreted factors.
Ida R. Hansen, Barbara Cannon and Jan Nedergaard
Manuscript

Contents

Abbreviations

UCP1	Uncoupling protein 1
BAT	Brown adipose tissue
WAT	White adipose tissue
AR	Adrenergic receptor
NE	Norepinephrine
BMI	Body mass index
HFD	High-fat diet
GLUT	Glucose transporter
MAPK	MAP kinase
PPARγ	Peroxisome proliferator-activated receptor γ
cAMP	Cyclic adenosine monophosphate
ERK 1/2	Extracellular signal regulated kinase 1/2

1 Introduction

During the past few years, brown adipose tissue has received much attention due to the acceptance of its presence in adult humans (Nedergaard et al., 2007). Previously, brown adipose tissue was believed to be present mainly in small rodents and hibernating mammals- and in infants.

The history of brown adipose tissue starts in the 17th century when it was thought to be a part of the thymus. About a hundred years later it was thought to be an endocrine organ involved in blood formation or a fat store of special nutrients. It was in 1961 that brown adipose tissue was shown to be thermogenic (reviewed in Cannon and Nedergaard, 2004). Rothwell and Stock (1979) were the first to associate effects of energy expenditure with brown adipose tissue, when feeding rats cafeteria diet and describing increased energy inefficiency (Rothwell and Stock, 1979).

The brown adipocytes are the smallest functional constituents of brown adipose tissue, identified by a large amount of mitochondria and small lipid droplets scattered in the cell. UCP1 (Uncoupling protein 1) is located in the inner membrane of the mitochondria - and when stimulated - uncouples respiration from oxidative phosphorylation. Briefly, activation of UCP1 starts with norepinephrine being released from sympathetic nerves, interacting via G-protein coupled β3-adrenoreceptors, activating adenylate cyclase and increasing cAMP levels in the brown adipocyte. The second messenger cAMP signals via protein kinase A (PKA), activating lipolysis and the release of free fatty acids from triglycerides. Free fatty acids are the acute substrate in thermogenesis; free fatty acids combusted in the respiratory chain results in a proton gradient across the membrane. the proton-motive force drives protons back into the mitochondrial matrix

through UCP1, and energy is released as heat. Free fatty acids are also in some way a regulator of UCP1 activation (reviewed in Cannon and Nedergaard, 2004). For more details about brown adipose tissue, UCP1 and thermogenesis please see review (reviewed in Cannon and Nedergaard, 2004).

Recent studies by (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009) confirm that brown adipose tissue is indeed present in adult man and activated after cold exposure. Studies also show an increase of active brown adipose tissue in lean subjects compared to obese (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009). Histological studies of the human BAT depots show high capillary density, sympathetic innervation and the presence of UCP1 (Zingaretti et al., 2009).

Active brown adipose tissue presents an opportunity to counteract obesity in humans. To evaluate the potential obesity-reducing function of brown adipose tissue, the tissue and its function need to be thoroughly studied.

1.1 The origins of brown, brite and white adipose tissue

BAT is a highly specialized tissue, which clearly differs from the energy-storing white adipose tissue. Indeed, BAT is characterized by its thermogenic function because it has the ability to dissipate energy and to provide heat.

Brown adipocytes were earlier thought to share a common precursor with white adipocytes but recent studies show that brown adipocytes share a common progenitor with myocytes. There is also a different cell-type that is comparable to both white and brown adipocytes and that is the brite adipocyte (brown-like-in-white) or beige. The brite adipocyte is suggested to

come from a type of white progenitor cell but shares common features with brown adipocytes such as the ability to express UCP1.

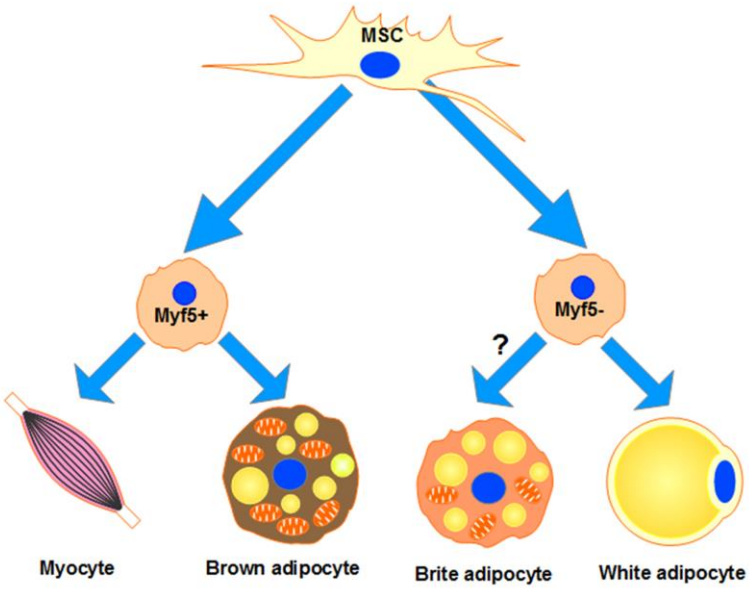

Figure 1. The adipocyte cell-lineage shows that brown adipocytes originate from a different cell lineage than white adipocytes. Brown adipocytes are more closely related to myocytes, and white and brite adipocytes perhaps derived from a common linage.

Gene analysis indicated that brown and white adipocytes derived from distinct precursor cell lineages that at some point in early development express the muscle-specific gene myf5 (Timmons et al., 2007), and it was established with lineage tracing that classical brown fat depots emerge from a muscle lineage (Atit et al., 2006; Seale et al., 2008). The transcription factors PRDM16 and C/EBPβ play a major role in promoting brown adipocytes from myoblast-like precursors (Seale et al., 2008; Seale et al., 2009).

The existence of a third type of adipocyte, found in white adipose tissue, that became brown-like after cold stress was early suggested (Loncar, 1991).

However, more recently the brite adipocytes were established as separate cells that are found in classical white adipose tissue depots and the brite adipocytes have a distinct expression signature that resembles brown adipocytes (Petrovic et al., 2010). The origin of the different adipocytes is a complex question; some studies show that some white adipocytes can emerge from myf5-positive progenitors (Sanchez-Gurmaches et al., 2012) and there are findings of myf5-positive cells in white adipose tissue that express very low levels of both brown and brite marker genes (Shan et al., 2013).

Table 1. Discussed primary features of the different adipose tissues and skeletal muscle, their similarities and differences.

Tissue	Lipid content	Mitochondria	Energy expenditure	Origin
BAT	Multilocular	+++	+++	Myf5 +
Skeletal muscle	Small lipid droplets	+++	+++	Myf5 +
Brite adipocytes	Multilocular	++	++	MYf5 -
WAT	Unilocular	+	+	Myf5 -

Brite adipocytes are found mainly in the inguinal white fat in mice i.e. subcutaneously (see fig. 2). In subcutaneous adipose tissue, Prdm16 can be is increased and induce a brown-like phenotype (Seale et al., 2011). The occurrence of brite cells and where they can be found in the adipose organ varies with genetic background, sex, age, nutritional status and environmental conditions (Frontini and Cinti, 2010). In this thesis, cells that appear in white adipose tissue with brown features and thermogenic properties will be named as brite cells.

The recent knowledge of cell lineage gives us an opportunity to maybe change how we think of muscle and adipose tissue. One usually says that there are two types of adipose tissue, white and brown. Nowadays maybe it is more correct to say that there are four types of muscle: skeletal, heart,

smooth muscle and brown adipose tissue (Nedergaard, personal funny comment worth thinking about

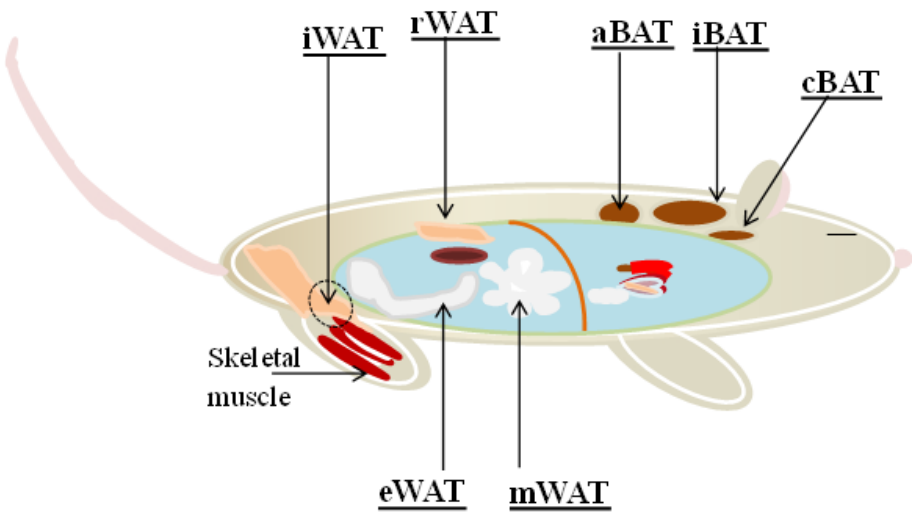

Figure 2. The figure shows localisation of brown and white adipose tissue depots in mice. Classical brown adipose tissue consists of the axillary (aBAT), cervical (cBAT) and interscapular (iBAT) depots. Classical white adipose tissue consists of the epididymal (eWAT) and mesenteric (mWAT) depots. Brite depots are suggested to be the inguinal (iWAT) and retroperitoneal (rWAT). Skeletal muscle used is gastrocnemius (picture adapted and modified from paper II).

1.2 Secretory role of brown adipose tissue, skeletal muscle, white and brite adipose tissues

1.2.1 Brown adipose tissue

Brown adipose tissue was earlier thought to play a minor role as an endocrine organ, due to the low expression and secretion of leptin and adiponectin (reviewed in Cannon and Nedergaard, 2004). Recent studies show effects on secretion after adrenergic stimulation, which could change the attitude towards brown adipose tissue function (reviewed in Villarroya et

al., 2013). So far little is known about the secretory role of brown adipocytes.

1.2.2 Skeletal muscle

Skeletal muscle comprises about half of the human body mass and is the largest contributor to resting energy expenditure and insulin-induced glucose disposal in adults. There is increasing evidence that skeletal muscle is an important secretory tissue with a secretome of hundreds of peptides. Myokines are secreted during different physiological conditions and can communicate with other tissues (reviewed in Pedersen and Febbraio, 2012; Trayhurn et al., 2011).

1.2.3 Brite adipose tissue

Brite adipose tissue has no established secretome yet, as little is known about brite cells in general. As brite cells are suggested to be white adipocytes with brown features, it is tempting to suggest that they behave similarly to brown or white adipocytes or maybe as an intermediate with both brown and white features.

1.2.4 White adipose tissue

White adipose tissue is energy storing and a highly active endocrine organ with leptin being one of the most important secreted proteins (Halaas et al., 1995). White adipose tissue is located in depots organized throughout the body, giving each depot specific metabolic functions. Adipokines are involved in energy metabolism and inflammation, and there are constantly new reports of new adipokines.

2 Secreted factors from brown adipose tissue

The major aim of the present thesis has been to identify secreted factors from brown adipose tissue and discuss their potential effects throughout the body. To evaluate brown adipocyte secreted factors, I have studied the literature found on brown adipose tissue secreted factors and also my own results in the study of secreted proteins (Paper I, III, VI and V). The information concerning each factor will be presented as follows. First, the general knowledge; the section will contain information about the main secretory organs and what main actions the factor has. Secondly, I will write about the receptor, if it is known and where the receptor can be found. The third section discuss if there is any connection of the factor to obesity or its comorbidities.

After this introduction about the factor itself, the focus is on how brown adipose tissue expresses and secretes the factor. This section will discuss regulation of the factor and what targets the factor might have. After the discussion about brown adipose tissue, the factor will briefly be considered in skeletal muscle, "brite" adipose tissue and white adipose tissue. This discussion is brief as the main focus is to compare the secretory manner to the secretion in brown adipose tissue.

The tissues discussed are those closely related to brown adipose tissue. These thus include skeletal muscle as brown adipocytes come from the same progenitor cell as myocytes.

The muscle section is then followed with information, if any can be found, on the factor secretion and effect in "brite" adipose tissue. The brite

cells are characterized as white cells with brown adipocyte features so it is interesting if their secretory ability is similar to brown or white adipose tissue. Since brite adipose tissue is recently defined, information is scarce.

The last tissue discussed is white adipose tissue. White adipose tissue is interesting to compare to, due to the fact that up until recently brown and white adipocytes were thought to come from the same progenitor. In the end of each section, I will evaluate if brown adipose tissue secretes the discussed factor in an auto-, para- or endocrine manner (or if the factor is a basement membrane protein). I will also to a lesser extent discuss if the manner of secretion and effects of the factor from brown adipose tissue are similar to these in any of the other tissues discussed above.

The factors will be divided into four groups where the division is dependent upon the secretion manner of the factor, although to define the different manners of how a factor can be secreted and place a factor in a given section is difficult, as one factor can have multiple ways of action.

The first group is basement membrane proteins; these factors are secreted from brown adipocytesand used in basement membranes surrounding the tissue. Thereafter come the autocrine factors, which are those factors secreted from brown adipocytes, used to stimulate the cells themselves. The following group is the paracrine factors that are secreted from brown adipocytes and stimulate nearby but different cells. The last section is about the endocrine factors, these are the factors secreted into the blood stream to have their effect on distant organs.

Within each group, the factors are arranged as follows, first the factors other than classic proteins e.g. fatty acids, and after that the factors that are proteins. The proteins are then arranged in alphabetical order, or depending on their main function.

3 Basement membrane proteins

Basement membrane proteins form extracellular matrices and consist of proteins such as laminins, collagens and proteoglycans. These components can be found in association with each other and together with a variety of other macromolecules. Basement membrane architecture is important to ensure tissue- and site-specific processes. Basement membrane also possesses cell-binding sites that interact with specific receptors. Some evidence suggests that such interactions are involved in controlling cell behaviour (Timpl, 1989). This section will first discuss basement membrane proteins known in brown adipose tissue and that is e.g. collagen III, which was identified in my microarray study (Paper I). Further basement membrane proteins, collagen VI, laminin, heparan sulphate proteoglycan and fibronectin were not identified up in our study

3.1 Collagen type III alpha 1

Collagen type III alpha 1 (Col3a1) is a fibrillar collagen; three copies of the gene product make up the molecule type III pro-collagen, which organises itself into a long and thin fibril and is found around cells (Sterling, 2011). A rare disease called Ehler-Danlos syndrome is caused by a mutation in the COL3A1 gene causing fragile connective tissue that ultimately results in premature death by arterial, intestinal and uterine rupture (Eder et al., 2013). Col3a1 is reported to be found in smooth muscle cells and skin (reviewed in Vuorio and de Crombrugghe, 1990). Col3a1 is up-regulated in subjects in response to weight loss (Dankel et al., 2010).

Data from signal sequence trap (SST) and microarray indicate that Col3a1 may be secreted from brown adipocytes (Paper I). Further data suggest increased gene expression of Col3a1 in brown adipose tissue obtained from animals after diet-induced obesity (Paper III). In gene expression studies, Col3a1 levels increase dramatically in primary brown adipocytes in response to norepinephrine stimulation, as well as in brown adipose tissue following diet-induced obesity (Paper I). Data indicate a higher expression of Col3a1 in brown adipocytes compared to white adipocytes (Paper I) and increased levels during brown adipocyte differentiation (Paper V).
Col3a1 is expressed in muscle (Heinemeier et al., 2009). Col3a1 is expressed in both white adipocytes (Paper I) and in white adipose tissue (Divoux et al., 2010; Nakajima et al., 1998), and col3a1 is secreted from adipocytes (Kratchmarova et al., 2002). The type III collagens are enriched in the stromal vascular fraction of adipose tissue (Divoux et al., 2010).
Thus, firm data regarding the function of Col3a1 as a brown adipocyte-secreted protein are lacking, but Col3a1 is a basement membrane protein in skeletal muscle, brown and white adipose tissue.

3.2 Collagen VI

Collagen VI is an extracellular matrix protein and it is composed of three major polypeptide chains – α1, α2 and α3 (Chen et al., 2013). It is suggested that Collagen VI provides structure and support for the cells, as well as triggering signalling pathways that regulate apoptosis, proliferation, angiogenesis and inflammation (Chen et al., 2013). Collagen VI is expressed in several tissues including skin, skeletal muscle, blood vessels and adipose tissue (Chen et al., 2013).

Col6a3 is increased in diabetic mice while obese (ob/ob) mice lacking the col6a3 gene have a better metabolic profile and gain less weight when fed a

high-fat diet (Khan et al., 2009). Col6a3 expression is positively correlated with BMI and fat mass (Pasarica et al., 2009).

In brown adipose tissue, Collagen VI is a secreted protein and an early marker in cell differentiation (Cousin et al., 1996; Haraida et al., 1996). Col6a2 expression is increased in brown adipose tissue after acute cold, and this reflects cell proliferation and differentiation (Cousin et al., 1996).

Collagen VI is present in skeletal muscle (Gara et al., 2011), and dysfunction of Col6a1 leads to metabolic changes and muscle weakness (De Palma et al., 2013). In the C2C12 muscle cell-line, Col6a2 expression increases during differentiation; this occurs concomitantly with other myogenic regulatory factors e.g. myogenin and MyoD. Col6a2 is a marker of the myoblast state (Ibrahimi et al., 1993).

Collagen IV is enriched in the extracellular matrix of white adipose tissue (Pasarica et al., 2009). In white adipose tissue, Col6a2 is a marker of the pre-adipocyte state (Ibrahimi et al., 1993) and is homogenously present around mature white adipocytes (Haraida et al., 1996). In paraovarian and inguinal white adipose tissue, Col6a2 is not increased after acute cold exposure (Cousin et al., 1996). Collagen VI is found surrounding parenchymal adipocytes (Divoux et al., 2010).

Collagen VI seems therefore to be a protein important in differentiating brown adipocytes working in the extracellular basement membrane similar to both white adipocytes and myoblasts (Chen et al., 2013; Khan et al., 2009).

3.3 Laminin

Laminin is a prominent basement membrane protein and plays a crucial structural and functional role in basement membranes (Reviewed in Timpl, 1989). The basement membrane is important in adipogenesis and constitutes a specialized layer surrounding the extracellular matrix, regulating

differentiation, migration and adhesion. Laminin also plays a significant role in several other biological processes such as cell adhesion, differentiation, and migration (Joo et al., 2011). Laminin can be found in the basement membrane in almost all animal tissues.
Laminin receptors are increased in interscapular brown adipose tissue in obesity-prone rats compared to obesity-resistant fed a high-fat diet. However, the exact role remains to be elucidated (Joo et al., 2011).

Laminin protein is found in brown adipose tissue (Haraida et al., 1996), as well as in skeletal muscle (Miura et al., 2010; Sanes et al., 1986). White adipose tissue also contains laminin protein, although at lower levels compared with brown adipose tissue (Haraida et al., 1996).

As indicated above, laminin has a role in several biological activities; however, more detailed information about the exact role in brown adipose tissue is lacking. Laminin is probably a major component of the basement membrane in brown and white adipose tissue, as well as in skeletal muscle.

3.4 Heparan sulfate proteoglycan

Heparan sulfate proteoglycan has a widespread occurrence in all mammalian tissues as am extracellular matrix component or as a cell-membrane-bound protein (Reijmers et al., 2013). Studies of various model organisms have demonstrated that heparan sulphate proteoglycans are of importance in development and normal physiology (Bishop et al., 2007). They are suggested to bind and present proteins to regulate biological processes, such as cell growth, adhesion and migration (Reijmers et al., 2013).

Heparan sulfate proteoglycans may also have a role in fatty acid transport across the adipocyte membrane and in lipid accumulation (Wilsie et al., 2005).

Heparan sulfate proteoglycan can be found in brown adipose tissue basement membranes (Haraida et al., 1996), and the distribution in the

basement membrane is constant; here is, however, no suggested specific role.

There is heparan sulphate proteoglycan in skeletal muscle, and heparan sulphate proteoglycans are key components of the skeletal muscle cell membrane and extracellular matrix and can modulate growth factor activities (Gutierrez and Brandan, 2010).

Some studies show that white adipose tissue basement membranes do not express heparan sulfate proteoglycans (Haraida et al., 1996). However, a more recent study shows a high expression of heparan sulfate proteoglycans in adipocytes, and inhibition of heparan sulfate proteoglycans decreased intracellular lipid accumulation (Wilsie et al., 2005).

There are no studies investigating the function of heparan sulfate proteoglycans in brown adipose tissue. Most likely, brown adipose tissue contains heparan sulfate proteoglycan in the basement membrane similar to what is the case in white adipose tissue and skeletal muscle.

3.5 3.5. Fibronectin

Fibronectin is a large glycoprotein with adhesive properties and is reported to play a role in tumour development (Boeuf et al., 2001; Wan et al., 2013). Fibronectin can interact with structures in the connective tissue (Haraida et al., 1996) and mediates several interactions with the extracellular matrix (Pankov and Yamada, 2002). Fibronectin is found in body fluids, soft connective tissue matrices and most basement membranes and can be produced by a variety of cells in vitro such as macrophages, hepatocytes and epithelial cells (Bradshaw and Smith, 2013; Hynes and Yamada, 1982). The fibronectin that is found in the plasma is mainly produced by the liver (Pankov and Yamada, 2002).

There is decreased fibronectin expression in white adipose tissue in obese subjects compared to the control group (Lee et al., 2013b).

Fibronectin expression is three times higher in brown than in white preadipocytes (Boeuf et al., 2001), and fibronectin can be detected in brown adipose tissue (Haraida et al., 1996).

There is fibronectin protein in skeletal muscle (Sanes et al., 1986), and the expression is increased after exercise (Heinemeier et al., 2013).

Fibronectin expression is found in both subcutaneous and visceral adipose tissue (Lee et al., 2013b). However, in one report, fibronectin protein in mature white adipocytes was not detectable at all (Haraida et al., 1996).

Very little is known about actions of fibronectin in brown adipocytes. I suggest that fibronectin in the basement membrane conduct interactions with the extracellular matrix in all three tissues.

4 Autocrine factors

An autocrine factor is secreted from one cell type and affects the cell type itself.

This section will first discuss the non-proteins, adenosine and prostaglandins. The proteins that are secreted in an autocrine manner from brown adipose tissue are adipsin, adrenomedullin, basic fibroblast growth factor, bone morphogenetic protein-8b (BMP8b), chemerin, insulin-like growth factor 1, lipocalin 2 and Niemann Pick type C2. In our study, we identified and further studied adrenomedullin, chemerin, lipocalin 2, Niemann Pick type C2 (Paper I, III, IV, V).

I will discuss their appearance in brown adipose tissue, muscle, brite and white adipose tissue and evaluate if they have similar actions in the different tissues.

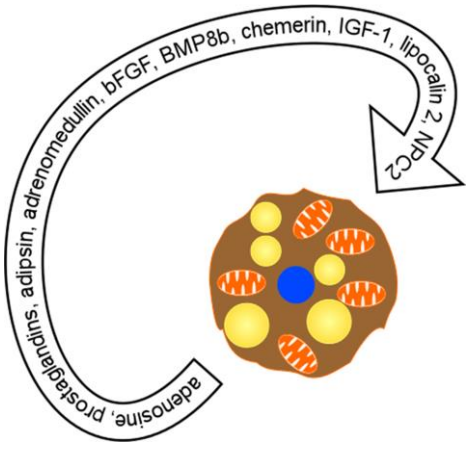

Figure 3. My current view of brown adipose tissue's autocrine factors. Adenosine, prostaglandins, adipsin, adrenomedullin, basic fibroblast growth factor (bFGF), bone morphogenetic protein-8b (BMP8b), chemerin, insulin-like growth factor 1 (IGF-1), lipocalin 2 and Niemann Pick type C2 (NPC2) are suggested autocrine factors.

4.1 Adenosine

Adenosine is an endogenous purine nucleoside that has the ability to affect many biological systems such as the nervous, reproductive, cardiac, renal, hepatic and respiratory systems. Adenosine levels are also increased under metabolically stressful conditions such as inflammation and cancer (Kumar, 2013). Adenosine can be found throughout the body and has a plethora of actions.

Adenosine signals through adenosine receptors which are G-protein coupled receptors with several subtypes (A1, A2A, A2B and A3); the different subtypes have the ability to stimulate or to inhibit adenylate cyclase activity (Kumar, 2013). Adenosine receptors are widely distributed throughout the body, but, for example, adenosine A1 receptor is especially prominent in brain, adipose tissue and kidney (LaNoue and Martin, 1994).

Studies on obese animal models suggest that an excessive activity of the adenosine A1 receptor has an impact and might induce obesity (reviewed in LaNoue and Martin, 1994). It is also suggested that increased signalling by adenosine A2B receptors increases insulin resistance in diabetes (Figler et al., 2011).

Adenosine is a regulator of metabolic processes in brown adipocytes. Brown adipocytes release adenosine and contain the adenosine A1 receptor (Schimmel et al., 1987) and the A2 receptor to a smaller extent (reviewed in LaNoue and Martin, 1994). Adenosine can inhibit adenylate cyclase activity, lipolysis and respiration in brown adipocytes (Unelius et al., 1990).

Adenosine is secreted from skeletal muscle (Ballard, 1991) and might have a regulatory role in skeletal muscle blood flow (Tabrizchi and Bedi, 2001). Skeletal muscle has both adenosine A1- and A2 receptor so adenosine probably works in an autocrine manner (reviewed in LaNoue and Martin, 1994).

Adenosine has been shown to be an important regulator of metabolic processes in white adipose tissue and can as a autocrine agent inhibit

lipolysis (LaNoue and Martin, 1994). White adipocytes contain the A1 type receptor (Saggerson and Jamal, 1990).

Adenosine has autocrine actions affecting energy homeostasis, similar between brown adipose tissue, white adipose tissue and skeletal muscle.

4.2 Prostaglandins

Prostaglandins are lipid mediators produced from arachidonic acid metabolism by the enzyme cyclooxygenase (COX) and prostaglandin type-specific synthases. COX exists in at least two isoforms where COX-1 is constitutive and COX-2 is inducible. Classical prostaglandins synthesised via COX are PGD2, PGE2, PGF2α, PGI2 and TXA2 (Sang and Chen, 2006). Prostaglandins elicit a wide range of important physiological functions regulating inflammation, immune response, tissue injury and repair. Prostaglandins are never endocrine, only autocrine or paracrine (Tootle, 2013). Almost all organs contain enzymes to produce prostaglandins but some tissues demonstrate greater capacity. Prostaglandins are involved in a variety of mammalian functions such as reproduction.

Prostaglandins exert their signals via the prostaglandin receptors that belong to the G protein-coupled receptor gene family (Fujimori, 2012). Prostaglandin E2 receptors have four subtypes (EP1, EP2, EP3, EP4) expressed in a variety of tissues such as endothelial cells, smooth muscle and blood cells (Foudi et al., 2012). Prostaglandin F2α predominantly acts via the type F prostanoid receptor (FP receptor) which is abundantly expressed in skeletal muscle (Markworth and Cameron-Smith, 2011).

In diabetic mice, the PGE2 receptor EP3 is upregulated and decreases intracellular cAMP and blunts glucose-stimulated insulin secretion. The production of PGE2 is increased in these mice (Kimple et al., 2013).

There are several reports of prostaglandin synthases and their occurrence in brown adipose tissue; however, there is less information

concerning the presence of prostaglandins and their possible secretion and physiological function. Some 30 years ago, Portet and colleagues reported the occurrence of prostaglandin E2 (PGE2) and prostaglandin Fα (PGF2α) in brown adipose tissue (Portet et al., 1980; Portet et al., 1982). Recent studies show that expression of lipocalin prostaglandin D synthase (L-PGDS) - which can produce D-series prostaglandins- is positively correlated with brown adipose tissue activity and might play a role in glucose utilization (Virtue et al., 2012).

The prostaglandin F2α receptor (FP receptor) is abundantly expressed in skeletal muscle, and in vitro studies couple FP receptor activation with myotube growth via a PI3K-, ERK- and mTOR-dependent pathway (Markworth and Cameron-Smith, 2011). There is also PGE2 and PGF2α production suggested, in an autocrine manner, to affect muscle growth (Beaulieu et al., 2012; Trappe et al., 2013).

In inguinal white adipose tissue (brite), COX activity and prostaglandin E2 are important factors in the induction of UCP1 expression (Madsen et al., 2010). Activation of β-adrenergic receptors enhances COX2 expression and the release of WAT-derived prostaglandins, and inducible brown adipose tissue (brite cells) is increased in intra-abdominal white adipose tissue (Vegiopoulos et al., 2010).

Prostaglandins are suggested to work in a paracrine manner and to be involved in white adipocyte differentiation regulation and to work as PPARγ modulators (Fujimori, 2012). In vitro studies show enhanced prostaglandin E2 production in differentiating white adipocytes (Hyman et al., 1982). Prostaglandin F2α treatment is a potent antiadipogenic factor in cultured preadipocytes (Casimir et al., 1996).

Prostaglandins, if secreted from brown adipose tissue, probably work as autocrine factors to control brown adipose tissue activity.

4.3 Adipsin

Adipsin (or complement factor D) is a serine protease (Cook et al., 1987) that has a role in the innate immune response where it is a key regulatory enzyme in the alternate complement pathway (Cianflone et al., 1999). The alternative complement cascade leads to a membrane-attack complex that creates pores in the cell membrane and hence results in apoptosis. Adipsin, together with complement factor B and C3, can generate acylation-stimulating protein (ASP) that has anabolic effects on glucose and FFA storage (Cianflone et al., 1999). Adipsin is abundantly expressed and secreted from adipocytes but can also be found in muscle, lung and macrophages/monocytes (White et al., 1992).

Circulating levels of adipsin and adipsin gene expression are deficient in adipose tissue in several animal models of obesity (Flier et al., 1987). Adipsin plasma levels are increased after high-fat diet (Blogowski et al., 2013; Kwon et al., 2012).

Adipsin is abundantly expressed in brown adipose tissue (Cook et al., 1987). Expression of adipsin in brown adipose tissue is decreased after β3-adrenergic agonist treatment but not after acute cold (Napolitano et al., 1991).

Although adipsin has a detectable expression in muscle (Flier et al., 1987; Wernstedt et al., 2006), there are no reports on function. White adipose tissue is the dominant producer of adipsin (Flier et al., 1987). Expression and secretion of adipsin is decreased after β3-adrenergic agonist treatment in mice (Napolitano et al., 1991).

There are similarities in adipsin expression between white and brown adipose tissue, suggesting that adipsin secreted from brown fat has a similar function to adipsin from white adipose tissue.

4.4 Adrenomedullin

Adrenomedullin was isolated from pheochromocytoma (Kitamura et al., 2012), a tumour of the medulla of the adrenal glands (Washington et al., 1946).

Adrenomedullin is a multifunctional protein with active vasodilation properties and may participate in blood pressure homeostasis (Kitamura et al., 2012). Adrenomedullin is found in a variety of tissues, such as adrenal medulla, lung and kidney (Kitamura et al., 2012).

Adrenomedullin carry outs its actions via the calcitonin-receptor-like receptor (CRLR), which is only stimulated by adrenomedullin when the receptor-activity-modifying protein-2 (RAMP2) is expressed (McLatchie et al., 1998).

Adrenomedullin is also an adipokine, strongly correlated to obesity and its comorbidities (reviewed in Li et al., 2007). In obese mouse models and diet-induced obesity, adrenomedullin gene expression is elevated (Nambu et al., 2005).

Brown adipose tissue shows adrenomedullin gene expression (Paper I, (Go et al., 2007; Nambu et al., 2005). Microarray data on primary brown adipocyte cell culture show that adrenomedullin gene expression is decreased after norepinephrine stimulation (Paper I). Our in vivo studies show a decrease of adrenomedullin in cold-acclimated mice but that a high-fat diet increases adrenomedullin gene expression (Paper IV). Other studies in brown adipose tissue show no effect on adrenomedullin expression, protein or receptor components when the tissue is stimulated with either α- or β-adrenergic agonists separately. However, a combination of α- and β-agonists stimulate expression of adrenomedullin and its receptor (Go et al., 2007). Adrenomedullin is suggested to increase UCP1 expression and lipolysis in brown adipocytes; this indicates that adrenomedullin has an autocrine role in brown adipose tissue (Go et al., 2007). A full understanding of how adrenomedullin is regulated has not been attained as yet.

No adrenomedullin expression is found in skeletal muscle (Cameron and Fleming, 1998).

There is a higher expression of adrenomedullin in white than in brown adipose tissue (Go et al., 2007). However, our data suggest that brown adipocytes have a higher expression of adrenomedullin than white adipocytes; this is discussed as a possible effect of poorly differentiated cell cultures (Paper I). In white adipose tissue, adrenomedullin gene expression is increased after high-fat feeding and in obese mouse models (Nambu et al., 2005), similarly to brown adipose tissue (Paper V). Adrenomedullin has a suggested role to in lipid metabolism (Iemura-Inaba et al., 2008).

The physiological role of adrenomedullin in brown adipose tissue remains to be clarified. It is suggested that the secretion of adrenomedullin from brown adipose tissue is autocrine, possibly stimulating lipolysis and thermogenesis. The secretion of adrenomedullin from white adipose tissue seems also to be autocrine and to stimulate lipolysis.

4.5 Basic fibroblast growth factor

Basic fibroblast growth factor (bFGF or FGF2) is a potent angiogenic growth factor and is thought to be involved in metabolic homeostasis (Cao, 2007). bFGF is secreted from adipocytes and macrophages during adipose tissue hypertrophy (Cao, 2007), and from smooth muscle cells and T-cells (Segev et al., 2002).

bFGF acts via tyrosine kinase membrane FGF-receptors. There are four identified; bFGF can signal via FGF receptor 1, 2 and 3 (Jaye et al., 1992).

It is suggested that bFGF regulates metabolism of adipocytes via GLUT1 and attenuates the insulin signal in adipocytes (Kihira et al., 2011). Studies indicate an increase of serum bFGF in type 2 diabetes (Zimering Eng 1996).

Insulin and NE increase expression of bFGF in cultured brown adipocytes and levels of bFGF in media (Lindquist and Rehnmark, 1998; Yamashita et al., 1995). Treatment of cultured brown adipocytes with bFGF leads to ERK phosphorylation indicating that bFGF has a role in cell survival (Lindquist and Rehnmark, 1998). In vivo studies have shown that cold acclimation increase bFGF expression in brown adipose tissue (Asano et al., 1999), as well as the levels of plasma bFGF (Yamashita et al., 1994). The same study shows that bFGF stimulated the growth of brown adipocyte precursor cells, indicating an autocrine mode of action (Yamashita et al., 1994).

In skeletal muscle, bFGF is a factor important for wound-healing and muscle regeneration (DO et al., 2012; Yun et al., 2012). In skeletal muscle, bFGF and the FGF receptor 1 are increased after injury and contribute to the increased myoblast proliferation during the early stage of muscle regeneration (Zhang et al., 2012).

There is expression of bFGF in white adipose tissue and bFGF can induce phosphorylation of p44/p42 in cultured adipocyte (Mejhert et al., 2010).

It seems that bFGF acts in an autocrine manner in brown adipocytes, as well as in white adipocytes and skeletal muscle and stimulates cell growth through this.

4.6 Bone morphogenetic protein-8b

Bone morphogenetic protein-8b (BMP8b) is involved in the production of sperm and oocytes. Non-functional BMP8b cause defects in spermatogenesis (reviewed in Ying et al., 2002). BMP8b can be found in adipose tissue, liver, brain, kidney, heart, skeletal muscle and testis (Whittle et al., 2012).

BMPs can bind two types of serine-threonine kinase receptors, that is the BMP- type I and -type II receptors. There is seven BMP type I receptors, i.e. activin receptor-like kinase 1-7 (ALK 1-7). BMP type II has three identified receptors called BMPR-II, activin receptor-II and IIB (ActT-II and

ActR-IIB) (reviewed in Miyazono et al., 2010). It is not yet clear which receptor BMP8b signals through, although the activin receptor-like kinase 7 (ARK7) has been suggested (Whittle et al., 2012).

BMP8b is suggested to have a role in controlling energy metabolism (Whittle et al., 2012). Its expression is induced in brown adipose tissue with feeding, high-fat diet and cold acclimation. BMP8b knockout mice have impaired thermogenesis. Expression of BMP8b is increased in brown adipocytes with differentiation. It is suggested that BMP8b sensitizes brown adipose tissue to sympathetic stimulation and therefore regulates energy homeostasis (Whittle et al., 2012). Several candidate BMP-receptors are expressed in brown adipose tissue but only activin receptor like kinase 7 (ALK7) shares a similar expression profile as BMP8b (Whittle et al., 2012).

Skeletal muscle has low levels of BMP8b expression, as does white adipose tissue (Whittle et al., 2012).

The role of BMP8b is not clear but it is suggested that brown adipose tissue- released BMP8b has a role in overall energy homeostasis. This function is then mainly in an autocrine way. BMP8b is expressed in skeletal muscle and white adipose tissue as well, but the function is unknown.

4.7 Chemerin

The active substance, tazarotene, in the drug Tazorac® is a retinoid that modulates the pathogenesis of psoriasis. One of the tazarotene-induced genes is chemerin (tazarotene induced gene 2/ TIG-2 or retinoic acid receptor responder 2/ RARRES2) (Duvic, 1997; Nagpal et al., 1997), which is a small soluble protein secreted in an inactive pro-form that after proteolytic cleavage can exert local biological actions. Chemerin functions as chemoattractant for antigen-presenting cells (APCs) (Wittamer et al., 2003), and circulating chemerin is associated with chronic inflammation. Elevated levels can be observed in various diseases (Rourke et al., 2013).

Thus, chemerin may play an important role in the control of inflammatory processes, although whether it exhibits pro- or anti-inflammatory properties is still under discussion. Chemerin is highly expressed in a variety of tissues such as adipose tissue, liver, kidney (Bozaoglu et al., 2007), placenta (Goralski et al., 2007), lung (Roh et al., 2007), pancreas and adrenal glands (Zabel et al., 2005).

Chemerin mainly signals through the chemerin receptor (CMKLR1), which is a G-protein coupled receptor that plays an important role in adaptive and innate immunity. Two more G-protein coupled receptors are identified (GPR1 and CCRL2), but affinity to chemerin is low and non-existents (Mattern et al., 2014). Little is known about the signal transduction connected to CMKLR1 but it is suggested that the Gi-protein is involved, as pertussis toxin inhibit the effects of chemerin stimulation (Wittamer et al., 2003). Chemerin treatment of different cell types is reported to promote ERK 1/2 phosphorylation, as well as p38 MAPK phosphorylation, Akt phosphorylation and PI3K signalling (reviewed in Rourke et al., 2013). CMKLR1 is mainly expressed in immature plasmacytoid dendritic cells (pDC), macrophages and in tissues such as spleen, lymph node (Wittamer et al., 2003), white adipose tissue, lung (Bozaoglu et al., 2007), kidney, heart (Roh et al., 2007) and skeletal muscle (Sell et al., 2009).

Chemerin is an adipokine and may be involved in the pathogenesis of obesity as it correlates with several markers of the metabolic syndrome, including BMI, leptin, abdominal visceral fat accumulation and obesity (Bozaoglu et al., 2007; Shin et al., 2012). Furthermore, increased chemerin serum levels positively correlate with overall adiposity and inflammatory markers and are increased in obese animal models (ob/ob and db/db) and in diet-induced obesity (Rourke et al., 2013). Chemerin is often elevated and associated with diseases with chronic inflammation. The associations, however, give little insight into bioactivity.

Chemerin and its receptor CMKLR1 are expressed in brown adipose tissue (Paper III; (Goralski et al., 2007; Takahashi et al., 2011; Vernochet et al., 2009), although both at significantly lower levels than in white adipocytes. We identified chemerin in norepinephrine-treated brown adipocytes in 2001 using the signal-sequence trap technique, although at that point the gene received a different name from BLAST (Paper I). Gene expression of chemerin in primary brown adipocytes is increased with increasing differentiation but is unaffected by norepinephrine treatment (Paper V). Our findings on chemerin in brown adipose tissue indicate a possible autocrine role in the tissue, as dramatic effects in gene expression in brown adipose tissue do not lead to increased chemerin levels in plasma (Paper III).

There is gene expression of chemerin in skeletal muscle but expression is low (Paper III; (Rourke et al., 2013). Skeletal muscle expresses the chemerin receptor CMKLR1, and chemerin treatment induces insulin resistance in skeletal muscle (Sell et al., 2009). Chemerin is suggested to increase myoblast proliferation and decrease myoblast differentiation via mTOR and ERK1/2-pathways (Issa et al., 2012), suggesting that chemerin secreted from myocytes may act in both an autocrine and a paracrine manner (Yang et al., 2012).

In brite adipose tissue (inguinal white fat), chemerin expression is significantly increased with high-fat diet and suppressed in the cold, similar to the expression pattern found in brown adipose tissue. The effect of increased expression can, however, not be detected as an increased chemerin plasma level, so an autocrine effect is suggested also here (Paper III).

Chemerin is highly expressed in white adipose tissue (Paper III), which is considered to be the main source of circulating chemerin levels. The chemerin receptor CMKLR1 is also expressed at high levels in white adipose tissue (Goralski et al., 2007), but expression of the receptor is highest in early differentiation stages of white adipocytes, indicating a paracrine action

(Bozaoglu et al., 2007). Data on regulation of chemerin gene expression are conflicting but nutrient intake could potentially control chemerin expression in white adipose tissue (Stelmanska et al., 2013). Chemerin signalling is important during the early expansion phase of adipocyte differentiation, and PPARγ increases chemerin expression (Muruganandan et al., 2011).

Chemerin targets various tissues, thus the potential endocrine effects throughout the body may be several. Chemerin secreted from brown adipocytes and possibly brite cells may act in an autocrine manner, similar to that from skeletal muscle, rather than that from white adipose tissue that possibly has paracrine and endocrine effects, as well influencing cell differentiation and energy homeostasis.

4.8 Insulin-like growth factor I

Insulin-like growth factor I (IGF-1) has multiple physiological effects with endocrine, paracrine and autocrine actions and affects cell proliferation, transformation and apoptosis. IGF-1 is produced by almost all tissues throughout the body, but IGF-1 in the circulation is primarily secreted by the liver and the secretion is under the control of growth hormones (reviewed in Delafontaine et al., 2004). IGF-1 serum concentrations parallel those of growth hormone, and IGF-1 inhibits the secretion of growth hormone by the pituitary (Le Roith, 1997).

IGF-1 exerts all its known physiological effects via the IGF-1 receptor, which is ubiquitously expressed. IGF-1 receptors signal via insulin receptor substrates (IRS-1, 2, 3 and 4) that can activate multiple signalling pathways, including PI3K, Akt and MAPK. The different biological actions of IGF-1 receptors include cell growth, differentiation, migration and survival (reviewed in Delafontaine et al., 2004).

It is suggested that IGF-1 has an effect in metabolism, as infusion of recombinant human IGF-1 is associated with increased insulin sensitivity

and glucose uptake (reviewed in Sandhu et al., 2002). Due to this, IGF-1 is suggested as a therapy for several disorders including diabetes and obesity (in Le Roith, 1997; Xie and Wang, 2013).

There is high expression of IGF-1 receptors in brown preadipocytes (Lorenzo et al., 1993), and the receptors are also detected on mature brown adipocytes (Desautels et al., 1996). In brown adipose tissue, IGF-1 is increased after cold exposure (Yamashita et al., 1994) and IGF-1 treatment increases gene expression of UCP1 in brown adipocytes in vitro (Guerra et al., 1994). IGF-1 treatment increases GLUT4 expression and total GLUT4 protein in the membrane fraction in foetal brown adipocytes (Valverde et al., 1999). In addition, IGF-1 can work as a mitogen for brown adipocytes (Lorenzo et al., 1993; Valverde et al., 2005). Mice lacking IGF-1 receptors and insulin receptors have impaired thermogenesis and tissue growth (Boucher et al., 2012).

Skeletal muscle has both IGF-1 and IGF-1 receptors and IGF-1 is an important mediator of muscle growth, enhancing myoblast fusion (Mavalli et al., 2010).

IGF-1 and the IGF-1 receptor are expressed in white adipocytes. IGF-1 is important in adipocyte differentiation and is suggested to regulate cell proliferation, differentiation and metabolism (Bluher et al., 2005).

IGF-1 is a major factor significant for cell growth and proliferation and has metabolic effects (reviewed in Delafontaine et al., 2004). It is questionable if brown adipose tissue secretes IGF-1, as only one study reports expression of IGF-1 in brown adipose tissue. If IGF-1 is secreted from brown adipocytes, all three tissues secrete IGF-1, plausibly having an autocrine action.

4.9 Lipocalin 2

Lipocalin 2 (Lcn2 or neutrophil gelatinase-associated lipocalin, NGAL or 24p3) is a small secreted protein with a wide range of biological functions due to its ability to bind a variety of ligands involved in, for example, apoptosis and innate immunity (Flo et al., 2004). Lipocalin 2 can be found in various tissues but more abundantly in epididymal adipose tissue, liver, lung and kidney (Wang et al., 2007)

Lipocalin 2 signals through the lipocalin 2 receptor 24p3R which is expressed mainly in heart, lung, liver, spleen, skeletal muscle and testis (Devireddy et al., 2005).

Circulating serum levels of lipocalin 2 are strongly associated with obesity, and adipose tissue and liver are suggested to be the main sources of lipocalin 2 (Wang et al., 2007). Obese mouse models and mice fed a high-fat diet display increased expression of lipocalin 2 in white adipose tissue and elevated lipocalin 2 protein levels in serum (Wang et al., 2007; Yan et al., 2007). Expression of Lcn2 is increased in white adipose tissue after acute cold exposure (Guo et al., 2010). The results from studies with lipocalin 2 knockout mice are inconclusive, as some knock-out mice enhance diet-induced obesity (Guo et al., 2010), while other studies show no effects or that the lipocalin 2 knockouts are protected against diet-induced obesity (Jun et al., 2011; Law et al., 2010). Thus, lipocalin 2 might not have a major impact on energy homeostasis.

Lipocalin 2 is expressed in brown adipose tissue according to my studies (paper IV) while an other report failed to observe any expression in brown adipose tissue (Yan et al., 2007). Using signal sequence trap, lipocalin 2 was one of the genes frequently identified (Paper I), which led us to speculate that it is highly secreted from brown adipocytes after norepinephrine stimulation in vitro. To test our hypothesis, brown adipocytes stimulated with norepinephrine, and brown adipose tissue from mice exposed to cold and from mice showing diet-induced obesity were examined on a designed

microarray. The results in vitro did not show the increase with NE that we expected, and cold-acclimated mice induced lipocalin 2 gene expression only slightly (Paper I, Paper IV). Lipocalin 2-knockout mice are suggested to be cold sensitive and display lower body temperature during cold stress; however; there are no effects on UCP1 gene expression in these animals (Guo et al., 2010; Jin et al., 2011). The cold-intolerance might rather be from decreased heat production from muscle shivering (Guo et al., 2010).

One report suggests that lipocalin 2 is not expressed in murine skeletal muscle (Yan et al., 2007). However, the human equivalent to lipocalin 2, NGAL, is highly expressed in human skeletal muscle and is suggested to participate in iron uptake (Polonifi et al., 2010).

Lipocalin 2 is abundantly expressed in and secreted from white adipose tissue (Wang et al., 2007; Yan et al., 2007). However, data obtained from in vivo studies on weight gain and insulin sensitivity with lipocalin 2-knockout mouse are inconclusive (Guo et al., 2010; Jun et al., 2011; Law et al., 2010). Reports exist showing no effects on glucose tolerance, inflammatory markers or serum adipokines (Jun et al., 2011). Other reports show increased fat mass, increased glucose intolerance and increases in inflammatory markers (Guo et al., 2010), as well as increased fat mass, with attenuated inflammatory markers and increased insulin sensitivity (Law et al., 2010). This might, however, be an effect of confounding factors in the studies performed, as different high-fat diets have been used, as well as different backcrossing of the mice and different length of the studies.

No firm conclusions can be drawn from the present studies concerning the effect of lipocalin 2 on brown adipose tissue. I suggest that lipocalin 2 is an autocrine factor in brown adipose tissue but with as yet unknown function.

4.10 Niemann Pick type C2

Niemann Pick type C2 (NPC2) is a small cholesterol-binding protein responsible for intracellular trafficking of lipoprotein-associated cholesterol (Klein et al., 2006). Mutations in the NPC-genes are responsible for Niemann-Pick type C disease that is fatal due to cholesterol accumulation in liver, spleen and the central nervous system (Klein et al., 2006). NPC2 is expressed in liver, neurons, epididymis and astrocytes (Klein et al., 2006).

One study shows an association between the NPC2 genotype and obesity in a Korean population (Kim et al., 2010).

In our study with the signal-sequence trap, NPC2 was frequently identified, indicating that NE stimulates NPC2 gene expression (Paper I). However, in further studies on NPC2, we found that high-caloric diet suppressed NPC2 expression in brown adipose tissue, but the expression was unchanged in response to cold stress (Paper IV). We also saw that NPC2 was increased in brown adipocytes during cell differentiation, but gene expression was unaffected by NE treatment (Paper V). There are no published results about NPC2 in brown adipose tissue.

Data concerning NPC2 in skeletal muscle are also lacking. Unpublished data from the department show expression of NPC2 in skeletal muscle, but expression was not affected by diet or cold exposure.

White adipocytes transfected with an NPC2 siRNA become more metabolically similar to brite cells, with increased lipolysis and insulin sensitivity (Csepeggi et al., 2010).

NPC2 is expressed in 3T3-L1 adipocytes, and it has been suggested that NPC2 plays an autocrine role in adipocyte differentiation and the maintenance of mature white adipocytes (Csepeggi et al., 2010).

NPC2 is suggested to have an autocrine role in brown and white adipose tissue. The expression and role of NPC2 in brite adipocytes and skeletal muscle remain unsolved.

5 Paracrine factors

A paracrine factor signals to nearby cells, without entering the circulation, and modifies their performance or differentiation.

This section includes the non-protein nitric oxide. The proteins discussed in this section are angiotensinogen, nerve growth factor, vascular endothelial growth factors and lipoprotein lipase. In our studies, lipoprotein lipase was identified with signal-sequence trap (Paper I) but its expression was not significantly affected in our further studies.

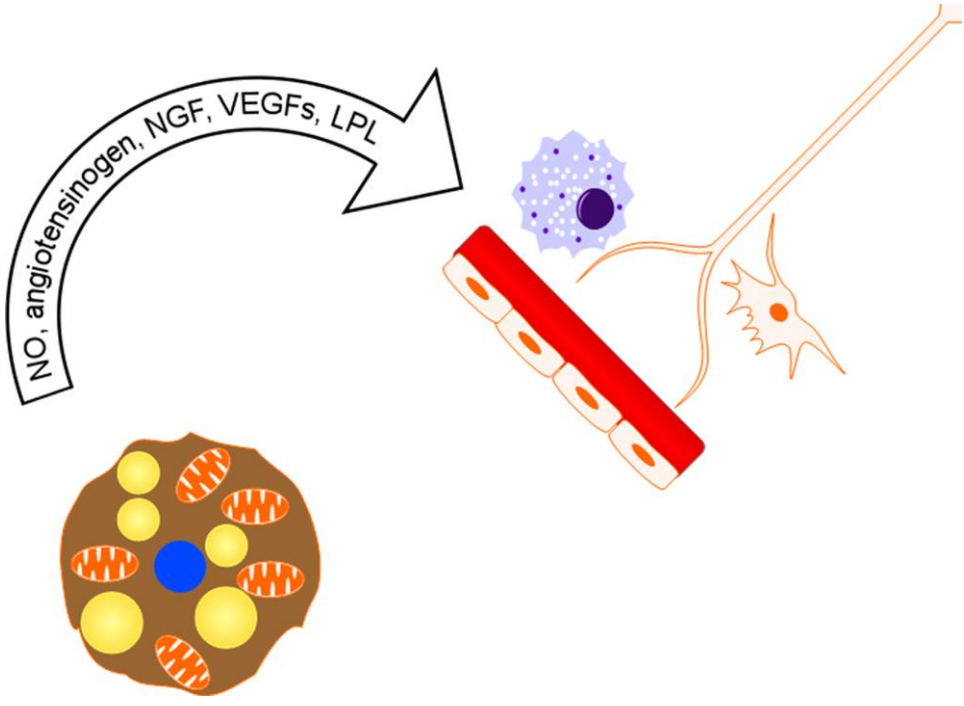

Figure 4. My current view of brown adipose tissue's paracrine factors. Nitric oxide (NO), angiotensinogen, nerve growth factor (NGF), vascular endothelial growth factors (VEGFs) and lipoprotein lipase (LPL) are suggested paracrine factors.

5.1 Nitric oxide

Nitric oxide synthase (NOS) has three isoforms (endothelial eNOS, neuronal nNOS and inducible iNOS) and produces nitric oxide (NO) from L-arginine. Nitric oxide exhibits several physiological functions, e.g. cell signalling with vasodilator action, and controlling cell proliferation and differentiation. eNOS and nNOS are constitutively present, whereas iNOS expression is increased under certain conditions such as inflammation (Wort et al., 2001). Nitric oxidase synthase and nitric oxidase production can be found in various cell types and tissues, such as macrophages, brown adipose tissue and skeletal muscle, and NO displays several physiological effects throughout the body (reviewed in Bredt and Snyder, 1994).

Nitric oxide has many functions and has been proposed to play a role in obesity by affecting lipolysis, glucose uptake and leptin signalling (Mehebik et al., 2005).

Brown adipose tissue can produce and secrete nitric oxide, suggested to be through induction of inducible NOS (iNOS) (Nisoli et al., 1997) but brown adipose tissue also contains detectable expression of endothelial NOS (eNOS) (Kikuchi-Utsumi et al., 2002). In vitro studies show that NO decreases cell proliferation and increases differentiation in cultured brown adipocytes, thus suggesting that NO acts in a autocrine/ paracrine manner during proliferation and differentiation (Nisoli et al., 1998). NO can inhibit mitochondrial respiration in an autocrine manner (Koivisto et al., 1997). In brown adipose tissue, adrenergic activation stimulates NO production to mediate vasodilation and increase blood flow (Nagashima et al., 1994).

Skeletal muscle produces NO, which then affects contraction and muscle function in an autocrine fashion (Kobzik et al., 1994). NO can also work in a paracrine way to affect blood flow.

Leptin induces NO production in white adipose tissue, and NO affect white adipocytes in an autocrine manner and is important for proper leptin

signalling (Mehebik et al., 2005). NO can also regulate the blood flow to mediate the metabolic an endocrine roles of white adipose tissue.

Nitric oxide has several effects in and on brown adipose tissue, suggesting that it may function in an autocrine and paracrine manner. NO from skeletal muscle and white adipose tissue seems to work in a similar way.

5.2 Angiotensinogen

Angiotensinogen is the starting factor in the renin-angiotensin system (RAS) where angiotensinogen is an inactive hormone that via a cascade is converted by the enzyme renin into the active form angiotensin II. Angiotensin II in its turn is involved in blood pressure homeostasis. Many tissues possess the renin angiotensin system components, and it is suggested that members of the RAS could control local functions. There is a linkage between local production of the renin angiotensin system and hypertension, atherosclerosis and kidney disease (Cassis et al., 2008). Liver is the primary source of circulating angiotensinogen but it can also be found in kidney, brain (Menard et al., 1983) and brown adipose tissue (Cassis and Dwoskin, 1991).

Angiotensin can signal through two G protein-coupled receptors (AT1, AT2) (Stegbauer and Coffman, 2011). These receptors can be found in a variety of tissues such as heart, epididymis, intestine, white and brown adipose tissue (Paul et al., 2006).

Several hormones and metabolic changes that are associated with obesity are reported to affect angiotensinogen expression in adipocytes, but confounding factors produce controversies around the results. Experiments suggest that the renin angiotensin system could be involved in the regulation of body fat (Weisinger et al., 2007). There are also implications that

alterations in the renin angiotensin system contribute to human insulin resistance (Underwood and Adler, 2013).

Angiotensinogen can be found in brown adipose tissue, at about 60% of the liver expression level. Liver is the main source of angiotensinogen (Cassis and Dwoskin, 1991). There is no renin expression in brown adipose tissue (Shenoy and Cassis, 1997); however, renin protein is found in brown adipose tissue, as well as angiotensin II (Shenoy and Cassis, 1997), and the angiotensin type 2 receptor (Cassis et al., 1996; Galvez-Prieto et al., 2008). Angiotensin II is increased after cold exposure and is suggested to enhance sympathetic activity during cold-induced thermogenesis (Cassis, 1993). Angiotensinogen gene expression is unaffected by high-fat diet (Rahmouni et al., 2004).

Skeletal muscle contains angiotensinogen and can produce angiotensin II; however, there is no detectable renin. Skeletal muscle also expresses angiotensin receptors but primarily the angiotensin type 1 receptor (Johnston et al., 2011). It is suggested that locally produced muscle angiotensin II has no endocrine role (Goossens et al., 2007).

In white adipose tissue, all renin-angiotensin system components can be found, as well as both angiotensin type 1 and type 2 receptors (Cassis et al., 2008; Galvez-Prieto et al., 2008). Angiotensinogen expression is at a similar expression level as the expression of angiotensinogen in brown adipose tissue (Cassis et al., 2008). It is suggested that local angiotensin II may increase leptin release from adipocytes (Cassis et al., 2004).

Brown adipose tissue seems to have the same capacity to produce and secrete angiotensinogen as white adipose tissue but the overall role seems different, as angiotensin secreted from white adipose tissue might have an endocrine role, while that from brown adipocytes does not. Brown adipocytes do not possess many angiotensin receptors so effects are rather paracrine than autocrine. Angiotensinogen secreted from muscle and white

adipose tissue might have an autocrine role as well as a paracrine. It seems that brown adipose tissue is fairly similar to both these tissues.

5.3 Nerve growth factor

Nerve growth factor (NGF) is essential for the development and the maintenance of sympathetic, sensory neurons and cholinergic neurons in the central nervous system (Aloe et al., 2012). NGF can be found in many tissues e.g. heart, skin, skeletal muscle kidney, intestine and lung (Maisonpierre et al., 1990),

There are two receptors identified for NGF signalling. Tropomyosin kinase receptor A (trkA) has a high affinity for NGF, and p75 has a low affinity and can be found in various tissues (Peeraully et al., 2004). TrkA demonstrates typical tyrosine kinase receptor signalling via MAPK, ERK, PI3K and phospholipase C (PLC), and p75 is a non-selective neutrophin receptor (Aloe et al., 2012). NGF-receptors are important in the development, maintenance, survival and plasticity of peripheral nervous system neurons.

Circulating NGF is increased in obesity, type 2 diabetes and metabolic syndrome; the connection to weight gain is, however, not elucidated (Bullo et al., 2007).

NGF secreted by brown adipocytes is involved in modulating sympathetic innervation. It is suggested that there is a relationship between NGF synthesis and proliferation activity (Nechad et al., 1994), and regulation of sympathetic innervation during perinatal and adult periods (Nisoli et al., 1998). Secretion of NGF from brown adipose tissue is increased in genetically obese animals (Nisoli et al., 1996), during stress and diabetes (Sornelli et al., 2009). Cold exposure decreases NGF expression in brown adipose tissue and this is mimicked by norepinephrine in brown adipocytes in vitro (Nisoli et al., 1996). It is remarkable that NGF is decreased by

sympathetic activity; one would guess that the stimulation would induce innervation. NGF deprivation produce low norepinephrine content in sympathetically innervated peripheral tissues such as brown adipose tissue (Gorin and Johnson, 1980). Interscapular brown adipose tissue shows gene expression of both trkA and p75 receptors; however, the high affinity trkA is expressed to a lesser extent (Peeraully et al., 2004). The p75 receptor has been detected with immunostaining (Nisoli et al., 1996), the implications of the presence of neuronal receptors in brown adipose tissue are probably not on the adipocytes itself but on nerves within the tissue.

NGF is expressed in skeletal muscle (Maisonpierre et al., 1990), and normal exercise increase NGF in soleus muscle in diabetic rats (Chae et al., 2011). Skeletal muscle expresses both high (TrkA) and low (p75) affinity NGF receptors, and inhibition of TrkA but not p75 decreases cell proliferation in vitro (Rende et al., 2000). Through chronic treatment of C2C12 cell cultures with anti-NGF antibody, myoblast differentiation was decreased. This is suggested to occur via the p75 receptor, as no TrkA was detected (Ettinger et al., 2012). It is still unclear what effects NGF has on myocytes but it is suggested that NGF may increase myotube fusion (Rende et al., 2000).

NGF is expressed in white adipose tissue and secreted from white adipocytes in vitro. There is an increase of NGF in white adipose tissue after stress and with diabetes (Sornelli et al., 2009). White adipose tissue has expression of both NGF receptors trkA and p75 and the suggested function is the development and survival of sympathetic neurons within the tissue or as a part of the inflammatory response (Peeraully et al., 2004).

Brown adipose tissue secretes NGF in a paracrine manner to stimulate innervation. The action is similar to the actions reported for NGF on skeletal muscle and white adipose tissue.

5.4 Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is found as several homologues; however, only VEGF-A, VEGF-B, VEGF-C have been reported to be present in brown adipose tissue (Asano et al., 1997; Asano et al., 1999; Asano et al., 2001). VEGF was first called, and described as, vascular permeability factor, which had a critical role in tumour angiogenesis (Dvorak et al., 1979). VEGF proteins are important angiogenic factors but can also stimulate endothelial cell proliferation and migration. All VEGF family members are secreted as dimeric glycoproteins with a so-called cysteine knot-motif (Kliche and Waltenberger, 2001).

The effects of VEGF-A, -B and -C are mediated via tyrosine kinase receptors, where VEGFR-1 also has a soluble form (sVEGFR-1) that works as an angiogenic inhibitor (Saito et al., 2013). VEGFR-2 is the main receptor with angiogenic signals, and VEGF-A signalling via VEGFR2 is a major regulator of blood vessel formation and function (Nakayama and Berger, 2013).

5.4.1 VEGF-A

VEGF-A is an essential factor in embryonic development and is a specific mitogen for vascular endothelial cells in vitro and plays a central role in the formation of embryonic blood vessels and angiogenesis in vivo (Carmeliet et al., 1996; Ferrara et al., 1996). VEGF-A is expressed in several different tissues such as liver, lung, and brown adipose tissue (Lagercrantz et al., 1998), white adipose tissue, skeletal muscle, heart, and kidney (Asano et al., 1997; Hagberg et al., 2010).

VEGF-A signals through VEGFR-1 or VEGFR-2 (Kliche and Waltenberger, 2001), where VEGFR-2 can induce for example proliferation, migration, NO release or modulate gene expression (Kliche and Waltenberger, 2001).

Mice fed a high-fat diet and performing exercise increase VEGF-A expression in white adipose tissue (Baynard et al., 2012). Mice with repressed VEGF-A are lean and resistant to diet-induced obesity (Lu et al., 2012). VEGF-A could therefore have some function in energy metabolism. However, Elias et al. showed that overexpression of VEGF-A protects against diet-induced obesity and systemic insulin resistance (Elias et al., 2012). VEGF-A may also have pro- and anti-inflammatory properties, decreasing pro-inflammatory cytokines and increasing recruitment of M2 macrophages (Elias et al., 2012).

In brown adipose tissue, there is abundant expression of VEGF-A, and expression is increased after cold exposure (Asano et al., 1997), as well as in cell cultures after norepinephrine treatment (Asano et al., 2001). Gene expression of VEGF-A is enhanced via the beta-adrenergic pathway (Asano et al., 1997; Fredriksson et al., 2000) and the increase of VEGF-A expression is independent of thermogenic oxygen consumption (Fredriksson et al., 2005). VEGF-A signals to increase angiogenesis via the VEGFR-2, as blocking the VEGFR-2 receptor impairs nonshivering thermogenesis and blocks angiogenesis (Xue et al., 2009) as well as increasing brown adipocyte apoptosis (Bagchi et al., 2013). VEGF-A stimulates proliferation of the surrounding vascular endothelial cells (Asano et al., 1997) and increases brown adipocyte cell proliferation in vitro (Bagchi et al., 2013). Overexpression of VEGF-A increases brown adipose tissue size, vascularization and UCP1 expression (Elias et al., 2012). This suggests a role for VEGF-A in brown adipocytes to promote survival, proliferation and development (Bagchi et al., 2013).

VEGF-A is secreted from skeletal muscle upon contraction (Hoier et al., 2010). Skeletal muscle VEGF-A is localized in vesicles and the amount is increased after exercise, suggesting that muscle secretes VEGF-A to extracellular fluids to control capillary growth (Hoier et al., 2013). VEGF-A protein is increased in the circulation after training, indicating that skeletal

muscle has a secretory role for VEGF-A (Breen et al., 1996; Wahl et al., 2011). Training increases the expression of both VEGF-A and the receptor VEGFR-1 (Birot et al., 2003).

There is VEGF-A gene expression and protein in white adipose tissue (Zhang et al., 1997). Gene expression of VEGF-A is lower than in brown adipose tissue (Asano et al., 1997; Asano et al., 1999). However, expression is increased after norepinephrine stimulation, and the effect is adenylate cyclase-mediated (Mick et al., 2002). According to Lu et al. mice with repressed VEGF-A have elevation of UCP1 in gonadal white adipose tissue. The VEGF-A repressed mice are lean and show resistance to diet-induced obesity (Lu et al., 2012).

VEGF-A seems to mainly work in a paracrine factor in all three tissues.

5.4.2 VEGF-B

VEGF-B is reported to have poor angiogenic activity under normal physiological conditions in most tissues and is redundant in embryonic development, as VEGF-B-knockout mice are healthy and fertile (Aase et al., 2001). VEGF-B is highly expressed in brain, heart, testis, brown adipose tissue (Lagercrantz et al., 1998) and skeletal muscle (Hagberg et al., 2010).

VEGF-B specifically binds to and signals via the VEGFR-2 receptor and promotes endothelial cell proliferation (Aase et al., 1999).

VEGF-B is reported to increase expression of fatty acid transport proteins and is suggested to have effects on energy metabolism (Hagberg et al., 2010). Inhibition of VEGF-B restores peripheral insulin sensitivity and muscle glucose uptake and prevents type 2 diabetes (Hagberg et al., 2012).

Gene expression of VEGF-B is abundant in brown adipose tissue and is unaffected by cold exposure (Asano et al., 1999), the same effect can be seen with norepinephrine in brown adipocyte cell cultures (Asano et al., 2001).

Gene expression of VEGF-B in skeletal muscle is abundant (Aase et al., 1999; Olofsson et al., 1996) and VEGF-B is reported to have a role in fatty

acid uptake (Hagberg et al., 2010). Decreasing VEGF-B signalling improves insulin sensitivity in muscle; VEGF-B is therefore an interesting anti-diabetic target (Hagberg et al., 2012).

In white adipocytes, VEGF-B expression is increased when VEGF-A is repressed, and repression of VEGF-A induces white adipocytes to become brown-like. A question is therefore if VEGF-B participates in the regulation of white adipocytes becoming brite (Lu et al., 2012).

VEGF-B is suggested to signal in a paracrine fashion via endothelial VEGFR-1 receptors and increase fatty acid uptake with fatty acid transport proteins (Aase et al., 1999; Hagberg et al., 2010), and this might be similar in all three tissues.

5.4.3 VEGF-C

VEGF-C is in vitro a mitogen and in vivo an angiogenic factor. VEGF-C is mainly involved in lymphangiogenesis (Breen, 2007). VEGF-C expression is found in many tissues but is most prominent in heart (Lagercrantz et al., 1998), placenta, muscle, ovary and small intestine (Joukov et al., 1996).

VEGF-C signals through the receptors VEGFR-2 or VEGFR-3 (Joukov et al., 1996; Kliche and Waltenberger, 2001; Kociok et al., 1998).

VEGF-C is undetectable in brown adipose tissue (Asano et al., 1999; Lagercrantz et al., 1998) but in immortalized brown adipocyte cell cultures, there is VEGF-C expression, and upon norepinephrine stimulation, expression is attenuated (Asano et al., 2001).

There is VEGF-C expression in skeletal muscle but its function is not fully known (Kivela et al., 2007a; Kivela et al., 2007b). There is VEGF-C expression in white adipose tissue (Coin Araguez et al., 2013); expression is not modulated in obesity models (Voros et al., 2005) .

There is not much reported concerning VEGF-C but I would suggest a similar paracrine action as the other two VEGFs in all three tissues.

5.5 Lipoprotein lipase

Lipoprotein lipase is secreted to the capillary lumen to hydrolyze triglycerides from circulating chylomicrons and therefore has a central role in overall lipid metabolism and transport (Havel and Gordon, 1960). Lipoprotein lipase shows tissue-specific regulation in a number of physiological states, e.g. fasting, feeding, exercise and thermogenesis, and the changes are mediated through hormone action, e.g. through insulin, glucocorticoid and noradrenaline (reviewed in Mead et al., 2002). The most dominant producers of lipoprotein lipase are adipose tissue, cardiac and skeletal muscle, but there is also a lower production in other cells, such as macrophages, spleen, testis, lung and kidney (reviewed in Mead et al., 2002).

In one study in adipocyte-specific lipoprotein lipase knockout mice, it is shown that the knockout mice have increased de novo lipogenesis. When knockout mice were fed a high-fat diet, they were characterized by reduced adiposity but were not protected from metabolic disease (Bartelt et al., 2013).

In brown adipose tissue, lipoprotein lipase activity is increased after cold exposure (Radomski and Orme, 1971) through β-adrenergic receptors (Carneheim et al., 1984), but also insulin increases lipoprotein lipase expression (Mitchell et al., 1992). In our signal-sequence trap, lipoprotein lipase was identified as a secreted factor but was however, after the following microarray-studies not affected to fit our criteria and was never further investigated (Paper I). Recent studies ascribe brown adipose tissue an active role as a main lipid-clearing organ with its local lipoprotein lipase (Bartelt et al., 2011).

Skeletal muscle has known lipoprotein lipase gene expression, and exercise increases the expression (Ladu et al., 1991), improving blood lipids in obese humans (Greene et al., 2012).

White adipose tissue has a high activity of lipoprotein lipase, and this is an important determinant of adipose triglyceride storage. Physical activity

decreases expression of lipoprotein lipase in white adipose tissue (Ladu et al., 1991), as well as cold, NE-infusion (Trayhurn et al., 1995) and stress (Casanovas et al., 2007). There are no increased britening in mice with adipocyte-specific loss of lipoprotein lipase (Bartelt et al., 2013).

Lipoprotein lipase is regulated so that energy can be directed where it is needed. Lipoprotein lipase is definitely paracrine, as it is transported out to the luminal surface of vascular endothelia where it is bound or released into the circulation. This is probably similar to both skeletal muscle and white adipose tissue.

6 Endocrine factors

This section contains those factors that are or might be secreted from brown adipose tissue in an endocrine manner. Note that even if the factor could be secreted in an endocrine manner from brown adipose tissue, this does not imply that the endocrine effect is of greater impact. This section contains the factors free fatty acids, heat, adiponectin, fibroblast growth factor 21 (FGF21), interleukin-1α (IL-1α), interleukin-6 (IL-6), leptin, retinol binding protein 4 (RBP4), resistin and triiodothyronine (T3) and the possibility of an unknown "anti-obesity" factor. I will discuss their appearance in brown adipose tissue, muscle, brite and white adipose tissue and evaluate if they have similar actions according to literature.

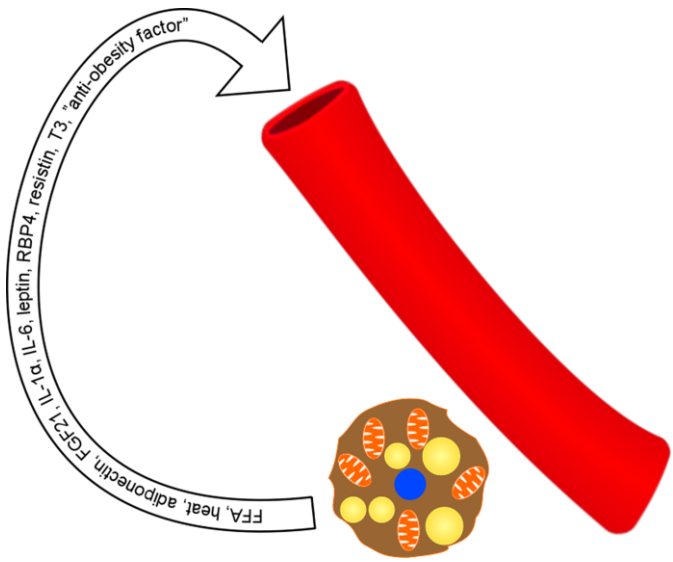

Figure 5. My current view of the endocrine factors from brown adipose tissue. Free fatty acids (FFA), heat, adiponectin, fibroblast growth factor 21 (FGF21), interleukin-1α (IL-1α), interleukin-6 (IL-6), leptin, retinol binding protein 4 (RBP4), resistin and triiodothyronine (T3) are suggested endocrine factors.

6.1 Free fatty acids

Free fatty acids are produced by lipolysis when circulating triglycerides are hydrolysed, by e.g. lipoprotein lipase (LPL), to glycerol and free fatty acids and when intracellular triglycerides are hydrolysed by adipose tissue triglyceride lipase (ATGL) and hormone-sensitive lipase (HLS). The free fatty acids can then be used as an instant source of energy or re-esterified and stored as triglycerides (Dragojevic et al., 2013). Free fatty acids have been demonstrated as a receptor-ligand for G-protein coupled receptors and are suggested to play a role in glucose homeostasis. There are some known receptors that are known to bind free fatty acids i.e. FFA1 (previously GPR40), FFA2 (previously designatedGPR43) and FFA3 (previously named GPR41) that bind short chain fatty acids, the receptors GPR84 and GPR120 is activated by medium and long chain fatty acidsbut the function is not yet known (Milligan et al., 2014; Rayasam et al., 2007).

Fatty acids play a role in the development of insulin resistance, as fatty acids are negatively involved in insulin action in the liver and skeletal muscle and in glucose-stimulated insulin secretion. Increased circulating free fatty acids in plasma, and delivery to the liver, are factors that may underlie many metabolic disturbances.

Brown adipocytes can secrete free fatty acids in vitro when stimulated with norepinephrine and lipolysis produces more free fatty acids than the brown adipocyte can combust (Nedergaard and Lindberg, 1979). Brown adipose tissue does not release fatty acids in the basal state, but maximal stimulation with norepinephrine stimulates free fatty acid release (Ma and Foster, 1986). The physiological importance of fatty acid release is unclear as they are only secreted at maximal stimulation. Only the fatty acid receptor FFA2 (GPR43) is reported to be expressed in brown adipose tissue (Regard et al., 2008).

Free fatty acids can be released from skeletal muscle, and release is lowered in type 2-diabetes (Blaak et al., 2000). White adipose tissue

lipolysis and secretion of free fatty acids supplies fuel to tissues and organs that can oxidize fatty acids. Only the fatty acid receptor FFA2 (GPR43) is reported to be expressed in skeletal muscle and white adipose tissue (Regard et al., 2008).

All tissues can release free fatty acids, although white adipose tissue does this to a higher extent. Free fatty acids are secreted and can be taken up by cells to be used as fuel or can be used as signalling molecules. The release of free fatty acids from brown adipose tissue and skeletal muscle could be endocrine but the physiological effect needs to be further studied.

6.2 Heat

The main function of brown adipose tissue is to produce heat. Brown adipose tissue was initially recognized to have thermogenic properties in the early 1960´s and functioned to help hibernating animals to regain their normal body temperature (Smith and Roberts, 1964).

Heat is produced via uncoupling protein 1 (UCP1) in brown adipose tissue. The heat produced is then transmitted via the blood stream to spread heat throughout the rest of the body (reviewed in Cannon and Nedergaard, 2004).

Any type of metabolism generates some heat. Skeletal muscle activity hydrolyses ATP to create movement of myosin and actin, and thus heat is produced. This is called shivering thermogenesis. The total heat released from skeletal muscle can be measured (Fales and Zierler, 1967). Although it has been suggested that heat can be produced in muscle thermogenesis via uncoupling protein 3 and that the ability to lose weight successfully can be linked to muscle thermogenesis and UCP3 (Lee et al., 2013c), there is as yet no evidence that UCP3 have any uncoupling action (Nabben et al., 2011).

Brite cells can also produce heat as they have adrenergically induced UCP1 expression (Okamatsu-Ogura et al., 2013; Petrovic et al., 2010; Shabalina et al., 2013).

All metabolic activity releases some amount of heat and therefore white adipose tissue probably also produces some heat.

Heat is definitely an endocrine product released from brown adipose tissue, as the heat is spread throughout the body. Heat production in brown adipose tissue and skeletal muscle is quite similar in that they both have the function to produce heat to sustain body temperature.

6.3 Adiponectin

Adiponectin, also known as AdipoQ (Hu et al., 1996), ACRP30 (Scherer et al., 1995) or GBP28 (Nakano et al., 1996), is a hormone involved in insulin function and energy homeostasis. In serum, adiponectin exists in different forms of multimers, up to 18-mers or possibly more, comprised of homotrimers (reviewed in Whitehead et al., 2006). Adipose tissue abundantly expresses and secretes adiponectin (Hu et al., 1996; Scherer et al., 1995; Zhang et al., 2002), but adiponectin may also be found in skeletal muscle (Delaigle et al., 2004) and liver (Yoda-Murakami et al., 2001).

The effects of adiponectin are mediated by two specific receptors, adipoR1 and adipoR2, which are ubiquitously expressed. However, adipoR1 is highly expressed in skeletal muscle whereas adipoR2 is predominantly expressed in liver (Yamauchi et al., 2003).

Adiponectin is often negatively correlated with obesity (Arita et al., 1999). Mice deficient in adiponectin develop insulin resistance and subsequently type 2 diabetes (Maeda et al., 2002). There is a well-established insulin-sensitizing effect of adiponectin induced by increased fatty acid oxidation and suppression of hepatic glucose production (reviewed in Kadowaki et al., 2006).

Adiponectin (Viengchareun et al., 2002) and the receptors adipoR1 and adipoR2 (Fu et al., 2007) are expressed in brown adipose tissue. Expression of adiponectin is reduced by acute β3-adrenergic stimulation, whereas AdipoR2 is increased, while AdipoR1 remains unchanged (Fu et al., 2007; Zhang et al., 2002). The regulation of adiponectin in brown adipocytes is still under discussion, although in vitro studies have shown a slight increase in expression of adiponectin after insulin stimulation (Viengchareun et al., 2002) and a slight decrease in expression after acute cold exposure (Puerta et al., 2002).

Adiponectin expression can be induced in skeletal muscle in response to LPS-injection in vivo (Delaigle et al., 2004). Skeletal muscle contains high levels of AdipoR1 and its expression correlates with insulin secretion (Staiger et al., 2004). In skeletal muscle, adiponectin also stimulates fatty acid oxidation and glucose transport (Yamauchi et al., 2002).

White adipose tissue abundantly expresses and secretes adiponectin (Hu et al., 1996; Scherer et al., 1995; Zhang et al., 2002) as well as expressing the receptors AdipoR1 and AdipoR2 (Fu et al., 2007). White adipocytes constitute the main source of adiponectin, and studies have shown that adiponectin has pleiotropic actions involved in inflammation and insulin sensitivity (reviewed in Li et al., 2011). Adiponectin can also promotes adipocyte differentiation by acting in a autocrine fashion (Fu et al., 2005).

Adiponectin is present at high levels in plasma and is thus an endocrine factor. The expression levels of adiponectin in brown adipose tissue are low compared with white adipose tissue, so it remains questionable whether adiponectin secreted by brown adipocytes constitute a significant part of total circulating protein levels (Zhang et al., 2002). However, brown adipocyte adiponectin could exert metabolic effects through paracrine or autocrine mechanisms, similar to the secretion of adiponectin from skeletal muscle. The effects of adiponectin are suggested to be several, and the exact function of brown adipocyte-secreted adiponectin remains unknown.

6.4 Fibroblast growth factor 21

The majority of the fibroblast growth factor family members are associated with mitosis, development and angiogenesis but only three FGFs (FGF19, 21 and 23) are "hormone-like" (Itoh, 2010). Fibroblast growth factor 21 (FGF21) induces glucose transporter-1 (GLUT1) to promote glucose uptake in adipocytes (Kharitonenkov et al., 2005). FGF21 is a metabolic hormone that, in response to fasting, may stimulate gluconeogenesis, fatty acid oxidation and ketogenesis (Woo et al., 2013). FGF21 is expressed in multiple tissues such as liver, adipose tissue and pancreas. The expression is affected by different physiological factors such as cold exposure and fasting (Fisher and Maratos-Flier, 2013).

To bring about its metabolic effects, FGF21 signals through cell-surface complexes of FGF-receptors together with the transmembrane protein β-klotho (Chartoumpekis et al., 2011). FGF-receptors are expressed in liver and at high levels in brown and white adipose tissue (Diaz-Delfin et al., 2012). β-klotho is a co-factor in liver, pancreas, ileum and adipose tissue (Adams et al., 2012).

FGF21 improves glucose tolerance (Muise et al., 2008) and may therefore represent a promising candidate for therapeutic intervention in obesity (Kharitonenkov et al., 2005). FGF21 treatment shows beneficial effects in obese mice and diabetic monkeys (Woo et al., 2013).

Adrenergic stimulation of brown adipose tissue, via a cAMP-mediated pathway, induces gene expression and secretion of FGF21 (Hondares et al., 2011). Brown adipose tissue expression of FGF21 changes in response to cold, and FGF21 increases the browning of white adipocytes (Fisher et al., 2012). In humans, FGF21 is expressed in brown but not white adipose tissue (Hondares et al., 2013). In human neonatal brown adipose tissue there is a positive correlation between UCP1 and FGF21 expression (Hondares et al., 2013).

Gene expression of FGF21 in muscle is elevated in patients with type II diabetes. The exact consequence of the increased expression is not known but it is suggested that FGF21 is a myokine secreted from muscle with autocrine functions (Lindegaard et al., 2013).

FGF21 is thought to play a physiological role to activate and expand the thermogenic machinery by browning of white adipocytes (Fisher et al., 2012). FGF21 is expressed and secreted from human brite adipocytes, and the secretion is increased by norepinephrine (Lee et al., 2013a).

White adipose tissue expresses and secretes FGF21 in an autocrine and paracrine manner, influencing lipid and glucose homeostasis as well as body weight. FGF21 induces subcutaneous white adipose tissue depots to become brite and acquire a metabolic phenotype similar to brown adipocytes (Fisher et al., 2012). It is suggested that FGF21 is secreted into the blood stream. However, the function of FGF21 needs further investigation (Muise et al., 2008).

In general, FGF21 is expressed and secreted by both white and brown adipocytes, as well as by muscle. FGF21 is present in the plasma; however, detailed information regarding effects of FGF21 secreted in an endocrine manner from brown adipocytes is lacking. Brown adipose tissue also seems to be able to secrete FGF21 in an autocrine manner. The secretion of FGF21 from white adipose tissue seems to be endocrine in some extent but is mostly autocrine and paracrine. FGF21 secreted from skeletal muscle is suggested to act in an autocrine fashion. As white adipose tissue and skeletal muscle mainly secrete FGF21 as an autocrine or paracrine factor, there is a possibility that FGF21 released from brown adipose tissue has an endocrine role influencing overall energy metabolism.

6.5 Interleukin-1α

Interleukin 1 is a pro-inflammatory cytokine and exists in two forms, IL-1α and IL-1β. IL-1α is a pro-inflammatory cytokine that induces other inflammatory cytokines and has pyrogenic activity (reviewed in Rider et al., 2013). IL-1α is likely to have several cell functions, as it is expressed in a variety of tissues and cells. IL-1 is most often secreted from activated monocytes and macrophages (Durum et al., 1985). IL-1α has been found in different cell types and tissues e.g. brown adipose tissue, spleen (Burysek and Houstek, 1996), white adipose tissue, and liver (Um et al., 2011).

IL-1 binds and signals via the IL-1α- and IL-1β-receptor (IL-1R1) and the receptor can be found in a variety of immune cells and in tissues such as brown adipose tissue (Burysek et al., 1993). The activated IL-1R1 receptor activates (NF)-κB and Jun NH2-terminal kinase and mitogen-activated protein kinase (JNK, MAPK), which are reported to affect insulin signalling (He et al., 2006). IL-1 has an antagonist, the IL-1 receptor antagonist (IL-1Ra) that binds the receptor and blocks IL-1α and IL-1β.

IL-1α is often increased in diabetic patients, as well as in obesity, rheumatoid arthritis or cancers (Um et al., 2011).

IL-1α is expressed in brown adipose tissue and cold-acclimation increase IL-1α expression (Burysek et al., 1993). IL-1α gene expression is enhanced in vitro by β3-adrenergic stimulation but not by α1-adrenergic stimulation (Burysek and Houstek, 1997). IL-1β can induce gene expression of IL-1α, indicating the existence of IL-1R1 receptors on brown adipocytes (Burysek and Houstek, 1996). In brown adipose tissue, the IL-1Ra antagonist is expressed at thermoneutrality and cold exposure (Burysek et al., 1993), and obesity slightly increases the IL-Ra antagonist expression (Juge-Aubry et al., 2003; Um et al., 2011). Diet-induced obesity has no significant effect on either IL-1α or its receptor (Um et al., 2011). IL-1α secreted from brown adipose tissue could contribute to the IL-1α levels in the plasma or have local effects on brown adipose tissue function.

There are no reports of IL-1α expression or protein in skeletal muscle (Dorph et al., 2006).

There are different reports of IL-1α in white adipose tissue. There is IL-1α expression in white adipose tissue (Um et al., 2011). However, IL-1α protein is not found in white adipose tissue (Juge-Aubry et al., 2003). IL-1α expression was decreased in white adipose tissue in diet-induced obesity. White adipocytes are affected by IL-1α and secrete the interleukin-1 receptor antagonist (IL-1Ra) that has an anti-inflammatory effect (Juge-Aubry et al., 2003), which is increased with obesity (Um et al., 2011).

The function of IL-1α secreted from brown adipocyte is not understood. Little is known about IL-1α secretion in general. Brown and white adipose tissue secretion of IL-1α could contribute to the IL-1α levels in plasma or the IL-1α could have autocrine actions.

6.6 Interleukin-6

Interleukin-6 (IL-6) is produced by a variety of cell-types and has pleiotropic functions on e.g. B-cells, T-cells, blood vessels and heart (Hirano et al., 1990; Kishimoto et al., 1995). IL-6 plays a central role in the immune response and in the pathogenesis of several diseases. IL-6 is a pyrogenic cytokine and is therefore an important mediator of fever.

IL-6 signals through the IL-6 receptor (IL6R). As IL-6 has many functions, the IL-6 receptor is expressed on a variety of lymphoid and non-lymphoid cells.

Elevated plasma levels of IL-6 are often associated with obesity, BMI, stress and inflammation (reviewed in Yudkin et al., 2000)

In our signal-sequence trap, interleukin 6 was identified with a reasonably high frequency (Paper I). Interleukin 6 was, however, not studied further as it was never significantly affected in the microarray studies. In a study by Stanford et al. 2013, grafts of brown adipose tissue were surgically

transplanted into IL-6-knockout mice and the results strongly indicate that IL-6 derived from this transplant is important for glucose homeostasis and insulin sensitivity (Stanford et al., 2013). This study is, however, questionable and results should be further scrutinized. IL-6 expression and secretion is increased in brown adipocytes stimulated with norepinephrine in vitro (Burysek and Houstek, 1997). It is suggested that IL-6 secreted from brown adipose tissue could be secreted into the circulation to reach the thermoregulatory centre and stimulate fever (Cannon et al., 1998), although, the physiological relevance of IL-6 secreted from brown adipose tissue needs further attention. However, there are no effects on brown pre-adipocytes of chronic IL-6 treatment (Mracek et al., 2004).

IL-6 expression and secretion into plasma increases in response to muscular exercise (reviewed in Pedersen and Febbraio, 2008). Muscle IL-6 release is increased when glycogen in the muscle is low, suggesting that IL-6 may function as an energy sensor. Stimulation of muscle with IL-6 in vitro increases insulin-stimulated glucose uptake in (reviewed in Pedersen, 2012).

White adipocytes display IL-6 secretion into plasma in vivo and may represent an endocrine loop aimed to control energy homeostasis (Mohamed-Ali et al., 1997). IL-6 is also increased in white adipose tissue after acute stress, the function of this is not clarified yet (Speaker et al., 2013).

IL-6 is found in the plasma, but the origin of the secretion, as well as the target tissue, is hard to deduce. IL-6 secreted from brown adipose tissue might be of relevance in an endocrine manner, as well as the IL-6 secreted from white adipose tissue and skeletal muscle.

6.7 Leptin

Leptin is encoded by the ob gene and was identified by Friedmann and co-workers (1994) and is mostly expressed and produced by mature white

adipocytes. Physiologically, leptin is secreted to control energy homeostasis, repressing food intake, appetite and increasing energy expenditure. First it was thought that leptin mainly controlled appetite. However, leptin receptors have been detected in various tissues, suggesting that leptin has a wide spectrum of peripheral functions (Adamczak and Wiecek, 2013). Leptin is primarily expressed and secreted from adipocytes but it is also produced in muscle, gastric epithelium and placenta (Baile et al., 2000).

Leptin carries out its actions via the leptin receptor. The leptin receptor exists in several alternative splice variants where one is thought to be soluble (Baile et al., 2000). Leptin receptors are present in the hypothalamus and in a variety of peripheral tissues, such as adipose tissue (Siegrist-Kaiser et al., 1997), kidney, lung, ovaries, testis, heart and skeletal muscle (Baile et al., 2000).

The lack of functional leptin receptors (db/db) or leptin deficiency (ob/ob) in mice generates morbid obesity and type-2 diabetes (Tartaglia et al., 1995; Zhang et al., 1994).

Leptin is expressed and secreted from brown adipocytes; however, in brown adipocytes, leptin expression is increased in conditions of inactivity, i.e. is negatively regulated via β-receptors (Buyse et al., 2001; Deng et al., 1997) and is down-regulated in acute cold (Puerta et al., 2002). Both metabolic and molecular effects of leptin on brown adipose tissue function are reported, such as increase of insulin-stimulated glucose utilization and increase of expression of certain target genes such as lipoprotein lipase and leptin receptors (Siegrist-Kaiser et al., 1997), indicating a function in energy homeostasis.

There is leptin gene and protein expression in skeletal muscle (Wang et al., 1998; Wolsk et al., 2012). Cultured myocytes have been found to release leptin, and human leg skeletal muscle has documented leptin secretion. Human muscle leptin is strongly suppressed during adrenaline infusion

(Wolsk et al., 2012). Muscle-secreted leptin might have autocrine or paracrine effects but the secretory role of muscle leptin is under discussion.

White adipose tissue is the primary site of leptin expression and the tissue expresses and secretes leptin to control energy homeostasis in an endocrine manner. There are also direct effects of leptin on white adipocytes, such as stimulation of basal lipolysis and activation of target genes (Siegrist-Kaiser et al., 1997).

The physiological significance of brown adipocyte-secreted leptin is open for speculation. Brown adipocytes, like muscle, have decreased leptin expression during adrenergic stimulation, which might indicate their common heritage. The total mass of skeletal muscle in the body suggests that skeletal muscle could nonetheless contribute to whole body leptin production. Brown adipose tissue leptin secretion is lower than that from white adipose tissue, raising the question of an endocrine function of the secreted leptin. It might be that leptin is secreted from brown adipose tissue when brown adipocytes are fat-replete, which they are after extensive housing in thermoneutrality or following a high-fat diet. Leptin might then signal to reduce appetite and stimulate thermogenesis. When norepinephrine stimulates thermogenesis, fat stores are not replete and thus leptin secretion is decreased.

6.8 Retinol binding protein-4

The main function of the retinol binding protein-4 (RBP4) is to transport retinol (vitamin A) from the liver via the circulation, to peripheral tissues. RBP4 has been identified as an adipokine with suggested linkage to type 2 diabetes (Yang et al., 2005). RBP4 is mainly produced in the liver and in white adipose tissue, but liver is the main source of plasma RBP4 (Rosell et al., 2012).

RBP4 binds directly to cell-surface receptors, with a high affinity receptor called STRA6. Expression of the STRA6 receptor is found in various tissues such as brain, eye, kidney, muscle, brown and white adipose tissue (Zemany et al., 2014). Also a second RBP4 receptor is suggested (RBPR2) which is expressed primarily in liver and intestine but also in adipocytes (Alapatt et al., 2013).

RBP4 expression is high in white adipose tissue. Increased serum levels are measured in insulin resistance in mice and humans. Serum RBP4 levels can be decreased with insulin-sensitizing drugs (Yang et al., 2005).

In brown adipose tissue, RBP4 expression is induced in cold-acclimated mice. In primary brown adipocyte cell cultures, norepinephrine induces RBP4 expression and secretion (Rosell et al., 2012). RBP4 serum levels are decreased in cold-acclimated mice, as well as the liver expression of RBP4 (Rosell et al., 2012); this suggests that RBP4 secreted from brown adipose tissue has a minor endocrine role. Brown adipose tissue contain the STRA6 receptor, and in obese mouse model (ob/ob) expression is increased (Zemany et al., 2014).

There is RBP4 expression in skeletal muscle and skeletal muscle shows STRA6 receptor expression, which is increased in ob/ob mice (Zemany et al., 2014).

RBP4 expression is high in white adipose tissue and is induced in white adipocytes on norepinephrine treatment (Rosell et al., 2012). The receptor STRA6 is expressed in white adipocytes, and RBP4 is suggested to have a role in differentiation (Muenzner et al., 2013).

Adipose tissue stores vitamin A derivatives and is involved in the release of retinol. RBP4 is found in the plasma, and expression of RBP4 is increased in active brown adipose tissue. Even so, RBP4 secreted from brown adipose tissue is suggested to have a more local than endocrine role. RBP4 secreted from white adipose tissue is suggested to be autocrine. Skeletal muscle contains both RBP4 and the receptor STRA6 expression; however, no role is

suggested. I suggest RBP4 is secreted in an autocrine way from skeletal muscle.

6.9 Resistin

Resistin is a hormone that is expressed and secreted from adipocytes. It can be found in the circulation and is suggested to be involved in modulating metabolism and energy balance. It is suggested that resistin is involved in human inflammatory conditions, as circulating levels are reported in chronic inflammation and autoimmune disease (Al-Suhaimi and Shehzad, 2013). Resistin is expressed differently between species, where humans express resistin in a variety of tissues such as spleen, stomach, lung, spleen and bone marrow (Nohira et al., 2004; Patel et al., 2003). In mice, resistin is mainly found in adipose tissue (Steppan et al., 2001).

It is unclear what receptor resistin binds to, but some possible receptors have been proposed (Benomar et al., 2013; Onuma et al., 2013).

Resistin has been indicated to be a link between obesity, insulin resistance and inflammation. Resistin in serum is positively correlated with changes in BMI, and it is suggested that resistin plays an important role in obesity-related insulin resistance (Al-Suhaimi and Shehzad, 2013). It is, however, argued whether resistin is related to obesity or diabetes, and studies suggests that resistin is unlikely to play an endocrine role in insulin resistance (Lee et al., 2005; Nagaev and Smith, 2001).

In brown adipocytes, resistin gene expression is increased with differentiation. After day 7, when cells are differentiated, the gene expression drastically decreases (Viengchareun et al., 2002). There is resistin expression in brown adipose tissue, and expression is not affected by starvation (Nogueiras et al., 2003) or acute cold exposure (Puerta et al., 2002).

In humans, resistin is expressed in skeletal muscle (Patel et al., 2003; Steppan et al., 2001). Resistin is also expressed in rat skeletal muscle and in L6 muscle-cells (Adeghate, 2004). However, resistin is not expressed in mouse skeletal muscle (Steppan et al., 2001). There may be a role for resistin in skeletal muscle, as skeletal muscle is a major tissue to utilize glucose. It is suggested that resistin has an impact on glycometabolism. A recent study suggests that resistin rather suppresses myogenesis and stimulates myoblast proliferation and therefore impairs insulin sensitivity instead of previous suggestions of a direct role of resistin in insulin resistance (Sheng et al., 2013).

There is resistin expression and secretion from white adipose tissue. A recent report suggests that resistin in white adipocyte cell cultures may affect lipid metabolism in adipocyte differentiation (Ikeda et al., 2013). There is a greater amount of resistin expression in white adipose tissue than in brown adipose tissue (Nogueiras et al., 2003; Steppan et al., 2001). It is also suggested that resistin modulates glucose uptake and promote adipogenesis in vitro (Sanchez-Solana et al., 2012). The expression of resistin in white adipose tissue is decreased by starvation and it is only the expression in white adipose tissue that is affected (Nogueiras et al., 2003).

Resistin in brown adipocytes is differently hormonally regulated from white adipocytes (Viengchareun et al., 2002). One study using BAT-deficient mice saw increased circulating resistin with diet-induced obesity (Lee et al., 2005). This indicates that resistin secretion from brown adipose tissue is not irreplaceable and might only have minor endocrine effects. The major effects of brown adipocyte resistin might rather be autocrine and paracrine.

6.10 Triiodothyronine

Triiodothyronine, also known as T3, is a hormone that affects almost all physiological processes in the body. Thyroxine (T4) is the pro-hormone that is activated via two different deiodinases (1 or 2) and becomes T3. There is also a third deiodinase, that unlike the other deiodinases, produces an inactive T3 (Bianco and Kim, 2006). Expression of deiodinase type 2 is mainly found in brown adipose tissue, brain, placenta and pituitary glands (Bianco and Kim, 2006).

T3 acts primarily through specific isoforms of thyroid hormone nuclear receptors interacting with thyroid response elements (TREs). The nuclear thyroid receptors (TR) are encode by two genes, TRα and TRβ, and can be found in multiple isoforms (Brent, 2000); the main products are TRα1, TRα2, TRβ1 and TRβ2 (Brent, 1994). The different isoforms of the receptors are enriched at different levels in different tissues. TRα1, -α2 and –β1 are expressed in almost all tissues, TRβ2 is limited to the brain (Brent, 1994), TRβ1 is predominant in the liver and TRα1 is enriched in the heart but the heart expresses both receptors (Brent, 2000). TRα seems to be required for competent thermogenesis, and TRβ regulates cholesterol metabolism (Obregon, 2008).

Evidence suggests that deiodinase type 2 plays a role in the regulation of energy expenditure. Mice that lack deiodinase type 2 display significant metabolic consequences and gain more weight on a high-fat diet and become less responsive to insulin (Marsili et al., 2011).

Brown adipose tissue expresses the enzyme deiodinase type 2 that converts T4 to T3. Brown adipose tissue expresses more than one T3-receptor isoform (Hernandez and Obregon, 1996). TRα1 is predominant (Obregon, 2008) and actions of T3 are mainly through the TRβ1 isoform (Martinez de Mena et al., 2010). A T3-induced increase of UCP1 expression is TRβ-isoform specific (Ribeiro et al., 2010). In brown adipose tissue, T3 has a critical role in the synthesis of UCP1 during cold exposure (Bianco and

Silva, 1987; Bianco and Silva, 1988) and is required for a full thermogenic function (de Jesus et al., 2001). Cold exposure and norepinephrine increase deiodinase type 2 expression and activity through a synergistic α- and β-adrenergic pathway (Raasmaja and Larsen, 1989). The deiodinase activity can be blocked with α1-adrenergic antagonists but not with β-adrenergic antagonists (Silva and Larsen, 1983). The locally produced T3 binds to and activates the nuclear thyroid receptors to produce the adrenergic induction of UCP1 and lipogenesis (Christoffolete et al., 2004). Transgenic animals with inactivated deiodinase II exhibit impaired thermogenesis and a greater susceptibility to diet-induced obesity. The defect in thermogenesis derives from impaired brown adipose tissue development (Hall et al., 2010). Transgenic mice ablated of all nuclear hormone-binding thyroid receptors (TRα1(-/-)β(-/-) did not activate adrenergic thermogenesis normally (Golozoubova et al., 2004). This was shown to be due to adrenergic desensitization, rather than to an innate inability of brown fat or UCP1 to respond to stimulation. This indicates that the recruitment for thyroid hormone for brown adipose tissue recruitment is primarily to prevent non-liganded thyroid hormone receptors from functioning as suppressors of transcription. T3 from brown adipocytes might have autocrine actions, as well as endocrine functions. Theoretically, the T3 produced could be sufficient to affect the systemic level (Fernandez et al., 1987).

There is deiodinase type 2 expression and activity in skeletal muscle. However, there are different results concerning the activity of deiodinase type 2, where activity is reported to be so low that it is questionable if skeletal muscle is a source of plasma T3 (Grozovsky et al., 2009), and other reports that muscle can generate physiologically relevant amounts of T3 (Ramadan et al., 2011). Skeletal muscle is an important target for thyroid hormones, as muscle accounts for most variation in metabolic rate, and T3 regulates expression of a variety of genes (Clement et al., 2002; Visser et al.,

2009). However, the physiological relevance of these changes is not fully understood.

A recent study suggests that T3 treatment can induce brown-like adipocytes in white adipose tissue to increase UCP1 expression and mitochondrial biogenesis (Lee et al., 2012).

T3 has a profound effect in adipose tissue in regulating adipocyte differentiation, lipolysis and genes involved in lipid metabolism. White adipose tissue demonstrates expression of all thyroid hormone receptor isoforms (Obregon, 2008). A mutation in the TRα receptor signalling is associated with increased visceral adiposity due to reduction in lipolysis (Liu et al., 2003). Both deiodinase type 1 and type 2 are expressed in epididymal white adipose tissue. However, expression is less than 10% of that in brown adipose tissue. Deiodinase type 2 is regulated by thyroid status, and it is suggested that white adipose tissue has a role in regulating T3 bioactivity (Calvo and Obregon, 2011).

Brown adipose tissue secretes T3 in an endocrine manner as well as acting in an autocrine manner. Skeletal muscle and brown adipose tissue might have analoguous effects regulating energy balance, both tissues being responsible for energy expenditure. However, brown adipose tissue has higher expression and activity of deiodinase type 2 than both white adipose tissue and skeletal muscle, indicating a more pronounced role for brown fat in T3 production than the other two tissues.

6.11 ”anti-obesity factor”

The idea of a satiety factor or an “anti-obesity factor” arose from the fact that genetic ablation of brown adipose tissue generated obesity-prone mice (Lowell et al., 1993), while the UCP1-knockout mouse was apparently protected against diet-induced obesity (Enerback et al., 1997). It however,

later turned out that UCP1-knockout mice do become obese when housed at thermoneutrality (Feldmann et al., 2009).

However, there could still be some protein secreted from brown adipose tissue. For instance, in humans there is more brown adipose tissue in lean than in overweight subjects (Sacks and Symonds, 2013). This might indicate a unique factor only secreted from brown adipose tissue. There is also the fact that active brown adipose tissue actually exists in adult humans. The bare fact that it is not degenerated is an indication that brown adipose tissue could have other important functions than just produce heat. Although, to produce heat with brown adipose tissue could be enough when it comes to weight loss.

Table 2. The discussed potential secretory actions of the brown adipocyte-secreted factors and the manner that they possibly are secreted by, compared to white adipose tissue and skeletal muscle.

Factor	BAT	Muscle	WAT
adenosine	A	A	A
Prostaglandin	A	A	P
Adipsin	A	N	A
adrenomedullin	A	N	A
bFGF	A	A/P	A
chemerin	A	A/P	P/E
BMP8b	A	N	N
IGF-1	A	A	A
lipocalin 2	A	A	A
NPC2	A	N	A
NO	P	A	A
angiotensinogen	P	A/P	A
NGF	A/P	A/P	A/P
VEGF-A	P	P	P
VEGF-B	P	P	P
VEGF-C	P	P	P
LPL	P	P	P
FFA	E	E	E
heat	E	E	E
adiponectin	E	A	E
FGF21	A/P/E	A	A/P/E
IL-1α	E	N	A
IL-6	E	E	E
leptin	E	A/P	E
RBP4	E	A	A/E
resistin	E	A	A/E
T3	E	A	A
anti-obesity factor	N	N	N

A = autocrine
P = paracrine
E = endocrine
N = no data

7 Summary and conclusion

The field of secretomes has recently become augmented, and the subject of secreted factors from brown adipose tissue and their possible endocrine roles is under investigation (Villarroya et al., 2013). Classical brown adipocytes are nowadays recognized as being derived from precursor cells that can also generate skeletal muscle. A new type of adipose tissue i.e. the brite adipose tissue is also recognized. Skeletal muscle and white adipose tissue both display multiple functions and produce endocrine factors termed myokines and adipokines, respectively.

To evaluate the secretory role of brown adipose tissue, we isolated possible brown adipocyte secreted factors with a signal-sequence trap technique in paper I. The method is not without problems but gave us the ability to examine both known and novel genes with microarray studies. Studying gene expression in mice exposed to different physiological conditions and in primary brown adipocyte cell cultures gave us an indication of how the possible brown adipocyte secreted factors were regulated.

In papers III, IV and V, factors isolated from the signal-sequence trap were further analyzed. We discovered that chemerin gene expression is suppressed in cold acclimation but increased with high-caloric diet. The plasma levels of chemerin do not follow the same pattern as the gene expression in brown adipose tissue. Therefore, we suggest an autocrine and/or paracrine role for brown adipose tissue-secreted chemerin. It seems that norepinephrine does not control chemerin gene expression. Gene expression of adrenomedullin, collagen type 3 a1, lipocalin 2 and Niemann Pick type C2 was affected by cold and by high-caloric diet. However, also

for these factors, the gene expression does not seem to be under the control of norepinephrine.

In paper II, the molecular signatures of brown adipose tissue, skeletal muscle, brite- and white adipose tissue were examined to identify differences and similarities between the tissues. The results show that there is a specific gene expression profile between different depots, but three major types of tissues could be distinguished as the brown, brite and white adipose tissue.

In this thesis, I have reviewed all known brown adipose tissue-secreted factors. I have also briefly compared the secretion from brown adipose tissue to how the factors are expressed and secreted in skeletal muscle, brite- and white adipose tissue. After studying the literature of the secreted factors from brown adipose tissue, there are some points that I think need to be considered.

One important point is that secreted factors are studied both in vivo and in vitro. In vivo studies of brown adipose tissue includes brown adipocytes but also epithelial cells, migrated cells and nerves. Research in cell cultures is cleaner and there is mainly one cell type. However, when all natural external stimuli and surroundings from cells are removed, they might behave differently.

Another important point is that even if a given factor is expressed in tissue or culture, there is no evidence that the factor is translated into protein (Nedergaard and Cannon, 2013) and secreted. A secreted factor is suggested as secreted when reported as secreted. However, this does not take into account that from a particular cell-type or tissue, the factor could be secreted in a different manner. There are also complications with the target tissue. Sometimes receptors are unknown or equally distributed in the body. This makes it hard to deduce if a factor is secreted in an autocrine, paracrine or endocrine way. Another important question is the impact a factor from a specific tissue has. The suggested factor could be secreted into the circulation; however, the consequence of this factor could be minor. White

adipose tissue and skeletal muscle are two of the largest organs within the body and could therefore have a higher impact than e.g. brown adipose tissue. Brown adipose tissue is found in almost all mammals and is now generally thought to be present in adult humans, but compared to white adipose tissue and muscle, the size is substantially smaller. Of course, size is not everything, but it is good to bear the size in mind. Comparing the endocrine factors in the three tissues (Table. 2), brown adipose tissue rarely secretes a factor exclusively. The impact of the endocrine factor from brown adipose tissue is then questionable.

Looking at the known factors secreted from brown adipose tissue, the research field is not so large and much is yet to be discovered. Much more effort has been put into white adipose tissue and skeletal muscle, probably due to their size, availability and their impact on the human body and it was only recently that brown adipose tissue was recognized as an organ in adult humans.

Taken together, I have analyzed all known brown adipose tissue-secreted factors and tried to evaluate their effects and actions. I have compared the secreted factors in skeletal muscle, brite and white adipose tissue to get a further understanding in the similarities and differences between the tissues.

In conclusion, brown adipose tissue is not the largest organ in the body but has great capacity and still has unexplored actions. The fact that adult humans have brown adipose tissue demonstrates that the tissue might have other functions than neonatal thermogenesis. Our results give us four new brown adipose tissue-secreted factors and the understanding that there are probably more brown adipose tissue-secreted factors to be found. Brown adipose tissue is especially considered to have a role in energy homeostasis and is discussed as a target for obesity and co-morbidities.

8 Sammanfattning på svenska

Brunfett är en vävnad som omvandlar kemiskt bunden energi direkt till värme. Brunfett är alltså inte som vitt fett som främst används för att lagra energi. Den senaste tiden har det visat sig att brunfett, även om namnet indikerar annat, är besläktat med muskler. Studier har också visat att det även finns en tredje typ av fett, så kallat ”brite” (”britt”). Britt fett är besläktat med vitt fett men har vissa likheter med brunt fett och kan producera värme vid behov. I avhandlingens andra artikel (paper II) visar vi att dessa tre olika fett-typer, brunt, britt och vitt, har olika gen expression profil.

Det är numera allmänt vedertaget att både vitt fett och skelettmuskler har andra funktioner än lagring av energi respektive utförande rörelse. Både vitt fett och skelettmuskler utsöndrar faktorer som reglerar andra funktioner så som aptit och inflammation.

I artikel I utforskar vi faktorer eventuellt utsöndrade från brunt fett med metoden signal-sequence trap. Dessa faktorer analyserades sedan för att utröna hur genuttrycket regleras både i kroppen och i cellkulturer. Khemerin analyserades vidare i artikel III då uttrycket i brunfett var kraftigt reglerat av temperatur. Genen för khemerin i brunfett regleras av både temperatur och diet men i olika riktningar. Resultatet indikerar att khemerin från brunfett har en autoreglerande eller reglerar intilliggande celler.

Vidare analyserades adrenomedullin, khemerin, kollagen typ III a1, lipokalin 2 och Niemann Pick typ C2 i artiklarna IV och V. Resultatet indikerar att generna är reglerade utav både temperatur och diet i brunfett men inte noradrenalin utan via någon annan faktor.

I avhandlingen analyseras alla hittills kända brunfettsutsöndrade faktorer och även huruvida de finns och utsöndras från skelettmuskler och vitt fett.

Sammanfattningsvis har jag analyserat tidigare kända samt identifierat nya faktorer som utsöndras från brunt fett. Forskningen om brunfett ökar kontinuerligt, särskilt nu när det lokaliserats hos vuxna människor. Tankar kring brunfett som en behandling av fetma och följdsjukdomar är numera aktuellt. Hittills har dock ingen unik faktor identifierats.

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