Role of Adipocyte SLC7A10 and Amino Acid Metabolism in Obesity and Insulin Resistance

Regine Åsen Jersin

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Abbreviations

3-HIB	3-hydroxyisobutyric acid
Asc-1	Alanine serine cysteine transporter 1
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BCAA	Branched chain amino acid
BMI	Body mass index
BMS	BMS-466442
CAT	Cationic amino acid transporter
CoA	Coenzyme A
CVD	Cardiovascular disease
DG	Dysglycemic
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
FA	Fatty acid
FFA	Free fatty acids
FTO	Fat mass and obesity-associated
GLUT-4	Glucose transporter type 4
GO	Gene ontology
GSEA	Gene set enrichment analysis
GSH	Glutathione
HAT	Heterodimeric amino acid transporter
HDL	high density lipoprotein
HIBCH	Hydroxyisobutyryl-CoA hydrolase
HOMA-IR	Homeostasis model assessment-estimated IR
IR	Insulin resistance
KD	Knockdown
KLF14	Krüppel-like Factor 14
LPS	Lipopolysaccharide
LU	Lu AE00527
MMA	methylmalonyl-CoA
MMS	methylmalonate semialdehyde
mRNA	Messenger ribonucleic acid
mRNA	Messenger RNA
MSA	Multiple sequence alignment
NADPH	Nicotinamide adenine dinucleotide phosphate
NAFDL	Non-alcoholic fatty liver disease
NG	Normoglycemic

NMDA	N-methyl-D-aspartate
OCR	Oxygen consumption rate
OM	Omental
ORO	Oil Red O
PEP	Phosphoenolpyruvate
PPARγ	Peroxisome proliferator-activated receptor-γ
RNA-seq	Ribonucleic acid-sequencing
ROS	Reactive oxygen species
SAT	Subcutaneous adipose tissue
SC	Subcutaneous
SD	Standard deviation
SEM	Standard error of the mean
siRNA	Small interfering RNA
SLC7A10	Solute carrier Family 7 Member 10
SNP	Single nucleotide polymorphism
SSP	Secondary structure predictions
SVF	Stromal vascular fraction
T2D	Type 2 diabetes
TAG	Triacylglycerol
TCA	Tricarboxylic acid
TF	Transcription factor
UCP-1	Uncoupling protein 1
VAT	Visceral adipose tissue
WAT	White adipose tissue
WHO	World health organization
WHR	Waist-hip ratio
WT	Wild type
α-KG	α-Ketoglutarate

Scientific environment

This thesis was conducted in the period from September 2017 to October 2020 at the Department of Clinical Science, Faculty of Medicine, University of Bergen (UiB) and the larger Hormone Laboratory research group at UiB and Haukeland University Hospital (HUS). My main supervisor was Associate Professor/Research Scientist Simon E. Nitter Dankel at the Department of Clinical Science. My co-supervisors were Associate Professor Melina Claussnitzer at the University of Hohenheim, Stuttgart, Germany and the Broad Institute of MIT and Harvard, Boston, USA, and Professor dr. med. Gunnar Mellgren at the University of Bergen and HUS. Parts of the study were performed in collaboration with Prof. Lise Madsen and colleagues at the Institute of Marine Research (the former National Institute of Nutrition and Seafood Research (NIFES)).

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Regine Åsen Jersin

Abstract

Obesity is a rising global concern, associated with increased risk of developing metabolic syndrome, insulin resistance and type 2 diabetes. Chronic availability of excess nutrients may promote adipocyte dysfunction, especially in genetically susceptible individuals. Adipocyte amino acid transporter SLC7A10 and branched chain amino acid (BCAA) metabolism has recently been strongly associated with obesity and related disease, and dissection of the underlying molecular mechanisms may provide novel therapeutic targets.

The aim of the present study was to uncover novel mechanisms linking adipocyte SLC7A10, BCAA uptake and catabolism, and 3-hydroxyisobutyric acid (3-HIB) release to adiposity, insulin resistance and T2D.

In Paper 1, we performed a global transcriptome screen of adipose tissue in the context of obesity and insulin resistance, and identified SLC7A10 as a novel candidate gene in the regulation of adipocyte metabolism. We overfed Slc7a10 WT and loss-of-function mutant zebrafish, resulting in visceral adipocyte hypertrophy and higher weight gain for the mutants. Inhibition of SLC7A10 in adipocytes *in vitro* caused reduced serine uptake, total glutathione levels, insulin-dependent glucose uptake and mitochondrial respiratory capacity, while promoting increased ROS generation and lipid accumulation. Conversely, SLC7A10 overexpression showed the opposite effects on mitochondrial respiration and ROS compared to inhibition.

In Paper 2, we found that BCAA consumption and catabolism is increased during adipogenesis of human as well as brown and white mouse adipocytes *in vitro*. Knockdown of the 3-HIB generating enzyme HIBCH reduced adipocyte lipid storage and 3-HIB release. 3-HIB treatment affected respiration and ROS generation in opposite directions in white and brown mouse adipocytes.

In Paper 3, we performed a gene set enrichment analysis (GSEA) of RNA-sequencing data from SLC7A10 impaired human primary adipocytes, and found increased expression of genes related to lipid metabolism, TCA cycle and amino acid catabolism.

Upon SLC7A10 inhibition, adipocytes showed increased uptake of BCAAs, aspartate, glutamate, and increased efflux of 3-HIB.

Overall, we have identified SLC7A10 and 3-HIB as regulators of adipocyte metabolism. Our work points to activation of adipocyte SLC7A10 as a potential preventive and/or therapeutic strategy for metabolic diseases, and implicates 3-HIB as a strong marker reflective of increased lipid accumulation, obesity and comorbidities.

List of publications

Paper I

Regine Å. Jersin, Divya Sri Priyanka Tallapragada, André Madsen, Linn Skartveit, Even Fjære, Adrian McCann, Laurence Dyer, Aron Willems, Jan-Inge Bjune, Mona S. Bjune, Villy Våge, Hans Jørgen Nielsen, Håvard Luong Thorsen, Bjørn Gunnar Nedrebø, Christian Busch, Vidar M. Steen, Matthias Blüher, Peter Jacobson, Per-Arne Svensson, Johan Fernø, Mikael Rydén, Peter Arner, Ottar Nygård, Melina Claussnitzer, Ståle Ellingsen, Lise Madsen, Jørn V. Sagen, Gunnar Mellgren, Simon N. Dankel. Role of the neutral amino acid transporter SLC7A10 in adipocyte lipid storage, obesity and insulin resistance. **Manuscript (revised resubmission).**

Paper II

Mona S. Bjune, **Regine Å. Jersin**, Arve Ulvik, André Madsen, Adrian McCann, Per-Arne Svensson, Maria K. Svensson, Bjørn G. Nedrebø, Oddrun A. Gudbrandsen, Grethe S. Tell, C. R. Kahn, Per M. Ueland, Gunnar Mellgren, Simon N. Dankel. 3-Hydroxyisobutyrate, a strong marker of insulin resistance in type 2 diabetes and obesity that modulates white and brown adipocyte metabolism. **Diabetes 2020;69:1903-1916.**

Paper III

Regine Å. Jersin*, Divya Sri Priyanka Tallapragada *, Linn Skartveit, Sindre Lee-Ødegård, Christian Drevon, Adrian McCann, Melina Claussnitzer, Gunnar Mellgren, Simon N. Dankel. Impaired adipocyte SLC7A10 function promotes lipid storage in association with altered BCAA metabolism and TCA cycle activity in insulin resistance. **Manuscript to be submitted.**

*contributed equally

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1. Introduction

1.1 Overweight and obesity

1.1.1 Definition, classification, and prevalence of obesity

Obesity is a rising health challenge worldwide and the prevalence has nearly tripled in the last 45 years, reaching pandemic levels. In 2016, more than 1.9 billion adults were defined as having overweight and 650 million of these as having obesity, which corresponds to 39% and 13% of the world's adult population respectively (WHO, 2017). Obesity is also a major concern in Norway, and an ongoing Norwegian study named HUNT (Helseundersøkelsen i Nord-Trøndelag) found that the percentage of people with obesity in the county of Nord-Trøndelag increased from around 10% in 1986 to 21-23% in 2006 and 22.5-23.5% in 2018 (Krokstad *et al.*, 2013; *HUNT4 - NTNU*, 2019). The World Health Organization (WHO) defines overweight and obesity as anomalous or excessive fat storage that could negatively affect health (WHO, 2017). Body mass index (BMI), which is calculated by a person's body weight in kilograms divided by height in meters squared (kg/m^2), is the most common anthropometric measure used to classify both overweight and obesity. A person is considered as having overweight with a BMI ≥ 25, obesity with a BMI ≥ 30 (Garvey, 2018), and morbid obesity with a BMI ≥ 40 or ≥ 35 with at least one obesity-related disease/comorbidity (Heymsfield and Wadden, 2017).

Despite being the most widely used measure of obesity, BMI has several disadvantages, such as failing to consider ethnicity, gender, lean mass and body fat distribution (Jackson *et al.*, 2002; Nishida *et al.*, 2004; Cornier *et al.*, 2011). Therefore, other measures of obesity are also used, including waist-hip ratio (WHR), waist circumference (WC) and waist-height ratio, which are shown good, or better, predictors of obesity-related disease risk compared to BMI (Lam *et al.*, 2015).

1.1.2 Metabolic effects of obesity

In the context of obesity, several organs such as the adipose tissue, brain, gut, liver, muscles, heart and pancreas can be strongly affected by an imbalance in energy homeostasis. Consequently, their functions can be altered, raising the risk of disease and severe complications (Kim, 2016). Since the complete picture is complex and there are large variations between individuals, several mechanisms of disease development have been proposed over the years, with suggested dysfunctions in one or several of the tissues as primary causal events (Czech, 2020). Adipose tissue, the primary tissue responsible for storing excess energy as fat, is greatly expanded in obesity (Knight, 2018; Adami *et al.*, 2019). This may lead to dysfunctional adipocytes (See *1.2.3. Adipocyte dysfunction*), and at first, increased sensitivity to insulin, the hormone regulating glucose uptake from the blood (Mehran *et al.*, 2012). Likely, as a protective mechanism, local insulin resistance (IR) in adipocytes eventually occurs, which can further promote elevated circulating glucose levels (Ferrannini *et al.*, 2018; Czech, 2020). The reduced insulin response leads to increased lipolysis in adipose tissue, causing high circulating free fatty acid (FFA) levels, contributing to whole-body IR (Lusis, Attie and Reue, 2008). The brain reacts to the altered metabolic conditions by signaling to non-adipose tissues, affecting their metabolic functions and resulting in excess energy storage. Hormonal signals directly from the adipose tissue, such as leptin, serve to inform the brain on the status of lipid storage in adipose tissue (Das, 2010). Fat storage in tissues other than adipose tissue is referred to as ectopic lipid accumulation, and includes abnormal fat storage in the heart, skeletal muscle, pancreas and liver (Lusis, Attie and Reue, 2008). The latter might lead to development of diseases, such as non-alcoholic fatty liver disease (steatosis) (Sarwar, Pierce and Koppe, 2018). After a while, the normal function of these tissues also becomes impaired, and to combat these dysfunctions, local IR occurs also here (Perry *et al.*, 2015). Consequently, the pancreas increases the secretion of insulin, which initially causes a state of hyperinsulinemia. Eventually, as the beta cells that produce this peptide hormone no longer sufficiently compensate for IR, circulating glucose levels will be chronically elevated (hyperglycemia), which can result in type 2 diabetes (T2D) due to excessive pancreatic lipid storage and diminished insulin production (Gastaldelli, 2011; Kolb *et al.*, 2018).

Overall, impairments of important pathways and functions in all the metabolically active tissues may contribute to alterations in glucose homeostasis and disease development (Remedi and Nichols, 2009; Cersosimo *et al.*, 2015; Fazakerley *et al.*, 2019). However, in the present thesis, the role of the adipose tissue in relation to obesity and comorbidities will be in focus.

1.1.3 Metabolic syndrome and obesity-related diseases

High fasting blood sugar is one of the five risk factors characterizing metabolic syndrome. The other four are abdominal obesity, high blood pressure, low high density lipoprotein (HDL) and high triacylglycerol (TAG) levels in the blood (Lusis, Attie and Reue, 2008). To be diagnosed with metabolic syndrome, three out of the five risk factors must be present (Eckel, Grundy and Zimmet, 2005). Having metabolic syndrome is associated with a higher risk of developing T2D, which is characterized by IR in target tissues and insulin deficiency due to beta cell dysfunction (Association, 2017; Chatterjee, Khunti and Davies, 2017). A study of 433 people with morbid obesity found that around 23% had impaired glycemic control or T2D, while 39.5% had metabolic syndrome and 72% were IR (Janković *et al.*, 2012). In the study, an oral glucose tolerance test based index (clamp-like index (CLIX)) test was used, and IR was defined as CLIX ≤5 (Janković *et al.*, 2012). Studies often use homeostasis model assessment-estimated IR (HOMA-IR) to identify IR (Matthews *et al.*, 1985), but the cutoff values for it to be classified as IR often vary between different studies (Qu *et al.*, 2011). The gold standard for assessing IR *in vivo* is hyperinsulinemic-euglycemic clamp, where insulin levels are maintained constant by intravenous infusions of insulin (Kellar and Craft, 2020). Glucose is then infused, providing steady-state euglycemia, and the concentration of glucose taken up by all the tissues in the body can be calculated (Tam *et al.*, 2012). Progressive IR is strong a predictor of future T2D (Taylor, 2012). Other comorbidities linked to obesity include cardiovascular disease (CVD) and many types of cancers (Haslam and James, 2005; Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration (BMI Mediated Effects) *et al.*, 2014).

1.1.4 Treatment options for obesity

Lifestyle interventions are often used to treat obesity and comorbidities, and can in some cases lead to stable weight loss and a reduction in risk factors for CVD (Unick *et al.*, 2013; Chen *et al.*, 2015). However, studies often show a poor long-term subject adherence to dramatic lifestyle interventions, and bariatric surgery is currently considered the most effective way of treating morbid obesity and related diseases (Wing *et al.*, 1998; Courcoulas *et al.*, 2020). Bariatric surgery causes striking alterations in metabolism, resulting in a sustained weight loss, and often remission of T2D and decreased mortality (Sjöström *et al.*, 2007). Despite these advantages, bariatric surgery is very invasive, expensive and might cause negative psychological and physical side-effects, such as suicidal thoughts, alcoholism and micronutrient deficiency (Courcoulas *et al.*, 2014; Kratz, 2020). Hence, this surgery is only available for people with morbid obesity who meet specific criteria, most commonly a BMI above 40, or above 35 accompanied with significant morbidity (Sjöholm *et al.*, 2013). Therefore, more knowledge on the specific molecular mechanisms involved in development of obesity and related diseases is needed to develop new and better treatment options.

1.1.5 Heritability, lifestyle and epigenetics

The primary cause of obesity is an imbalance between the total energy consumed and expended (WHO, 2017), but individual environmental and genetic factors regulate, through complex mechanisms and pathways, how the body tackles the intake of surplus calories (Haslam and James, 2005). Genetics can influence obesity either by a single mutation (monogenic obesity) or by an interplay between several loci (polygenic obesity), the latter being the most common genetic contributor to obesity and hence referred to as common (multifactorial) obesity (Heymsfield and Wadden, 2017; Rohde *et al.*, 2019). Interestingly, the heritability value (h^2) for body weight was found to be 0.78-0.81 in a study of both mono- and di-zygotic twins (Stunkard, Foch and Hrubec, 1986). h^2 takes into account several genetic values, and describes the proportion of genetic variation based on these (Naomi R. Wray, 2008). In another twin study where the siblings were separated by birth, the h^2 for BMI was estimated to be 0.66 for women

and 0.70 for men (Stunkard *et al.*, 1990). Additionally, Rankinen and colleagues found that 25-70% of the variations in BMI and total body fat composition could be explained by genetics (Rankinen *et al.*, 2015). Moreover, research has identified over 870 single nucleotide polymorphisms (SNPs) that are strongly linked with BMI (Rohde *et al.*, 2019), and a study found that the heritability explained by common SNPs for BMI and WHR was 41% and 46%, respectively (Vattikuti, Guo and Chow, 2012). Further insight into the molecular mechanisms underlying this genetic contribution may enable us to predict an individual's risk of obesity and related diseases, and to develop new prevention and treatment strategies.

However, making the system even more complex, is the research showing that genetic risk can be strongly influenced by an obesogenic environment (Swinburn *et al.*, 2011). Therefore, a healthy lifestyle can prevent the development of obesity and related diseases, even if you are genetically predisposed for obesity (Hunter, 2005). WHO reports that there has in recent years been an increase in the intake of energy-dense foods and a decrease in physical activity, causing reduced expenditure of the excess calories consumed, thereby driving increased obesity (WHO, 2017). The combination of genetic predisposition and ready access to highly processed foods that can both promote increased appetite (Locke *et al.*, 2015; Hall *et al.*, 2019) may severely challenge people to maintain a healthy body composition. Other lifestyle factors that are suggested to be linked with risk of obesity and related diseases are sleeping behaviors, socioeconomic status, smoking, and suboptimal intake of vitamins (Ló Pez-Sobaler *et al.*, 2016; Justice *et al.*, 2017; Wulaningsih *et al.*, 2017). Recently, it has also been suggested that the influence of environmental factors on genetic predisposition might, at least in part, be mediated through epigenetic mechanisms in several organs, including the adipose tissue (Rohde *et al.*, 2019).

1.2 The Adipose tissue is more than just a fat storing unit

1.2.1 Adipose tissue functions and cellular composition

Adipose tissue was previously considered to be a passive tissue, with the sole purpose of storing energy surplus as TAG in lipid droplets. Now, adipose tissue is known to be an important endocrine organ, with complex and dynamic functions, ranging from protecting delicate organs (i.e., the eye), streamlining of aquatic mammals, providing insulation, and not least to regulate whole-body energy homeostasis (Pond, 1992; Rosen *et al.*, 2014). When communicating with other metabolic organs, adipose tissue secretes cytokines, known as adipokines, existing in vast numbers with diverse functions (Deng and Scherer, 2010). Leptin and adiponectin were the first signaling proteins discovered to be secreted from adipose tissue (Zhang *et al.*, 1994; Scherer *et al.*, 1995). Adiponectin is known to decrease inflammation in several tissues and to reduce the release of glucose from the liver, while leptin suppresses hunger by binding the leptin receptor in the brain and increases the breakdown of TAG in adipocytes (Ghaben and Scherer, 2019).

A variety of different cell types make up the adipose tissue, including immune cells such as macrophages and T cells, and preadipocytes and mature adipocytes (Hellman, Larsson and Westman, 1963; Schoettl, Fischer and Ussar, 2018). Based on the characteristics of the adipocytes residing in the tissue, the adipose tissue can be divided into three different primary groups, namely white, brown, and beige adipose tissue. White adipose tissue (WAT) is often thought of as the lipid storing tissue, consisting of unilocular cells with a single large lipid droplet and a relatively low abundance of mitochondria. By contrast, brown adipose tissue (BAT) is composed of multilocular cells with several smaller lipid droplets and a high mitochondrial content. Hence, BAT is a specialized energy-dissipating adipose tissue which converts chemical energy to heat in a process called non-shivering thermogenesis (Ghaben and Scherer, 2019). β-adrenergic stimulation activates this process, which is driven by a mitochondrial protein called uncoupling protein 1 (UCP-1). UCP-1 uncouples the proton motive force of the respiratory chain, localized in the inner mitochondrial membrane, causing a leak of protons into mitochondrial matrix (Klingenspor *et al.*, 2008). Further, this leads to the production of heat instead of adenosine triphosphate (ATP) (Rosen and Spiegelman,

2014). The other type of fat cell that express appreciable levels of UCP-1 are beige adipocytes. These cells are interspersed among the white adipocytes and have characteristics that resemble both the white and brown fat cells. When stimulated by exercise, chronic cold exposure or β-adrenergic receptor agonists such as isoproterenol (ISO), UCP-1 expression, and thereby thermogenesis, is initiated in the beige cells in a process often called beiging (Ikeda, Maretich and Kajimura, 2018). However, recent lineage tracing has contributed to development of an even more complex picture of heterogeneity, supporting the growing evidence for distinct adipocyte subpopulations existing in the different adipose tissue depots (Schoettl, Fischer and Ussar, 2018; Lee *et al.*, 2019; Ramirez *et al.*, 2020; Vijay *et al.*, 2020).

1.2.2 Adipose tissue depots and expansion

Body fat can be stored in several adipose tissue depots in the human body, largely divided into subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (Lee, Wu and Fried, 2013). SAT is located directly below the skin, and is in humans divided into different compartments including abdominal, retroperitoneal, gluteal and femoral SAT (Schoettl, Fischer and Ussar, 2018; Kahn, Wang and Lee, 2019) (**Figure 1**). Around 80% of all human body fat is stored in subcutaneous (SC) depots (Arner, 1997). In mice, the main SAT depots are retroperitoneal, inter- and sub-scapular, and posterior adipose tissue, the latter including inguinal, gluteal and dorso-lumbal WAT. Conversely, the VAT surrounds and shields the organs in the trunk, and is both in humans and rodents divided into subtypes such as pericardial, omental (OM) and mesenteric VAT (Kahn, Wang and Lee, 2019) (**Figure 1**). In most of the adipose depots, the main cell type is white adipocytes, but there are also some depots rich in BAT (Schoettl, Fischer and Ussar, 2018). Previously, human BAT was thought to only exists in the interscapular depot of infants but to be lost in adults. However, in 2009, independent studies reported that several adipose depots contain brown adipocytes in adults, such as those in the neck, supraclavicular and paravertebral regions (Cypess *et al.*, 2009; van Marken Lichtenbelt *et al.*, 2009; Virtanen *et al.*, 2009). Rodents have BAT in cervical, axillary and inter- and subscapular depots (*Adipose Tissue and Adipokines in Health and Disease*, 2007;

de Jong *et al.*, 2015) (**Figure 1**). On the contrary, zebrafish is thought to have no BAT, but adult fish have several anatomical sites containing WAT, including the cranium, esophagus, mandible, around the eyes, among the pancreatic cells, and SAT (the latter is illustrated in **Figure 1**). Zebrafish also have white adipocytes in VAT, which is for example located behind the swim bladder and by the visceral cavity (**Figure 1**) (Imrie and Sadler, 2010).

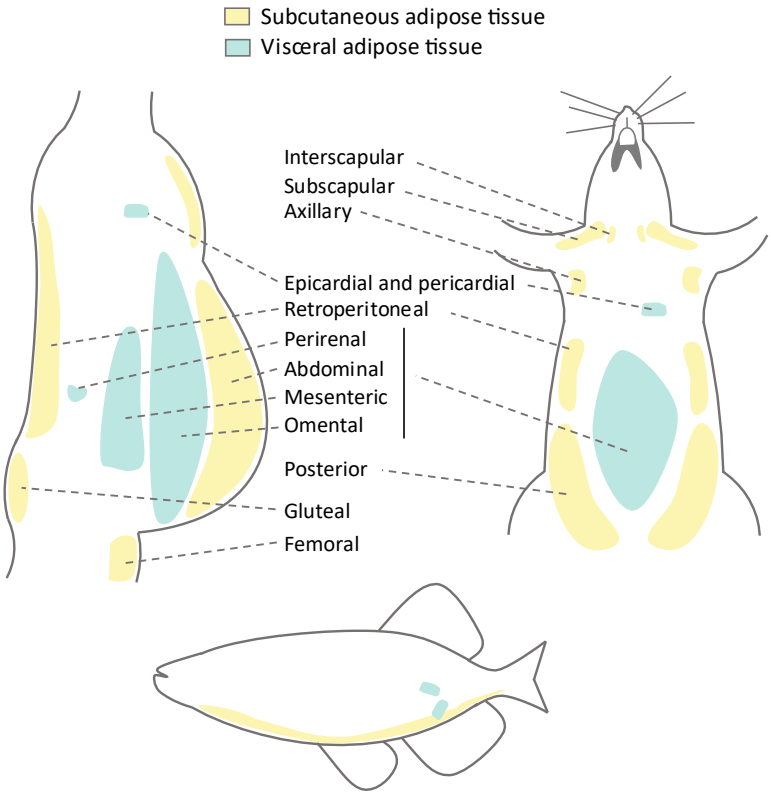

Figure 1. Adipose tissue depots in human, mouse and zebrafish. The location of several of the human and mouse adipose tissue depots are depicted in this figure, including epicardial and pericardial and retroperitoneal depots. In the human picture, perirenal, abdominal, mesenteric, and omental depot locations are all illustrated separately, while they are visualized together for the mouse. Gluteal and femoral location is depicted exclusively in the human figure, and interscapular, subscapular, axillary and posterior for mouse. Only the main zebrafish visceral and SC adipose tissues are visualized here. SAT depots are illustrated in yellow and visceral adipose tissue depots in green. This figure is based on (Imrie and Sadler, 2010), (Quail and Dannenberg, 2019) and (Schoettl, Fischer and Ussar, 2018).

For a long time now, it has been known that SAT and VAT distribution in humans is largely dependent on variables such as age and sex (Enzi *et al.*, 1986). Women typically have a higher fat storage in femoral and gluteal SAT than men, which is correlated with a healthier metabolic phenotype, compared to storing fat in the intra-abdominal region (Karastergiou *et al.*, 2012; Karpe and Pinnick, 2015). In men, VAT accounts for about 10-20% of the total fat mass, while it makes up only 5-8% in women (Wajchenberg, 2000). Moreover, VAT mass increases in both genders with increasing age (Ibrahim, 2010), concomitant with a higher risk of developing IR and metabolic diseases (Chen *et al.*, 2018).

How fat is stored in the different adipose depots might also affect disease risk. In conditions of excess nutrient availability, the adipose tissue is capable of expanding in two different ways, namely by hyperplasia or hypertrophy. Hyperplasia denotes when adipose tissue expands by increasing the number of adipocytes, and this process may represent a healthier way of storing surplus energy, compared to hypertrophy, which results from storing TAG in already existing adipocytes (Longo *et al.*, 2019). The final number of adipocytes may largely be decided during childhood and stays constant in both lean and obese adults, and 10% of fat cells are renewed each year (Spalding *et al.*, 2008). However, both obesity in early childhood and overfeeding later in life is associated with increased hyperplasia in specific adipose depots in adults (Tchoukalova *et al.*, 2010), and hyperplasia has been called a recovery mechanism responding to overnutrition (Longo *et al.*, 2019). Interestingly, studies in adult mice have shown that VAT has the ability to expand through both hyperplasia and hypertrophy, while SAT expands mainly through hypertrophy (Wang *et al.*, 2013; Kim *et al.*, 2014; Jeffery *et al.*, 2015). Hypertrophy is characterized by large lipid-laden adipocytes, which is found to be a strong determinant of metabolic disease risk (Kim *et al.*, 2014; Laforest *et al.*, 2015).

1.2.3 Adipocyte dysfunction

When adipocytes increase rapidly in size due to a high energy storing demand in obesity, the extracellular matrix (ECM), providing the structural foundation of the adipose tissue, is remodeled to support the adipose tissue's growing mass (Mariman and Wang, 2010). This remodeling is associated with several alterations in adipocyte metabolism (Ghaben and Scherer, 2019). For instance, hypertrophy may increase mechanical stress on the adipocytes, causing fibrosis and hypoxia, the latter due to reduced oxygen availability for each adipocyte in the growing adipose tissue (Halberg *et al.*, 2009; Khan *et al.*, 2009). These stressors might also increase lipogenesis and induce inflammation in the adipose tissue, further causing cellular changes leading to secretion of inflammatory signaling proteins such as tumor necrosis factor (TNF) or reduce production of anti-inflammatory adipokines, which are both events associated with IR (Klöting *et al.*, 2010; Ghaben and Scherer, 2019). In addition, the high demand to sequester lipids in the adipocytes may also cause mitochondrial dysfunction upon nutrient excess, and limited oxidative phosphorylation in murine fat cells has been described as a hallmark of obesity (Schöttl *et al.*, 2015). Adipocyte lipid accumulation is also linked to reactive oxygen species (ROS) levels, and while normal levels might regulate adipogenesis, too high or low ROS levels are thought to impair adipocyte function (Jankovic *et al.*, 2015; Ghaben and Scherer, 2019; Longo *et al.*, 2019).

1.2.4 Adipogenesis

When adipose tissue expands via hyperplasia, multipotent mesenchymal stem cells undergo adipogenesis to become mature adipocytes. This process of adipocyte differentiation, named adipogenesis, is divided into two steps. Most of the previous research is performed on the second phase *in vitro*, and we know little about the exact molecular mechanisms *in vivo* (Ghaben and Scherer, 2019). Briefly, the first phase is the commitment phase, where the fibroblast-like cell becomes restricted to the adipocyte fate without any changes in morphology. This is dependent on zinc-finger transcription proteins and bone morphogenic proteins (BMPs) that activate production of other transcription factors (TFs) (e.g. SMADs) needed to transcribe proteins important in the

second phase (Huang *et al.*, 2009; Gupta *et al.*, 2010; Quach *et al.*, 2011). Several pathways, such as the wingless-type mouse mammary tumor virus integration site family (WNT)/β-Catenin pathway, also need to be inhibited before the cells can commit to the adipocyte linage (Longo *et al.*, 2019). In the second step, the newly formed preadipocyte undergoes terminal differentiation. Firstly, SMAD4 activates transcription of the adipogenesis master regulator peroxisome proliferator-activated receptor-γ (PPARγ) (Ghaben and Scherer, 2019). Physiological ligands of this master TF with high affinity are not known, although several ligands with relatively low abundance or affinity are suggested, including long-chain polyunsaturated fatty acids (FAs) (Schupp and Lazar, 2010). There are several synthetic drugs called thiazolidinediones (TZDs) that are agonists of PPARγ, and they are used for activation both *in vitro* and *in vivo* (Rosen *et al.*, 1999). When activated, PPARγ induces expression of co-activator CCAAT/enhancer-binding protein-α (C/EBPα), which together with PPARγ trigger expression of other proteins important for the function of mature adipocytes, such as adipokines and proteins involved in insulin and lipid metabolism (Lefterova *et al.*, 2008).

1.2.5 Lipolysis, glucose uptake, lipogenesis and glyceroneogenesis

TAG molecules stored in lipid droplets in mature adipocytes are hydrolyzed into a glycerol molecule and three FAs when the body enters a state of fasting and thus needs energy to fuel vital functions. This process is called lipolysis. The glycerol and FFAs can then be released from the adipocytes and transported to other organs (e.g. myocytes) via the bloodstream, where FFAs can be broken down by β-oxidation and used to fuel the tricarboxylic acid (TCA) cycle in the mitochondria of liver or muscles (Yu and Ginsberg, 2005). Secreted lipolytic intermediates and products are also important in cellular signaling with other tissues (Zechner *et al.*, 2012). In addition, the FAs from adipocyte lipolysis can be converted to Acyl-Coenzyme A (CoA) in the fat cells, and enter β-oxidation in the mitochondria (Hankir and Klingenspor, 2018). β-oxidation however, mostly occurs in BAT to fuel thermogenesis, but it might also occur in WAT

during fasting or to induce beiging (Wang *et al.*, 2003; Lee, Ellis and Wolfgang, 2015; Gonzalez-Hurtado *et al.*, 2018).

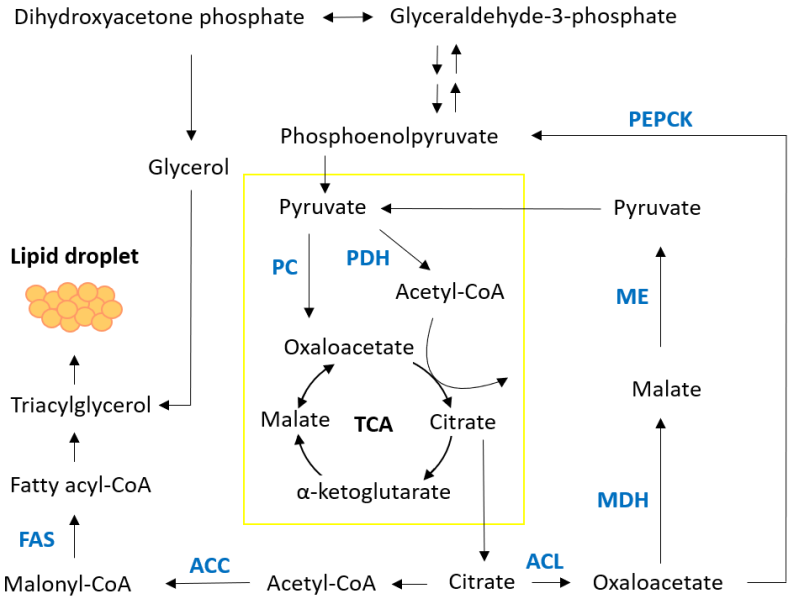

Figure 2. Basic overview of *de novo* lipogenesis, TCA cycle and glyceroneogenesis. Selected key enzymes are shown in blue/bold. The yellow square indicates processes in mitochondria. The figure is based on (Jitrapakdee, Vidal-Puig and Wallace, 2006).

Compared to BAT, WAT is more specialized towards storing excess nutrients as lipids to provide the body with energy when it needs it. This process is called lipogenesis, involving both de novo synthesis of FAs from precursors such as glucose and amino acids, and TAG synthesis where three FFAs are esterified to one molecule of glycerol-3-phosphate (G-3-P), forming one TAG molecule (Reshef *et al.*, 2003) (**Figure 2**). In adipocytes, FFA can be taken up from the circulation or be synthesized *de novo* from substrates such as glucose or branched chain amino acids (BCAAs) through acetyl-coenzyme A (Acetyl-CoA) and pyruvate (Reshef *et al.*, 2003; Crown, Marze and Antoniewicz, 2015; Green *et al.*, 2015; Ladeira *et al.*, 2016). G-3-P is either produced from glucose, or in the metabolic pathway glyceroneogenesis (Nye *et al.*, 2008). Glucose uptake in adipocytes is mostly insulin-dependent, which mechanistically means

that insulin is needed to induce the translocation of glucose trasporter-4 (GLUT4) from vesicles inside the adipocytes to the plasma membrane (Cushmans and Wardzalafj, 1980). Adipocyte GLUT4 is important to maintain whole-body glucose homeostasis (Abel *et al.*, 2001), and the glucose taken up into the adipocyte is used to fuel branches of glycolysis, the TCA cycle and pyruvate anaplerosis (Krycer *et al.*, 2017). The latter is a process where pyruvate is converted to oxaloacetate by pyruvate carboxylase (PC), and in adipocytes this enzyme is important for both the *de novo* FA synthesis and glyceroneogenesis (Jitrapakdee, Vidal-Puig and Wallace, 2006) (**Figure 2**). Another enzyme essential for glyceroneogenesis is PEPCK, which converts oxaloacetate to phosphoenolpyruvate (PEP) (**Figure 2**). Glyceroneogenesis has emerged as a major source of carbon for glycerol, and is now thought to be more important than glucose for this process in adipocytes (Nye, Hanson and Kalhan, 2008). The precursors that can be used to synthesize glycerol in this pathway are many, including TCA intermediates, pyruvate, lactate and certain amino acids (Ballard, Hanson and Leveille, 1967; Reshef, Niv and Shapiro, 1967; Reshef, Hanson and Ballard, 1969).

1.3 Alanine, serine, cysteine-transporter 1 (SLC7A10)

1.3.1 SLC7A10 in metabolic diseases and adipocytes

Several amino acid transporters have recently been implicated in the development of human disease (Kandasamy *et al.*, 2018). Among these is the small neutral amino acid transporter SLC7A10 (solute carrier family 7 member 10), also named Asc-1 (Alanine serine cysteine transporter 1), which has previously been associated to T2D risk (Small *et al.*, 2011). Small *et al.* studied a T2D-associated SNP by the *KLF14* gene, which appears to modulate the expression of this maternally expressed TF (Small *et al.*, 2011). This SNP was previously found to be associated with T2D risk (Voight *et al.*, 2010), and Small *et al.* suggested that KLF14 (Krüppel-like Factor 14) acts as a master *trans*-regulator of gene expression in SAT. Interestingly, in a cohort of 776 female twins, *SLC7A10* was the only gene that was down-regulated in SAT of people carrying this T2D diabetes associated risk allele, and showed the strongest heritable expression with

a h^2 of 0.79. In addition, SLC7A10 expression was found to correlate negatively with metabolic traits such as BMI, HOMA-IR and circulating TAG levels, and positively with circulating HDL and adiponectin levels (Small *et al.*, 2011). In 2018, Small and coworkers implicated *KLF14* T2D risk alleles in the regulation of female-specific body fat distribution and adipocyte size (Small *et al.*, 2018). Moreover, SLC7A10 has been found to serve as a cell surface marker of white adipocytes, with an expression ranging from 4-fold higher in mesenteric WAT than in interscapular BAT, to 79-fold higher in perigonadal WAT compared to subscapular BAT. In addition, the expression of SLC7A10 is 5-fold higher in WAT compared to any region of the brain, where this carrier has received a lot of attention due to its relatively high expression and its neutral amino acid transporting functions (Ussar *et al.*, 2014).

1.3.2 SLC7A10 transports small neutral amino acids

SLC7A10 is a sodium-independent amino acid transporter, localized in the plasma membrane, carrying small neutral amino acids such as serine, glycine, alanine, threonine, and cysteine. The transporter can transport both the L- and D-stereoisomers of these amino acids (Fukasawa *et al.*, 2000), but exhibits a higher affinity for the glycine, L-serine, D-serine and D-alanine (Nakauchi *et al.*, 2000). Moreover, SLC7A10 is a member of the SLC7 family of amino acid carriers, which consists of two subgroups, namely the cationic amino acid transporters (CATs) and the heterodimeric amino acid transporters (HATs) (Verrey *et al.*, 2004). Since SLC7A10 is linked to the glycoprotein (heavy chain) 4F2hc (SLC3A2) through a highly conserved disulphide bridge, which is required for the carrier to function, it is defined as a HAT (Fukasawa *et al.*, 2000; Nakauchi *et al.*, 2000). A trait that makes SLC7A10 unique among amino acid transporters, is its two different mechanisms of action. The main mechanism is exchange mode, where the carrier mediates bidirectional transport of amino acids coupled with transport of small neutral amino acids in the other direction, making it an antiporter (Fukasawa *et al.*, 2000). In addition, amino acids can be transported via facilitated diffusion, which is a form of passive transport, where small neutral amino acids

spontaneously move along their concentration gradient through SLC7A10 (Fukasawa *et al.*, 2000; Pineda *et al.*, 2004).

Glycine and D-serine have particularly received attention in studies of SLC7A10's roles in the brain, due to their functions as positive allosteric modulators of a variant of glutamate receptors entitled N-methyl-D-aspartate (NMDA) receptor (Rutter *et al.*, 2007). The NMDA receptor is linked to memory loss and to various disorders such as Alzheimer and schizophrenia, and SLC7A10 function in the brain has therefore been the focus of investigation (Fukasawa *et al.*, 2000; Helboe *et al.*, 2003; Matsuo *et al.*, 2004; Pineda *et al.*, 2004; Xie *et al.*, 2005; Burnet *et al.*, 2008; Rosenberg *et al.*, 2013; Safory *et al.*, 2015; Ehmsen *et al.*, 2016; Billard and Freret, 2018; Mesuret *et al.*, 2018). Hence, several inhibitors of SLC7A10 have been developed over the last 20 years, including ACPP (Sakimura *et al.*, 2016), BMS-466442 (BMS) (Brown *et al.*, 2014) and Lu AE00527 (LU) (Sason *et al.*, 2016), and their mechanisms and specificity have been thoroughly examined (Kutchukian *et al.*, 2017; Torrecillas *et al.*, 2019; Mikou *et al.*, 2020). Previous studies in non-adipose tissue have shown that SLC7A10 can transport both D- and L-serine, but directionality of the transport has been uncertain (Matsuo *et al.*, 2004; Rutter *et al.*, 2007; Safory *et al.*, 2015; Sakimura *et al.*, 2016; Sason *et al.*, 2016). Indeed, to this date, the preferred direction of amino acid transport through SLC7A10 in the brain *in vivo* is not yet resolved (Mikou *et al.*, 2020), and to our knowledge nothing was known about this transporter's direct and indirect functions in adipose tissue before the present project.

1.4 Amino Acids: Protein building blocks and beyond

1.4.1 Amino acid properties and functions

Amino acids consist of an amino group and a carboxyl group, and there are 20 different proteogenic naturally occurring amino acids, divided into essential and non-essential (Wu, 2013). The essential amino acids are only available in food, while the non-essential can be produced in the body from other amino acids or metabolites (Salazar, Keusgen and von Hagen, 2016). Previously, amino acids were only thought of as basic building

blocks used in protein translation. However, we now know that amino acids are also important for processes such as lipid metabolism, purine synthesis, biosynthesis of biomolecules and fueling of energy metabolism (Wu, 2010; Saha *et al.*, 2014).

Different amino acids have different entry points into the metabolic pathways. For example, aspartate and asparagine can be converted to oxaloacetate, a central intermediate of the TCA cycle, while the end products of BCAA degradation can be both propionyl-CoA and acetyl-CoA. Alanine, cysteine, threonine, tryptophan, glycine and serine are precursors of pyruvate, and glutamine, proline, arginine and histidine can all be converted to glutamate, which is a direct precursor of the TCA intermediate α-ketoglutarate (α-KG) (Berg, Tymoczko and Stryer, 2002). In addition to fueling metabolism, a recent study found that amino acids are more important than glucose for providing carbon for cell proliferation in mammalian cells (Hosios *et al.*, 2016). Due to the amino acids' many important cellular functions, their circulating levels and metabolic potential in various tissues have been linked to various diseases, including obesity and comorbidities (Felig, Marliss and Cahill, 1969; Drábková *et al.*, 2014; Martínez *et al.*, 2017; Gar *et al.*, 2018; Nie *et al.*, 2018).

1.4.2 One-carbon metabolism and disease

Both serine and glycine, which are known to have a high affinity for the amino acid transporter SLC7A10 in the brain ((Fukasawa *et al.*, 2000; Nakauchi *et al.*, 2000)), have central roles in one-carbon metabolism, where carbon units are circulated through the folate and methionine cycle (Matsuo *et al.*, 2004; Yang and Vousden, 2016). These cycles regulate several pathways and mechanisms, through biosynthesis of molecules such as lipids, nucleotides, proteins and substrates for methylation reactions, and through regeneration of functional metabolites important for maintenance of biological processes (Locasale, 2013; Maddocks *et al.*, 2016).

Closely coupled to the methionine cycle is the transsulfuration pathway, where serine, glycine and cysteine serve as key precursor for glutathione (GSH), the body's major redox-regulating molecule (Newman and Maddocks, 2017b). In addition, through one-

carbon metabolism, serine is particularly important for cellular respiration in mammalian cells (Lucas *et al.*, 2018), generation of ATP, and lipid metabolism (Vazquez, Markert and Oltvai, 2011; Tedeschi *et al.*, 2013; Gao *et al.*, 2018). Due to its regulatory function in many metabolic pathways and processes, altered or dysregulated one-carbon metabolism is implicated in several diseases such as CVD, Alzheimer and cancer (Ducker and Rabinowitz, 2017). Furthermore, serine consumption and metabolism has been implicated in the development of cancer, in part because of its role in fueling cell proliferation (Labuschagne *et al.*, 2014; Newman and Maddocks, 2017a), and has also been linked to non-alcoholic fatty liver disease (Mardinoglu *et al.*, 2014).

1.4.3 BCAAs in obesity, insulin resistance disease and adipocyte metabolism

The BCAAs, leucine, valine and isoleucine, have been at the center of attention in the past several years, partly due to observations that high BCAA plasma levels show a strong positive association with obesity and IR (Felig, Marliss and Cahill, 1969; McCormack *et al.*, 2013). In addition, there is now emerging evidence implicating defects in BCAA catabolism in the pathogenesis of metabolic diseases and metabolic syndrome (Zhou *et al.*, 2019), and in impairments in specific energy metabolic processes, such as mitochondrial respiration and glycolysis (Wang *et al.*, 2019).

Valine, isoleucine and leucine are all essential amino acids and there are over 40 mitochondrial enzymes responsible for their catabolism in mammals (Lynch and Adams, 2014). The first enzyme in the catabolic pathway is the branched-chain amino-transferase (BCAT), generating branched-chain keto acids BCKAs (Hutson, Sweatt and LaNoue, 2005), alpha keto isocaproate (from leucine), alpha keto methyl valerate (from isoleucine) and alpha keto isovalerate (from valine) (**Figure 3**) (Arany and Neinast, 2018). Subsequently, BCKA dehydrogenases (BCKDs) irreversibly decarboxylate the BCKAs, forming branched chain acyl-coenzyme As (CoAs) in a rate limiting step (Neinast, Murashige and Arany, 2019). Isoleucine catabolites are further metabolized through several steps into Acetyl-CoA, while leucine catabolites can also be used to form propionyl-CoA (Adeva-Andany *et al.*, 2017). Valine, on the other hand, has an additional fate. The enzyme hydroxyisobutyryl-CoA hydrolase (HIBCH), which is

responsible for removing the CoA moiety from 3-hydroxyisobutyryl-CoA, renders the 3-hydroxyisobutyric acid (3-HIB) free to either leave the cell or to be catabolized further to methylmalonate semialdehyde (MMS) (J. Letto, Brosnan and Brosnan, 1986; Taniguchi *et al.*, 1996). The latter can be used in pyrimidine metabolism, or be converted to propionyl-CoA or methylmalonyl-CoA (MMA), and be utilized in the synthesis of odd chain FAs or cholesterol, or fed into the TCA cycle (J Letto, Brosnan and Brosnan, 1986; Molloy *et al.*, 2016).

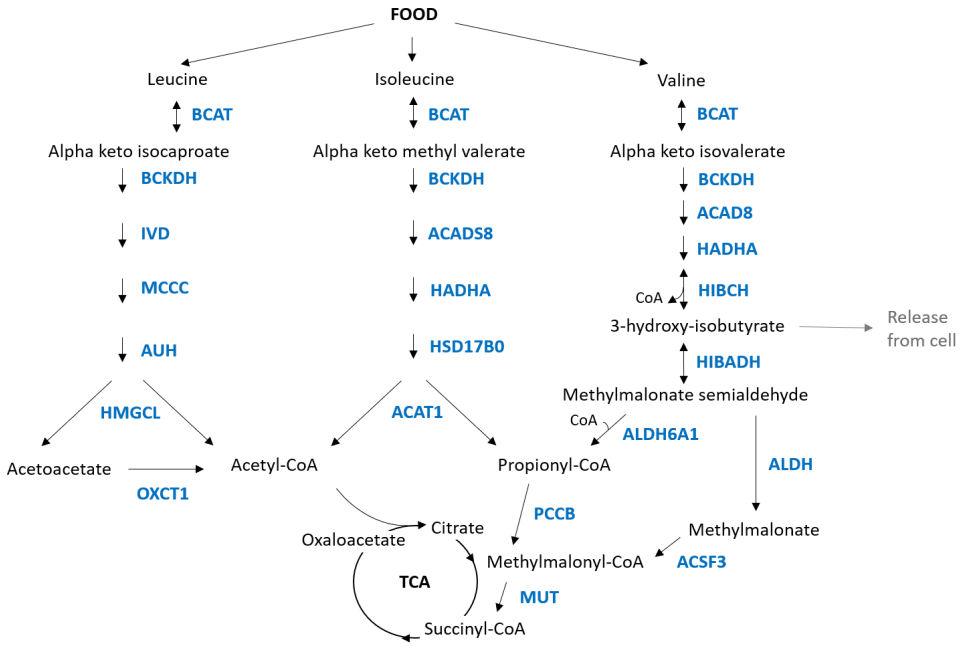

Figure 3. General overview of BCAA catabolism. This figure is based on (Neinast, Murashige and Arany, 2019) and (*KEGG PATHWAY: Valine, leucine and isoleucine degradation - Reference pathway*, 2020).

Interestingly, elevated circulating 3-HIB levels have recently been associated with IR, T2D and gestational diabetes (Harris *et al.*, 2017; Haufe *et al.*, 2017; Andersson-Hall *et al.*, 2018; Mardinoglu *et al.*, 2018). Several organs may contribute to the accumulation of BCAAs and 3-HIB observed in the blood related to obesity and metabolic syndrome, including the liver, heart, skeletal muscles, and adipose tissue (Kedishvili *et al.*, 1994; Lynch and Adams, 2014; Lyon *et al.*, 2019). Knowledge is lacking on how the complex interplay between each tissue affects net circulating BCAA concentrations, and on

which tissues have the metabolically strongest influence on disease pathogenesis. However, adipose tissue is emerging as a promising candidate, with a high basal uptake of the BCAAs, and impairments in BCAA catabolism in the context of obesity and comorbidities (Lackey *et al.*, 2013; Badoud *et al.*, 2014; Burrill *et al.*, 2015; Blanchard *et al.*, 2018). During adipogenesis, fat cells switch from using glutamine and glucose as primary substrates for Acetyl-CoA synthesis to utilizing more BCAAs, and impairment of BCAA catabolism has been shown to perturb adipogenesis (Green *et al.*, 2015). Moreover, the BCAAs provide carbons to generate FAs in white adipocytes (Crown, Marze and Antoniewicz, 2015), and a recent study found that the BCAAs are transported at a higher rate into the mitochondria of brown adipocytes that are exposed to cold-stimulus, thereby reducing the circulating BCAA levels and exerting an improved regulatory effect on energy homeostasis (Yoneshiro *et al.*, 2019). Yet, little is known about the differences between BCAA metabolism in WAT and BAT, and whether adipocytes secrete 3-HIB. It was previously shown that 3-HIB stimulates uptake of FAs from endothelial cells connected to muscle tissue, dependent on the PPARγ coactivator PGC-1α (Jang *et al.*, 2016). However, it is not known if this valine catabolite can also modulate adipocyte biology.

2. Aims of the study

The overall aim of the study was to expand our knowledge of the role of adipocyte SLC7A10 and amino acid metabolism in obesity.

Specific objectives were to:

1. Uncover novel functions of SLC7A10 in adipocytes.

2. Delineate mechanisms by which impairment of SLC7A10 might lead to obesity, adipocyte hypertrophy and IR.

3. Determine the association between circulating BCAAs and 3-HIB with obesity, IR and T2D, and how BCAA catabolism and 3-HIB are linked to adipocyte biology, including comparison of adipocyte subtypes.

4. Identify specific pathways and substrates involved in SLC7A10-dependent triacylglycerol storage in adipocytes and assess how SLC7A10 relates to amino acid flux and metabolism *in vitro* and *in vivo*.

3. Methodological considerations

The present work was based on data from different sources, including human cohorts, animal studies, and *in vitro* cultured human and mouse primary adipocytes, as well as immortalized murine cell cultures. Consistent results across these data sources has given a sound foundation on which to draw conclusions. Nonetheless, each of these sources of data has limitations that need to be carefully considered. Materials and methods used in this thesis are described in full in the methods section of each paper, while key models, assays and analyses are briefly presented and discussed below.

3.1 Clinical cohorts

Together with collaborators from different parts of the world, we have analyzed several human cohorts for the papers presented in this thesis. All studies were approved by the respective Regional Ethical Committees, and all participants gave written informed consent. Seven, three and one cohorts were used in Paper 1, 2 and 3, respectively. All the anthropometric data for the different cohorts are described in detail in the papers.

There are many strengths of using several human cohorts, including that the data from clinical cohorts reflect the current state of the organs and tissues in the body, which can be linked to the development of multiple diseases. Cohort data may therefore expose a higher clinical relevance compared to experiments performed in *in vitro* cell cultures and can be valuable as a starting point to systematically find candidate genes or biomarkers involved in human disease. For example, from the ADIPO cohort (Paper 1) we have obtained clinical information and RNA expression data from both stromal vascular fraction (SVF) and mature adipocytes isolated from different adipocyte depots of the study participants as described previously (Veum *et al.*, 2012). This gave us the opportunity to examine differences in gene expression between adipose depots, between SVF and adipose tissue, and between different people, which lead to the discovery of several candidate genes involved in metabolic syndrome, such as *SLC7A10*. Using

several different cohorts also serve to validate findings and support more robust conclusions.

Some of the analyses from our human cohorts are based on co- and anti-expression, or correlations between gene expression, various traits of metabolic syndrome and adipose tissue volume or adipocyte size. Therefore, it is important to distinguish association and co-expression from causality. Thus, conclusions of whether increased SLC7A10 expression contributes causally to reduced body weight and health improvements observed after bariatric surgery, or if it is a consequence of these changes, cannot be determined from these data alone. The same is also true for the associations observed between the plasma 3-HIB levels, and various metabolic risk factors and the glycemic status of the individual. Therefore, animal models with impairments in the function of the target protein or enzyme of interest, herein SLC7A10 and HIBCH, are pivotal for uncovering the biological processes involved, and whether these are important causal contributors in the development of obesity and related diseases.

3.2 Slc7a10b loss-of-function Zebrafish (Paper 1)

The overfeeding experiment in Slc7a10b loss-of-function zebrafish presented in this thesis was conducted at the Institute of Marine research (former NIFES). The Norwegian Food Safety Authority authorized experiments performed later than 5 days post fertilization (dpf) (FOTS ID 9199). The fish were raised and cared for in accordance with the guidelines of the Norwegian Animal Welfare Act, and the ethical principles of replacement, reduction and refinement (The Three Rs) were applied.

Homozygote wild type (WT) and Slc7a10b mutant (sa15382) zebrafish were bred from heterozygote eggs obtained from the Zebrafish International Resource Center. The mutant fish contain A→T point mutation in the conserved 3' splice site between exon 6 and 7 in the *Slc7a10* isoform b gene (ENSDART00000073398.5), causing a disruption in the splice site, prohibiting the removal of the intron in the mature mRNA. Consequently, the mRNA length was increased, producing a Slc7a10b protein that is

either rendered inactive or directly sent to degradation after translation due to impaired protein folding.

When performing animal experiments, it is important to limit possible stress factors. Towards this end, the adult zebrafish were housed in 1.5 or 3-liter tanks with a standard recirculating system, daily water exchange and electrical conductivity. In addition, the fish were kept in a 14h light/10h dark cycle, and fed with a complete nutrition product at 8am, noon and 4pm. The zebrafish were also fed freshly hatched *Artemia* one time per day, to ensure welfare and an enhanced diet variation.

During the overfeeding study, 3 fish were held per tank and the circulation system was shut down 5 minutes prior to and 30 minutes after feeding, to maintain a better control of the food intake. Importantly, before conducting the main overfeeding study, we performed a two-week pilot study in 6 WT and 6 loss-of-function 2-month old zebrafish, revealing a large variation in body weight between male and female zebrafish, most likely due to the production of eggs in the females. Thus, to limit variations in body weight and size, the final overfeeding study was performed using only male fish. The main 8-week overfeeding study was performed in 33 WT and 39 mutant 4-month old zebrafish, and length of each fish was recorded at the start and the end of the study, while weight was measured at the start and after 3, 6 and 8 weeks. Each tank held 3 fish, to improve their quality of life, and to limit the use of bench space and tanks. In addition, power calculations suggested that this number of tanks (and fish) would be sufficient (discussed further below).

Moreover, we learned that collecting blood from tiny fish such as zebrafish is often quite challenging, and several different methods exists. We ended up with performing a collection method modified from a previous study, where the tail fin of each fish was cut directly after sacrifice, and each fish was put into an Eppendorf tube containing ethylenediamine tetra acetic acid (EDTA) solution and spun in a centrifuge to use the centrifugal force to extract the blood into the bottom of the tube. Using heparin or EDTA coated tubes might have resulted in better quality of the collected blood, and/or less coagulation of blood on the tailfin of the fish. The use of more anticoagulant could

therefore have increased the volume of plasma obtained from each fish, and enabled more analyses, such as measuring metabolite and amino acid plasma levels. Unfortunately, we did not have sufficient blood to measure some of the key circulating parameters that are linked to obesity and T2D, and could not properly assess, e.g., the degree of whole-body IR in our WT and mutant zebrafish.

Recently, zebrafish have emerged as a valuable model for investigating adipose tissue biology, including glucose homeostasis and lipid metabolism (Zang, Maddison and Chen, 2018), as well as other mechanisms involved in the pathogenesis of metabolic diseases (Oka *et al.*, 2010; Schlegel and Gut, 2015), including NAFDL (Non-alcoholic fatty liver disease), IR and T2D (Zang, Shimada and Nishimura, 2017; Chen, Zheng and Zhang, 2018). It has frequently been used as a model for diet-induced obesity (Ran *et al.*, 2017) and recently also as a model to study effects of aging on mitochondrial respiration capacity (Salmi, Tan and Cox, 2019). In addition, it is important to note that zebrafish are thought to not possess any thermogenic beige or brown adipocytes, and they can therefore be a useful model organisms for studying effects of genetic modifications on exclusively WAT metabolism and function (Gesta, Tseng and Kahn, 2007; Seth, Stemple and Barroso, 2013). This is especially important in the context of SLC7A10, which was previously identified as a marker for white and not beige or brown adipocytes (Ussar *et al.*, 2014).

All teleostean fish, such as zebrafish, originate from a lineage that went through a whole genome duplication 250 million years ago. Thus, there might be several versions of the same gene in one species (Amores *et al.*, 1998; Jatllon *et al.*, 2004; Voldoire *et al.*, 2017). These genes are called paralogs, and usually develop different functions. Orthologs on the other hand, are genes in different species evolved from a common ancestral gene, and more often retain the same function (Stamboulian *et al.*, 2020). Whereas humans and mice express a single isoform of SLC7A10 exhibiting 93% protein sequence identity (Nakauchi *et al.*, 2000), zebrafish express two paralogs, Slc7a10a and Slc7a10b, showing 74% and 76% sequence identity with human SLC7A10, respectively (T coffee alignment tool). To further assess similarity of the functional SLC7A10 protein domains across the different species, we made secondary structure predictions

(SSPs) for the human, mouse and zebrafish orthologous SLC7A10 proteins using the software HMMTOP, and combined these results with our multiple sequence alignment (MSA) (Jersin, 2016) (**Figure 4**). SLC7A10 belongs to the APC superfamily of transporters, which prompted us to utilize HMMTOP, since this software has previously been shown to reliably predict the secondary structure of transmembrane proteins (Reddy *et al.*, 2014). Our MSA showed cross-species conservation of a large proportion of the amino acid residues, with 333 residues (around 64%) fully conserved the four species. All predicted secondary structures were in the same regions of the protein sequences of all species, thereof 12 transmembrane helices (Jersin, 2016) (**Figure 4**). This similarity supports the functional relevance of our zebrafish as a partial *Slc7a10* knockout model.

Figure 4. Mouse, zebrafish and human SLC7A10 are all predicted to have 12 transmembrane helixes located in the same regions of the protein sequence. To generate a multiple sequence alignment (MSA) of human, mouse and zebrafish Slc7a10 protein sequences we utilized the T-coffee alignment tool. Subsequently, secondary structure predictions (SSPs) were performed separately for all species using the HMMTOP tool, identifying 12 transmembrane helixes (black squares around the one letter code for the amino acids). The intracellular side of the cell membrane is indicated by a thick green line, and the extracellular side with a thick yellow line. Conservation of amino acids between the orthologs is indicated with the following symbols: *, fully conserved residue; :, conserved groups with strong similar properties; ., conserved groups with weak similar properties. This MSA was also presented in the Master Thesis "*Novel functions of the amino acid transporter SLC7A10 in adipocytes*" (Jersin, 2016).

Of note, our zebrafish study includes no measurements of energy expenditure or direct food intake. However, no differences between tanks were observed by a blinded lab technician, and all the food was consumed by the three fish in each tank (independent of the genotype) before the circulation system was turned back on. It is also worth noting that the loss-of-function of Slc7a10b in Zebrafish was global and not adipose tissue specific. Thus, we cannot conclude to what extent the obesity phenotype in the mutant zebrafish depended on effects in other less Slc7a10-enriched tissues, such as the brain, and the pathogenic relevance of impaired Slc7a10 function in an adipose-specific knockout animal model would be of great value (further discussed in *7. Future Perspectives*). On the other hand, a study showed that homozygote Slc7a10 mutant mice showed tremors, seizures, early postnatal death and 20-30% less body weight 14 days after birth compared to their WT counterparts (Xie *et al.*, 2005). In addition, another study directly injected Slc7a10 inhibitor LU, which cannot cross the blood-brain barrier, into the brain of mice and observed no difference in body weight compared to vehicle (Sason *et al.*, 2016). Due to the function and expression of Slc7a10 in the brain, other researchers have performed several studies with suppression of Slc7a10 expression and function in this tissue. However, none of these studies have to our knowledge reported increased weight gain compared to control, suggesting that the effects we observed in our zebrafish study were largely due to lack of Slc7a10 function in peripheral tissues (e.g., adipose tissue). A mouse or zebrafish study with animals harboring an adipose specific knockout is therefore warranted, but *in vitro* target tissue cell models can also be very helpful tools to examine effects and specific mechanistic pathways affected by modulation of target protein function and expression. Herein, we have modulated SLC7A10 by pharmacological inhibition and overexpression, and HIBCH by knockdown (KD), using cultured human and mouse adipocytes as our model systems.

3.3 Human primary adipocytes, and mouse and human cell cultures

All the adipose tissue samples utilized in this thesis were taken with approval from the respective regional ethical committees and all the human subjects gave written informed

consent. For the current projects, we have in total isolated human primary adipocytes from abdominal subcutaneous liposuction material collected from around 40 patients at Aleris or Plastikkirurg1 in Bergen, Norway. Briefly, we enzymatically digested the adipose tissue, isolating the stromal vascular fraction (SVF) containing preadipocytes as described previously (Stenson *et al.*, 2011; Lee and Fried, 2014) and seeded cells directly into cell culture plates. We also obtained immortalized human cells from Joslin Diabetes Center (Harvard Medical School, Boston, USA), isolated from SC neck adipose tissue of a subject undergoing thyroidectomy (Cypess *et al.*, 2013). In addition, perirenal WAT and BAT was collected from healthy human kidney donors (Svensson *et al.*, 2014). Moreover, preadipocytes from interscapular WAT and BAT were isolated from C57Bl/6 male mice and immortalized at Joslin Diabetes Center using SV40 virus. These immortalized preadipocytes and the commonly used cell line 3T3-L1 are genetically manipulated so they can proliferate indefinitely, and are often quick to grow, easy to culture, well characterized and theoretically homogeneous. However, because immortalized cells are genetically modified, they may not exhibit the exact same functions as the normal unmanipulated cells and could sometimes acquire altered phenotypes. Hence, immortalized cells might differ more in gene expression patterns and biological functions from *in vivo* cells, as well as from freshly isolated primary cells. Another reason for the fresh primary cells being more similar to the *in vivo* growing cells is that they are not expanded through passages, which can cause a selection towards a specific cellular subtype and reduce the heterogeneity seen in adipose tissue. In addition, passaging cells can allow for occurrence of spontaneous mutations, which may in theory alter important cellular functions. However, isolating primary cells is time-consuming and costly, and heterogeneity of the cell population seeded may cause variations between experiments, and the experiments cannot readily be repeated at a later time-point with fresh cells from the same subject. Nonetheless, primary adipocytes can be frozen, but this often requires some expansion first, which might alter cell composition and function. Also, the freezing alone might alter cell characteristics and thus their ability to differentiate, discussed further below.

Human primary, immortalized human, immortalized mouse WAT and BAT, and murine 3T3-L1 preadipocyte were all differentiated using adipogenic “cocktails” as outlined in

the respective papers. There are differences between the *in vitro* differentiation protocols used for mouse and human adipocytes, including the duration of the differentiation process. The murine cells reach mature levels after 8 days of differentiation, whereas the human cells are subjected to an adipogenic cocktail for around 12 days. In addition, the concentrations of substances used in the cocktails differ between the protocols, as well as some of the compounds that induce differentiation. The murine preadipocytes are induced to differentiate using medium complemented with fetal bovine serum (FBS), L-glutamine, insulin, dexamethasone, methyl-isobutyl-xanthinin (IBMX) and rosiglitazone (Dankel *et al.*, 2014). The human adipogenic cocktail contains FBS, biotin, triiodothyronine (T3), DL-Pantothenate, transferrin, cortisol, insulin, hepes and rosi, the latter only for the first 6 days (Veum *et al.*, 2012). Biotin is a coenzyme that regulates lipid and amino acid metabolism (Kuri-Harcuch, Wise and Green, 1978), and together with Pantothenate and transferrin, it increases lipid accumulation and adipogenesis (Wang *et al.*, 2018). T3 is an active form of thyroid hormone, which activates phosphatidyl inositol 3-kinase (PI3K), causing increased expression of adipogenic genes such as leptin and PPAR-γ (Obregon, 2008; Oliveira *et al.*, 2013). Insulin activates TFs that are important in regulating adipogenesis and increases GLUT4-mediated uptake of glucose into adipocytes (Laviola *et al.*, 2006), and the synthetic PPAR-γ agonist rosiglitazone enhances adipogenesis by enhancing the activation and expression of PPAR-γ (Albrektsen *et al.*, 2002). Dexamethasone, a synthetic glucocorticoid, promotes expression of, e.g., cAMP Respons Element Binding Protein (CREB) which is a TF responsible for expression of adipogenesis inducing genes (Reusch, Colton and Klemm, 2000), and the phosphodiesterase inhibitor methyl-isobutyl-xanthine (IBMX) also exerts its effect by stimulating expression of this TF (Petersen *et al.*, 2008).

Interestingly, frozen human primary preadipocytes did not differentiate well without addition of IBMX to the differentiation cocktail, which is surprising because the fresh cells did not need IBMX to induce adipogenesis. On the contrary, we found that addition of IBMX hampered differentiation of freshly seeded preadipocytes. Jia *et. al* have previously demonstrated that IBMX supplementation is critical for obtaining differentiation of Adipose-Derived Stem Cells (ASCs), however they do not mention whether the cells are frozen or passaged prior to adipogenesis (Jia *et al.*, 2012). In

addition, we surprisingly did not detect appreciable levels of *SLC7A10* mRNA in fully differentiated adipocytes from ASCs that had been frozen, even when supplementing the adipogenic cocktail with IBMX. Together, these lines of evidence suggest that passaging or freezing primary preadipocytes introduces alterations in cell characteristics and/or might exert a selection pressure towards one specific adipocyte progenitor subtype which needs IBMX to differentiate. Because of the many uncertainties with using the less characterized frozen cells, we restricted our *in vitro* human primary cell experiments to freshly isolated preadipocytes. The frozen and fresh cells are now being characterized by members of our group, since the exact nature of the cell models we are working with needs to be further resolved, e.g., by FACS sorting of specific subpopulation of progenitor cells.

It is also important to consider the difference in adipogenic potential of cells from different patients. Preadipocytes from some individuals might have a better genetic and epigenetic foundation to differentiate compared to others, with possible impact on risk of T2D (Claussnitzer *et al.*, 2014). When hASCs from people with both obesity and T2D was cultured and differentiated *in* vivo, they were found to retain their memory of the donor due to epigenetic reprogramming, which affected several genes known to regulate fat tissue function (Andersen *et al.*, 2019). Therefore, we did not utilize preadipocytes from donors having T2D in any of our *in vitro* studies. Moreover, the individual variations in cell composition in the donor adipose tissue might also play an important role in how well a pool of preadipocytes differentiate. Another issue is that highly differentiated adipocytes, containing a lot of lipid droplets, are more fragile and can detach more easily than less differentiated fat cells. This might, at least in part, be due to the changes in cell morphology throughout differentiation, when the cells go from being long and fibroblast-like to round and less attached to the surrounding surface. Hence, some methodological precautions must be taken to prohibit the mature adipocytes from loosening from the cell plate. One solution to this is to coat the plates with gelatin or collagen, ensuring that the cultured adipocytes have more than just a plastic surface to adhere to, making them attach more strongly to the bottom of the wells. Moreover, it is also of great importance to remove and add cell medium slowly, but not too slow, because this might cause the cells to dry up. The repeated medium changes

throughout adipogenic differentiation poses a greater risk of mechanical stress on the cells. To avoid generating differences in stress and number of detached cells between different treatment groups that might introduce undue technical variability, it may be important, especially in 96-well plates where all the medium is removed at the same time by inverting the plate, to vary between which wells are given medium first and last. Replicating findings also minimizes the impact of these potential biases on the scientific results. Therefore, a major strength of this study is that we have replicated our results from multiple metabolic and cellular assays both several times in the same model, but also across different human and mouse adipocyte cell models described above. Moreover, consistent effects on cellular functions across models and species support that the molecules of interest are in evolutionarily conserved and hence involved in important molecular processes.

3.4 Treatment of cultured cells using pharmacological and natural compounds, and transient overexpression or knockdown

Modulation of SLC7A10 for the functional assays in paper 1 and 3 was mainly performed by using the pharmacological inhibitor entitled BMS (Brown *et al.*, 2014), but we have also confirmed multiple key results using the inhibitor LU (Sason *et al.*, 2016) and by observing opposite results when overexpressing adipocytic SLC7A10. Inhibitors may however cause unspecific phenotypic effects. We therefore examined the effect of the two SLC7A10 inhibitors on cell viability by using the neutral red cytotoxicity assay, following the manufacturer's protocol (Abcam, ab234039). We found no differences in cell viability compared to cells treated with normal medium with neither inhibitor (**Figure 5**).

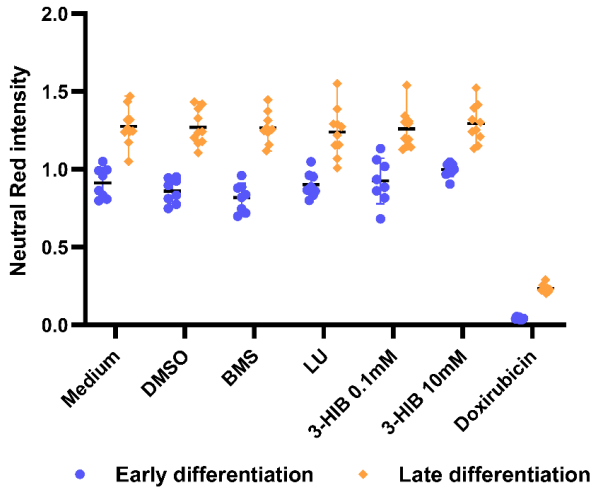

Figure 5. Slc7a10 inhibitors lack cytotoxic effects: 3T3-L1 cells were treated with either DMSO (vehicle), BMS (SLC7A10 inhibitor 1) (100 µM), LU (SLC7A10 inhibitor 2) (100 µM), or 3-HIB (0.1 or 10 mM) for 72 hours during different stages of differentiation to assess for effects on cell viability. Doxorubicin (5mM) was used as a positive control for known cytotoxic effects. Treatment lasted for 72 hours from D-2 to D0 and D2 to D5 of differentiation, and on the day of the assay (day 1 for early differentiation and day 6 for late differentiation), cells were washed and incubated in freshly diluted neutral red dye solution for 2 hours. Following solubilization, cell viability was measured as a function of neutral red dye taken up through active transport by live cells, as measured spectrophotometrically at 540nm. Data are presented as mean ± SD (n=8-10).

Also, both the inhibitors' specificity and mechanisms of action have been thoroughly examined previously (Brown *et al.*, 2014; Sason *et al.*, 2016; Torrecillas *et al.*, 2019; Mikou *et al.*, 2020). BMS is suggested to have two mechanisms of action. Firstly, it works by competitive inhibition, whereby it occupies the orthosteric (active) site of SLC7A10, where the transported amino acids usually are bound before they are transferred through the plasma membrane inside the transporter. Secondly, BMS inhibits the transport non-competitively, by also binding an allosteric site, blocking the movement of transmembrane helixes required for transport of amino acids (Torrecillas *et al.*, 2019). LU is suggested to work in a similar manner, also blocking the rocking of the moieties that inhibit the transport process (Mikou *et al.*, 2020). However, it is important to note that these studies are based on computational techniques, specifically homology modelling, since no X-ray crystallographic structure of SLC7A10 has yet been resolved. The structures of membrane proteins are generally difficult to elucidate, because they are often hard to express in large enough amounts, and to solubilize and

thereafter crystallize (Doerr, 2009). In addition, the studies that have examined mechanisms of SLC7A10 inhibitors have looked mostly at the influx and efflux of D-serine, and thus focused less on the flux of the other SLC7A10-transported amino acids. Furthermore, the authors do not mention whether the diffusion mode is also completely blocked, since SLC7A10 is able to work through facilitative diffusion, in addition to exchange mode (Fukasawa *et al.*, 2000). However, this seems very likely since the transporter is stuck in the open-outward facing confirmation when inhibited by both transporters, which indicates that the transporter is closed on the cytosolic side (Torrecillas *et al.*, 2019; Mikou *et al.*, 2020), blocking diffusion of amino acids through the transporter channel.

In addition to treating adipocytes with inhibitors, we attempted to knock down *Slc7a10* using several small interfering RNA (siRNA) constructs, transfection reagents and varying the experimental settings in both mouse and human cells. However, we did not manage to reduce the *Slc7a10* mRNA expression and lacking functional assays with KD is a limitation of our *in vitro* study. On the other hand, we successfully overexpressed Slc7a10 in 3T3-L1 adipocytes, causing opposite effects on several functional assays compared to inhibition.

For Paper 2, the main modulation of BCAA metabolism is performed by either treating cells with the valine breakdown product 3-HIB or knocking down the valine catabolizing enzyme HIBCH. Supra-physiological concentrations (10 mM) of 3-HIB were initially used in our experiments and had no effects on cell viability (**Figure 5**). In addition, we examined the cellular effects of supplementing cell cultures with multiple doses of 3-HIB, which also did not affect cell viability (**Figure 5**), and we observed similar results between concentrations in several of our function assays. Furthermore, the *Hibch* KD efficiency in murine WAT and BAT, and 3T3-L1 cells was high, and produced similar phenotypic effects across the cell models.

3.5 RNA sequencing and gene ontology analyses (Paper 1 and 3)

Paper 1 contains an analysis examining genes that are co- or anti-expressed with *SLC7A10* in adipocytes isolated from patients, RNA sequencing results from VAT of Slc7a10b WT and loss-of-function zebrafish, and from cultured Dimethyl sulfoxide (DMSO) and BMS-treated human primary adipocytes. Genes found to be highly co- or anti-expressed, or significantly different between treatment and control were subjected to ontology analyses, mainly using the freely available online tools Panther (Thomas *et al.*, 2003) and GOrilla (Eden *et al.*, 2007, 2009).

Gene ontology (GO) analyses are often performed to screen for patterns of biological processes or pathways strongly affected by a specific modulation. For the Zebrafish RNA-seq, we had to pool the collected tissues from the three fish in each tank together to obtain enough RNA for the analysis. This resulted in less samples, and hence a lower statistical power. Due to the vast number of genes tested in these types of analyses, it is important to correct for multiple testing when calculating p-values. After both reducing the number of samples and correcting for multiple testing, we obtained a more reliable list of genes that significantly varied in expression between WTs and mutants. When subjecting the up- and down-regulated genes to GO databases to screen for possible biological processes that were affected, there were few significant categories. However, since we had pooled RNA from 3 different fish for each sample we analyzed for RNA seq, the genes found to be significantly differing between the WT and mutants actually has a three times higher n than the number of tanks used. Therefore, the genes identified by analysis of the RNA sequencing results can be considered strong candidates for mediating the effects of impaired SLC7A10 function. This is however not taken into consideration in the statistical analysis, since the analysis only knows the total n of the samples (each tank), and we got a shorter list of significant genes differing between the WT and the mutant. We therefore performed an alternative analysis using GOrilla, which could take advantage of the large number of genes that showed a large fold difference between the WT and mutant fish. All genes were organized into a single ranked list based on fold change and subjected to a GO analysis in an made for ranked

lists in Gorilla. This alternative analysis is an advantage of Gorilla when samples show more variation in gene expression.

For the RNA-seq from the DMSO and BMS-treated human primary adipocytes, we had more samples and the effect of BMS on global gene expression was strong, which resulted in a large number of significant up and down-regulated genes compared to DMSO. Here we used a multiple testing-corrected p-value < 0.05 and looked at both significantly affected cellular pathways and biological processes. When deciding these cut offs, there are no definite rules to follow. However, these analyses are typically hypothesis-generating that need further validation with functional experiments. Rerunning GO analyses with different p-value cut-offs and tools can help to highlight the functional categories that are most robustly associated with the treatment/manipulation of interest, giving a greater likelihood that effects will be observed in corresponding functional assays. Also, applying different GO strategies to analyze transcriptome data, including gene set enrichment analysis (GSEA), can provide additional insight and confidence in functional predictions.

Generally when interpreting gene expression analyses, it is important to consider that even though the measured alterations in gene expression can indicate which pathways and mechanisms are affected by a specific treatment, they might not give a straightforward picture of the cellular condition. Firstly, even though a gene is highly transcribed into mRNA, it is not necessarily further translated into protein. Several down-stream regulatory mechanisms can either make the mRNA dormant or send it for degradation. Also, the protein sequence might not undergo the required post-translational modifications to be functional or be activated (e.g., by being phosphorylated by up-stream proteins in a cellular signaling cascades). In addition, the directionality of differentially expressed genes between BMS and DMSO might also be difficult to interpret. Up- or down-regulated genes might reflect an attempt to compensate for the reduced function of SLC7A10. This can be exemplified by the increased mRNA expression of *Slc7a10b* observed in adipose tissue of the Slc7a10b loss-of-function zebrafish, and in the BMS treated cells showing higher SLC7A10 protein expression compared to control. However, despite sometimes unclear directions

of the observed functional ontologies, transcriptome studies are useful for obtaining an overview of affected pathways and processes. Overlap of the same overrepresented biological processes in different data sets and organisms, where the same factor has been modulated, can further increase confidence in the relevant processes to investigate further. Therefore, the overlapping genes and GO results from the Zebrafish and BMS RNA-seqs are likely to be more biologically and functionally relevant, strengthening the individual results. To further confirm biological relevance, we have used Western blot to look into protein expression to confirm gene expression results and performed functional assays to more specifically investigate the metabolic pathways and biological processes affected.

3.6 Metabolomics, functional assays, and normalization

Several methods and assays are applied to the various cell models utilized in the present thesis. Among others, we have performed metabolomics measuring amino acids and metabolites in medium samples from cultured adipocytes by GC-MS at Bevital AS, and conducted multiple functional assays, measuring radiolabeled amino acid and glucose uptake, mitochondrial respiration, lipid staining by Oil Red O (ORO), ROS generation, total GSH concentrations, FA uptake, and total cholesterol and glycerol levels.

When collecting the medium from cells in culture for metabolomics analyses, there are several pitfalls to be aware of. Firstly, it is important to collect the medium at the same timepoint each day since the flux is calculated per hour and the measured amino acid and metabolite concentration will be inaccurate if the timing varies too much. We kept all collection within less than one hour away from 24 or 48 hours. Secondly, the medium should be collected in the same way every time, with the same number of resuspensions and tilting of the cell culture plate. This ensures that the amino acid concentrations measured at the different timepoints can be comparable. Lastly, all the medium should be removed before the cells are subjected to the fresh medium, since this might affect the total cellular uptake or synthesis of amino acids, or the leftover extracellular amino acids might add to the concentration of measured amino acid in the next measurement,

rendering the data imprecise. This is also important when considering that unconditioned medium is used as a reference when calculating the total flux. Another aspect to consider is the amino acids produced by the cells, which is a dynamic process dependent on the needs of the cells. Therefore, the results calculated reflect the net balance of fluxes in and out of the cell, i.e., the amount of amino acids and metabolites taken up from the medium into the cells (when calculating uptake) and released to the extracellular fluid or not taken up (when calculating influx). Hence, unless also collecting the cells, nothing is known about the intracellular amino acid and metabolite status in these experiments, and about the total contribution of cellularly synthesized amino acids to the result, except for the essential amino acids which cannot be produced in mammalian cells. In one of the metabolomics experiments in Paper 2 we also subjected intracellular cell lysates to metabolomics analysis. This was however not feasible in experiments where cells were treated with compounds, due to the large number of cells needed to have enough lysate for the measurement, requiring copious amounts of compound. For the intracellular experiments, cells from 6 confluent 15 cm dishes were typically combined for any timepoint of interest.

In general, functional assays are used to examine, in a controlled and specific manner, effects of treatments on various cellular pathways and biological processes compared to control, such as mitochondrial respiration, cell stress and fat storage (Simpson, 2006). In the present work we utilize the seahorse mitochondrial stress test assay to measure respiration in living cells. Briefly, this method is based on measuring oxygen consumption rate (OCR), while modulating the function of the molecules in the electron transport chain. For this assay, it is important that the pH of the Seahorse medium is adjusted to 7.4 at 37°C. If this is not done correctly, it might affect the oxygen measurements performed by the Seahorse machine, and different experiments cannot be directly compared. When measuring oxidative stress in adipocytes, we use a commonly used ROS probe, which emits fluorescence when exposed to oxidation. When conducting the ROS assay, the probe must be protected from light, to avoid excitation. In addition, when measuring difference in ROS generation between treatments, the compounds examined should be added to the cells in close vicinity to the spectrophotometer, so that the first measurement captures as little effect as possible

(representing the baseline ROS before treatment). Since the FA uptake assay is also fluorescence based, and uses a light sensitive FA substrate that emits fluorescence when taken up into the cell, many of the same considerations as for the ROS assay are important for this assay as well. Furthermore, both amino acid and glucose uptake measurements are performed with molecules labeled by carbon-14 or hydrogen-3. For all radioactive assays, it is important to keep as much distance to the radioactive source as possible, and to have detailed protocols in place describing which protective equipment to wear and how the experiment can be conducted in a safe and effective manner. It is also important to keep the number of radioactive experiments to a minimum, while having enough replicates to obtain the required power, and to have safe disposal routines in place before starting.

To control that the measured effects are due to the specific treatment and not artifacts of for example different cell number due to variation in attachment, growth or the amount of mechanical stress applied between wells, all results from functional assays should be normalized to a direct or surrogate measure of viable cells. This is not always straightforward and finding the best normalization method requires planning, as well as knowledge of both the methodology the assay utilizes and the cellular pathways affected. Firstly, in assays where the cells are lysed before readout, results cannot be normalized to the cell count in each well by Hoechst staining. Therefore, only ROS, Seahorse and FA uptake assay, which are all performed in live cells, can be normalized to cell number in the plates after the assay is finished. This is, in my opinion, the most robust way of normalizing results from functional assays. Nevertheless, there is also one issue with this normalization methods, namely that cells might loosen during the assay. The ROS, Seahorse and FA uptake assays are all run for several hours, but Hoechst staining can only normalize the measured result to the number of cells attached in each well at the end of the experiment. However, since these assays are all run in machines with a persistent temperature of 37 °C, to prevent cells from loosening, and all wells are treated the same way, this method of normalization should give reliable results. In addition, one should ensure accurate seeding of cells into each well and start treatment only after cells have stopped proliferating and started to develop into adipocytes. This will keep the cell number in each well as similar as possible and is thus important when

utilizing all normalization methods. Secondly, assays where cells are lysed before readout can be normalized to for example the protein or deoxyribonucleic acid (DNA) concentration measured in each well, as a surrogate measure of cell number. Assays that are normalized to protein concentration include glucose uptake, while total GSH levels are normalized to DNA concentration. However, when treating cells throughout differentiation, protein and DNA concentration in each cell could be affected. Nevertheless, treating terminally differentiated adipocytes for a short time (minutes to 24 hours) with various compounds should normally not affect these measures markedly. In addition, the amount of DNA is not affected in the cells that have started to differentiate, since they no longer undergo mitosis. DNA is also more robust compared to protein and can resist degradation during prolonged exposure to higher temperatures and other suboptimal conditions. Therefore, normalization to DNA is in my opinion the best way to normalize results from cells that are no longer attached to the surface of the wells. Ideally, we should have utilized DNA concentration to normalize results from all assays using lysed cells.

In conclusion, no normalization method is perfect, but if the effect of the compound is strong enough, the appropriate methodological measures and considerations are taken, and the results are replicated several times, the end results of the functional assay should reflect the cellular *in vitro* situation in response to a treatment of interest.

3.7 Statistics and sample variation

The specific statistical methods used in this thesis are described in detail in each individual paper. Briefly, we used two-tailed unpaired Student T-test when comparing two different groups, one-way ANOVA with Dunnett's or Sidak's correction for multiple comparisons when comparing more than two groups and DESeq2 for differential expression analysis of RNA-seq data. In Paper 1 and 3, data are presented as mean ± SD (standard deviation) or as geometric mean ± 95% confidence intervals (only Seahorse data). The latter was utilized when describing Seahorse data, due to a large number of replicates and higher variations between samples in this assay. In Paper

2, data are visualized by mean ± SEM (standard error of the mean) or mean ± 95% confidence intervals (HUSK data). For data with less than 2 replicates (n<2), no measure of dispersion is shown, and significance is always indicated by *P < 0.05, **P < 0.01, ***P < 0.001.

When performing experiments in human primary preadipocytes, there might be natural variation between the samples from different people in how the cells behave. This might, as mentioned previously, be due to genetics, epigenetics and the physiological state of the person and tissue the cells were isolated from. These cells, purified from liposuction aspirate from the abdomen, are not genotyped, and we only have access to clinical data limited to sex, BMI, relevant diseases history, and whether they previously have had bariatric surgery. To exclude sex-specific differences, we have mainly utilized primary preadipocytes from women (except from one man). However, it is important to take into account that these preadipocytes are all treated similarly when and after they are isolated, and a strength of the SLC7A10 inhibitor treatment is that it consistently leads to a similar response in cells from all of the different subjects. As discussed previously, cell cultures are more similar genetically, which makes different experiments more comparable. Correlation analyses of cohort data were adjusted for BMI, sex, and age. For the zebrafish used in the overfeeding study in Paper 1, they were all siblings, and therefore retain similar genetics. When working with biological data, it can therefore often be difficult to know whether a deviating data point is an outlier due to an actual error in the analysis or a biologically relevant finding caused by interindividual differences. Conducting standardized outlier tests helps to present the data in an unbiased and robust way. In the present thesis, outliers are consistently removed using a Whisker Tukey test before visualizing the data.

Overall, utilizing clinical data from several human cohorts, and making consistent observations in both *in vitro* cultured primary human adipocyte and mouse cell models, as well as *in vivo* zebrafish, strengthen the findings presented in this thesis.

4. Summary of results

4.1 Paper 1: Role of the neutral amino acid transporter SLC7A10 in adipocyte lipid storage, obesity and insulin resistance

In this study, we sought to investigate molecular mechanisms in adipocytes that affect IR and T2D risk. In multiple human cohorts, including the Western Norway Obesity Biobank (WNOB), SAT expression of the neutral amino acid transporter *SLC7A10* was consistently negatively associated with plasma TAG levels, IR, visceral adiposity and adipocyte size. To examine the isolated effect of SLC7A10 impairment *in vivo*, we overfed Slc7a10b loss-of-function and WT zebrafish for 8 weeks, and found that the transgenic fish gained more weight and developed larger adipocytes compared to their WT counterparts.

To investigate how impaired SLC7A10 function directly affects adipocyte metabolism, we treated cultured 3T3-L1 mouse and primary human adipocytes using two different SLC7A10 inhibitors. We observed that SLC7A10 protein and mRNA expression increased during adipogenesis, and when inhibiting SLC7A10 throughout differentiation, we found a potent increase in lipid accumulation in both the murine and the human adipocytes compared controls. By performing RNA-seq on differentiating human adipocytes treated with SLC7A10 inhibitor for 24 hours, we observed an overrepresentation of significantly affected genes related to multiple metabolic processes, such as ATP synthesis, TCA cycle, cholesterol biosynthesis and glycolysis. Furthermore, we found that SLC7A10 impairment reduced mitochondrial respiratory capacity in the cultured adipocytes. Conversely, overexpression of Slc7a10 significantly increased mitochondrial respiratory capacity in murine 3T3-L1 adipocytes.

Overlapping of RNA-sequencing data from VAT of the loss-of-function zebrafish with the inhibitor-treated human primary adipocytes revealed consistent changes in gene expression and in affected biological processes, such as triglyceride metabolic, oxidation reduction and alpha-amino acid catabolic processes. Since SLC7A10 is known to transport small neutral amino acids in the brain, we investigated how

inhibition of the carrier affected the flux of these amino acids in adipocytes. Impairment showed no significant effect on cysteine, alanine, threonine and glycine flux, but a marked reduction in serine uptake into mature adipocytes. Because serine is a known precursor for the body's primary antioxidant, GSH, the reduced uptake prompted us to assess total GSH levels. Adipocyte SLC7A10 inhibition decreased cellular levels of GSH, and consequently increased generation of ROS, while overexpression of Slc7a10 reduced ROS generation. Treatment with ROS scavenger decreased the fat accumulation in SLC7A10 inhibitor-treated adipocytes to levels similar to the control without scavenger present, but did not significantly counteract the lipid-storing effect of insulin, suggesting ROS-dependent lipid accumulation upon reduced SLC7A10 activity but not upon increased insulin stimulation. In addition, SLC7A10 impairment reduced insulin-stimulated glucose uptake, further indicating that increased glucose uptake did not promote the observed increase in fat storage and that SLC7A10 directly affects adipocyte insulin sensitivity.

In conclusion, we here identified SLC7A10 as a novel modulator of white adipocyte metabolism, inversely associated with obesity, IR and increased risk of common metabolic diseases.

4.2 Paper 2: 3-Hydroxyisobutyrate, a strong marker of insulin resistance in type 2 diabetes and obesity that modulates white and brown adipocyte metabolism

In this study, we examined how impaired adipocyte catabolism of BCAAs and stimulation with extracellular valine catabolite 3-HIB, affect metabolic mechanisms in white and brown adipocytes, and the possible role of 3-HIB in the development of IR and T2D. We analyzed several large cohorts including the Hordaland Health Studies (HUSK) and WNOB, and found a strong elevation of circulating 3-HIB in insulin resistance and a gradual increase from normoglycemia to hyperglycemia and established T2D. Moreover, we found a significant positive correlation between plasma 3-HIB concentration and metabolic risk traits including BMI, HOMA2-IR, glucose and TAG levels, and observed a reduction in serum 3-HIB levels in two different cohorts one year after bariatric surgery. Interestingly, one-week post-surgery we found an increase in circulating 3-HIB levels, which might reflect the acute metabolic adaptations to maintain whole-body energy homeostasis in a condition of reduced nutrient intake.

To gain new insight into BCAA catabolism and 3-HIB in adipocyte metabolism, we first measured amino acid and metabolite flux throughout differentiation of cultured human primary and 3T3-L1 murine adipocytes. We found increasing BCAA uptake and 3-HIB release during adipogenesis, with a concomitant increased expression of BCAA catabolic enzymes. The latter was also replicated in immortalized mouse WAT and BAT cells. Furthermore, knockdown of HIBCH, an enzyme responsible for catabolism of the valine metabolite 3-hydroxy-isobutyryl-CoA to 3-HIB, decreased lipid accumulation in and 3-HIB release from all the mouse cell models examined.

Lastly, we observed that 24-hour treatment with 3-HIB increased insulin-stimulated glucose uptake in both WAT and BAT cells, while shorter-term (3-hour) treatment reduced insulin-dependent glucose uptake. On the other hand, short-term 3-HIB addition increased FA uptake in both adipocyte subtypes, but decreased mitochondrial respiration and ROS generation in WAT, while increasing both of these cellular processes in BAT.

In conclusion, we identified 3-HIB as a novel adipocyte-released signaling metabolite, strongly linked to metabolic syndrome, that regulates nutrient uptake and important metabolic processes in both white and brown adipocytes.

4.3 Paper 3: Impaired adipocyte SLC7A10 function promotes lipid storage in association with altered BCAA metabolism and TCA cycle activity in insulin resistance

Having found reduced insulin-dependent glucose uptake with a concomitant increase in lipid accumulation in SLC7A10-inhibited adipocytes compared to control, we here hypothesized that extracellular amino acids served as substrates for the increased TAG synthesis and storage.

We subjected RNA-sequencing data from human primary adipocytes treated with SLC7A10 inhibitor to a gene set enrichment analysis (GSEA), after manually compiling comprehensive gene lists for metabolic pathways involved in lipid storage as well as for genes involved in metabolism of each of the 20 primary amino acids. The GSEA revealed that multiple processes related to lipid metabolism were significantly altered compared to control, such as glycerophospholipid metabolism, TCA cycle, cholesterol biosynthesis, FA transport and TAG metabolism. When treating mature 3T3-L1 adipocytes with SLC7A10 inhibitor for 24 hours, we observed no effect on FA uptake or cholesterol content, and a surprising reduction in glycerol content. However, in response to SLC7A10 impairment from day 2 to 8 of differentiation, all these processes increased significantly compared to control. Moreover, 24-hour SLC7A10 impairment during differentiation of human adipocytes markedly increased expression of genes involved in glyceroneogenesis, the key process for TAG synthesis in adipocytes, where glycerol is synthesized from the TCA cycle intermediate oxaloacetate.

The GSEA for amino acid metabolism revealed a significant enrichment for BCAA metabolism, with upregulation of several key enzymes in this biological process. A significant upregulation was also observed for several genes encoding enzymes in the phenylalanine and tyrosine, aspartate and asparagine, and lysine metabolic pathways. To explore if these changes in gene expression reflected altered amino acid consumption and changes in TCA-related metabolites, we performed GC-MS analysis on conditioned medium from human primary adipocytes treated with SLC7A10 inhibitor throughout adipogenesis. We observed increased consumption of all the BCAAs, no change in the

net flux of methyl malonic acid (MMA), and a potent increased efflux of valine catabolite 3-HIB compared to controls, the latter consistent with SLC7A10-mediated increase in protein expression of the 3-HIB generating enzyme HIBCH.

Furthermore, the comprehensive GC-MS analysis confirmed reduced serine uptake, but also indicated a decrease in alanine efflux and increased consumption of aspartate. Glutamate uptake was significantly increased in mature adipocytes, in line with decreased expression of the glutamate generating enzyme GLS, and increased uptake of the glutamate catabolic product and TCA cycle intermediate α-ketoglutarate.

Finally, we examined baseline data from the human cohort MyoGlu consisting of lean/overweight people with normoglycemia (NG) and dysglycemia (DG), to assess the *in vivo* relationship between adipose SLC7A10 and amino acid and metabolite plasma levels. In this cohort, *SLC7A10* mRNA expression in subcutaneous adipose tissue was inversely associated with BMI, circulating TAG levels and total fat mass and fat mass in several different body compartments. In addition, subcutaneous *SLC7A10* expression correlated positively with MMA, and negatively with plasma levels of all the BCAAs and 3-HIB. When dividing the study participants into NG and DG, we observed significantly reduced subcutaneous *SLC7A10* expression in DG compared to NG, and the opposite effect on circulating 3-HIB levels.

Overall, our study indicates that increased uptake and catabolism of BCAAs, aspartate and glutamate contribute to fueling FA and glycerol synthesis upon impaired SLC7A10 function, in the context of decreased insulin-stimulated glucose uptake. The high 3-HIB plasma levels, associated with IR, may partly reflect decreased adipocyte SLC7A10 function and concomitant dysregulation of fat cell metabolism.

5. General discussion

5.1 SLC7A10, 3-HIB and catabolism of specific amino acids in adipocyte biology

To look into the mechanisms by which adipocytic SLC7A10 and amino acid metabolism affect adipocyte biology and dysfunction, we performed transcriptome analyses and consequent functional assays and found consistent effects on multiple cellular processes, such as amino acid, glucose and FA uptake, mitochondrial respiration, ROS generation and fat storage (**Figure 6)**.

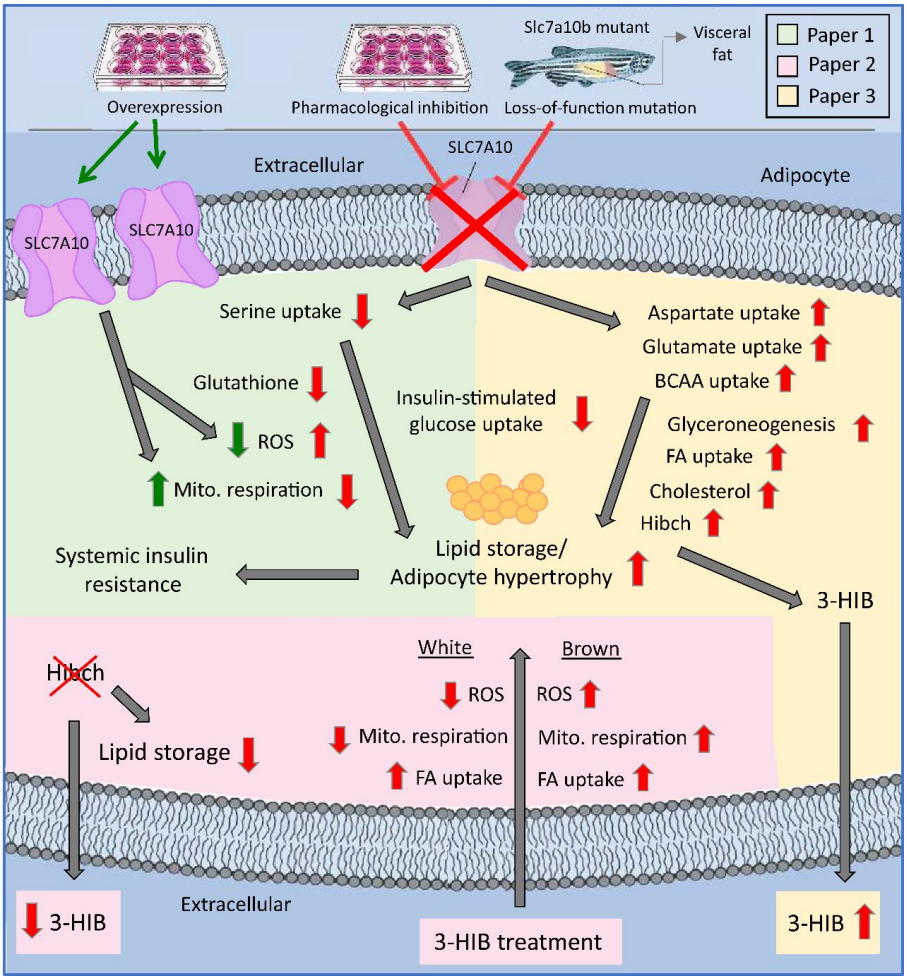

Figure 6. Overall findings of the functional studies in Paper 1, 2 and 3. The relationship between the main functional findings of Paper 1 (green background), Paper 2 (pink background) and Paper 3 (yellow background) are illustrated here, as well as the key results from each paper. ROS; reactive oxygen species, Mito. Respiration; mitochondrial respiration, FA uptake; fatty acid uptake.

5.1.1 Altered amino acid flux and catabolism regulates adipocyte metabolic processes

One of the main findings of this thesis is the reduced serine uptake when SLC7A10 is inhibited in adipocytes, and the increased influx and catabolism of other amino acid, in particular the BCAAs, with associated effects on several metabolic processes (**Figure 6**). The decreased serine influx we observe is consistent with previous findings of SLC7A10's function in the brain, where inhibition impairs flux of both L- and D-serine in neurons (Matsuo *et al.*, 2004; Rutter *et al.*, 2007; Sason *et al.*, 2016). In several cell types, serine catabolism has been found to be required for many critical cellular processes, including biogenesis of mitochondria and translation of proteins (Minton *et al.*, 2018), fueling lipid metabolism, oxidative phosphorylation and purine synthesis, and maintaining redox balance (Yang and Vousden, 2016; Newman and Maddocks, 2017a). These roles of serine are in line with the affected processes we observe in adipocytes *in vitro* when SLC7A10 function is impaired, however, not necessarily in the same direction as observed with serine deprivation in some other cell types.

Interestingly, several of the studies performed on the sodium-independent SLC7A10's carrier function in brain cells also find effects on glycine transport (Ehmsen *et al.*, 2016; Mesuret *et al.*, 2018). In adipocytes, we observe a small reduction in glycine uptake when performing radioactivity experiments using sodium-free assay buffer with and without SLC7A10 inhibitor present. This decrease in glycine influx supports that adipocytic SLC7A10 is capable of transporting glycine in fat cells, but our data suggest that this occurs only in experiments without sodium available (Verrey *et al.*, 2004). In more "physiologically relevant" conditions (here normal differentiation medium), we find no differences in net glycine flux when SLC7A10 function is impaired compared to vehicle. However, in contrast to the intracellular measurement of radiolabeled glycine, from these measurements in culture medium we cannot know whether both influx and efflux of glycine were altered to a similar extent, which would result in no change in the net uptake even though there might have been intracellular changes in glycine metabolism.

Upon SLC7A10 inhibition during adipogenesis, the uptake of the negatively charged amino acids glutamate and aspartate was increased. Glutamate can be converted into the TCA intermediate α-KG, while aspartate is catabolized to oxaloacetate (Martínez-Reyes and Chandel, 2020). Together with the increase in uptake and catabolism of the BCAAs, which can be directed towards the TCA cycle via generation of propionyl-CoA or acetyl-CoA (Ye *et al.*, 2020), the increased influx of these amino acids may fuel glyceroneogenesis and *de novo* FA synthesis (Reshef *et al.*, 2003). Together, the increase of these processes may, at least in part, promote the increased fat storage seen when SLC7A10 is perturbed. This mechanism helps to explain how the adipocytes could increase lipid accumulation despite the decreased insulin-stimulated glucose uptake upon SLC7A10 inhibition. Our data and previous studies in 3T3-L1 adipocytes particularly implicate increased uptake of BCAAs in lipid storage during adipogenesis (Green *et al.*, 2015; Nilsen *et al.*, 2020), where BCAAs may replace around 30% of the glucose and glutamine that is otherwise used to fuel proliferating cells, in order to provide substrates for lipogenesis (Green *et al.*, 2015). Another interesting finding in this thesis is the increase in cellular cholesterol levels and expression of cholesterol synthesis genes when adipocyte SLC7A10 is impaired. A previous study links cholesterol synthesis in late adipogenesis to increased catabolism of leucine (Halama *et al.*, 2016), which is consistent with our findings. Taken together, the effects of SLC7A10 inhibition on adipocyte metabolic processes is most likely caused by both the decreased serine influx, and also involves increased uptake and consumption of other lipogenic amino acids.

5.1.2 Altered ROS generation and lipid and energy metabolism

Strengthening the link between adipocytic SLC7A10 and fat storage, is our novel finding showing increased release of 3-HIB when SLC7A10 is impaired. We found that higher 3-HIB release follows the pattern of increased adipocyte differentiation, fat storage and mRNA expression of *HIBCH* which removes the CoA moiety of 3-HIB-CoA, allowing 3-HIB to leave the cell. When we knocked down Hibch in mouse adipocytes, we found a decrease in 3-HIB levels in the media, and a 35-40% decrease in lipid accumulation. This indicates that valine provides a major source of the carbons

that are used to fuel fat storage in adipocytes and/or that 3-HIB release is important to promote lipid accumulation. Consistently, studies have found that adipocytes switch from using glutamine and glucose as main fuel for TAG synthesis during differentiation to utilizing BCAAs (Crown, Marze and Antoniewicz, 2015; Green *et al.*, 2015). Conceivably, when 3-HIB exits the cell after Hibch removes the CoA moiety, there may be less carbon left from the degraded valine to enter the TCA cycle. On the other hand, this provides a free CoA (Taniguchi *et al.*, 1996) that can be used in other metabolic processes, for example to further catabolize leucine and isoleucine to propynyl-CoA or acetyl-CoA. Thus, future studies should investigate the effect of Hibch KD on the uptake of leucine and isoleucine, and possibly also other amino acids, and assess whether these may fuel the increase in fat storage associated with 3-HIB release. When impairing SLC7A10 we see increased 3-HIB release and HIBCH expression, which is consistent with the Hibch KD data. Moreover, we show that there is a somewhat higher relative net increase in uptake of leucine and isoleucine when SLC7A10 is inhibited compared to valine, while the levels of valine taken up by the cells per hour appear to be higher than leucine and isoleucine both with and without impairment of SLC7A10. Other studies have found that valine and isoleucine catabolism in adipocytes contribute to 100% of lipogenic propionyl-CoA (Crown, Marze and Antoniewicz, 2015), while valine and isoleucine are responsible for 25-30% of the lipogenic acetyl-CoA (Crown, Marze and Antoniewicz, 2015; Green *et al.*, 2015). Thus, the extra leucine and isoleucine uptake we observe may, independent of valine, contribute to fuel the excess fat storage or, since the valine influx is already high, the amount of valine that is not released as 3-HIB might be high enough to contribute to the increased lipid accumulation. This should however be further explored by future studies, tracing the metabolic route of the carbons from the three BCAAs taken up when SLC7A10 is impaired compared to controls. Of note, a recent *in vivo* study that administered radiolabeled BCAAs to mice showed that around 20% of 3-HIB in plasma was radiolabeled already within 3 minutes, indicating extremely fast turnover in the valine degradation pathway (Neinast *et al.*, 2019). A similar strategy can be applied to trace the intracellular fate of 3-HIB in adipocytes.

The link between adipocyte 3-HIB release and fat storage, seen in both in Paper 2 and 3, further strengthens the hypothesis that 3-HIB might function as an autocrine, paracrine and/or endocrine signaling molecule, reflecting a state of increased fat storage and/or nutrient availability in adipocytes. Previous studies have indicated that 3-HIB can increase FA uptake in skeletal muscles and thereby promote IR (Jang *et al.*, 2016), while adipose-derived microvascular endothelial cells did not respond to 3-HIB treatment (Mardinoglu *et al.*, 2018). In this thesis, we observe a novel 3-HIB-mediated FA uptake in both white and brown adipocytes, which might contribute to storing excess FAs as lipids in white adipocytes and to catabolizing them via mitochondrial respiration and thermogenesis in brown fat cells (Lee, Mottillo and Granneman, 2014). Surprisingly, we saw no clear effect on lipid accumulation by 3-HIB treatment in neither of the adipocyte subtypes (data not shown). The reasons for this is not clear, but it might be due to insufficient FA concentrations in the cell medium when treating with 3-HIB throughout adipogenesis, which might differ from the *in vivo* situation when extracellular 3-HIB levels are high. Both the FA uptake assay and the glucose uptake assay measure uptake after deprivation of FAs and glucose, respectively. Based on our studies we also could not assess how 3-HIB might contribute to increased lipid storage by affecting the TAG-FA cycle between adipocytes and other organs (e.g., the liver) (Reshef *et al.*, 2003), which would require *in vivo* studies. Of note, our data show that SLC7A10 inhibition, associated with increased 3-HIB release, can increase adipocyte glyceroneogenesis which is an important regulator of the TAG-FA cycle (Reshef *et al.*, 2003).

3-HIB treatment in white adipocytes caused decreased ROS and mitochondrial respiration, while the opposite was seen in brown fat cells (**Figure X.1**). This might be due to the large difference in the amount of expressed BCAA catabolic genes that we observe between the adipocyte subtypes, where brown adipocytes have the highest expression (Nilsen *et al.*, 2020), as well as the different basic functions of brown and white adipocytes. In addition, recent reports show that the BCAA catabolizing enzyme complex branched-chain 2-oxoacid dehydrogenase (BCKDH), localized in mitochondria, can produce O- and H_2O_2 (oxygen species) at higher rates than complex

I in the respiratory chain (Ribas, García-Ruiz and Fernández-Checa, 2014). We observe a 9-fold higher expression of *Bckdha* and 3-fold higher expression of *Bckdhb* in differentiated brown compared to white adipocytes, which might explain the increase in ROS seen in brown but not white adipocytes. Conceivably, the increased BCAA uptake and *BCKDHB* expression we observe when SLC7A10 function is impaired might therefore also have contributed to elevated levels of ROS.

Furthermore, the observation that 3-HIB treatment affects ROS and mitochondrial respiration is consistent with increased levels of ROS upon higher activity of mitochondrial oxidative phosphorylation (respiratory chain) (Rosen *et al.*, 2014; Liemburg-Apers *et al.*, 2015). Importantly, a recent study found that adipocyte respiration does not increase mitochondrial ROS unless glucose is available, and that adipocyte respiration is mainly fueled from other sources than glucose (Krycer *et al.*, 2020). It is also important to take into account that there are several other known sources of cellular ROS production (Engin, 2017) in addition to mitochondrial ROS, such as generation by NADPH (Nicotinamide adenine dinucleotide phosphate) oxidases, glyceraldehyde auto-oxidation and as by-products from several other cellular pathways (Manna and Jain, 2015; Hauck *et al.*, 2019). In SLC7A10-inhibited adipocytes, we observe an increased ROS generation (after 15-45 minutes with inhibition) and subsequent reduced mitochondrial respiration (24 hours inhibition). Conversely, we find that Slc7a10 overexpression in 3T3-L1 adipocytes decreases ROS levels and increases mitochondrial respiratory capacity. These data are consistent with a report showing that ROS generation decreases respiration in white adipocytes (Wang *et al.*, 2010). Other previous studies have reported that controlled ROS production in adipocytes contributes to intracellular signaling, and that short-term ROS generation can promote adipogenesis and lipid accumulation (Lee *et al.*, 2009; Tormos *et al.*, 2011; Wang *et al.*, 2015; Jones IV *et al.*, 2016; Hosios and Vander Heiden, 2018), and that stable high ROS and reduced antioxidant capacity are observed in obesity (Masschelin *et al.*, 2020). Thus, the order of events, nutrients available, and the amount as well as intracellular localization and reason for ROS generation, might all decide the outcomes and effects observed on adipocyte biological processes.

The reduced GSH production, and consequent increase in ROS generation when SLC7A10 function is inhibited, may also direct the metabolism away from oxidative phosphorylation and towards the increase in lipid accumulation we observe as a feedback mechanism to protect the cells against prolonged high ROS levels (Armstrong *et al.*, 2004; Quijano *et al.*, 2016). Previous studies have observed that high ROS levels in adipocytes can increase lipid synthesis (Jones IV *et al.*, 2016). Homeostatic levels of ROS are thought to contribute to adipogenesis and healthy fat cells, while too low or too high levels contribute to adipocyte dysfunction (Rosen and Spiegelman, 2014). We observe increased ROS levels when inhibiting SLC7A10, which are decreased when ROS scavenger is added in an insulin-free condition. However, when insulin is present, the ROS scavenger failed to significantly reduce lipid accumulation in SLC7A10-inhibited cells. These data suggest that insulin-dependent lipid accumulation might not be mediated via increased ROS, and that the lipid accumulation we observe upon SLC7A10 inhibition is at least partly independent of insulin and rather dependent on ROS, also consistent with the reduced insulin-stimulated glucose uptake upon SLC7A10 impairment. Taken together, our data support a central role for altered mitochondrial respiration and ROS generation in lipid accumulation.

Since serine is thought to be mainly taken up or synthesized from glucose or glycine (Amelio *et al.*, 2014), one might hypothesize that serine biosynthesis is increased when SLC7A10 is impaired. Previous studies in cancer cells have revealed that serine starvation diverts intermediates from glycolysis to generation of pyruvate and serine biosynthesis to support cell proliferation (Ye *et al.*, 2012; Maddocks *et al.*, 2017). Surprisingly, we do not observe increased expression of genes involved in serine biosynthesis (data not shown), suggesting that serine deprivation in adipocytes due to SLC7A10 inhibition diverts carbons from taken up nutrients mainly to pyruvate production. The increase in pyruvate generation seen in cancer cells, from other sources than glucose, is found to be utilized by cells to increase oxidative phosphorylation (Chaneton *et al.*, 2012), but since white adipocytes are specialized to store fat (Cohena and Spiegelmanb, 2016), synthesis of TAG may be prioritized. Such a prioritization of pyruvate for TAG synthesis independent of glucose contributes to explain the reduced insulin-stimulated glucose uptake we find when SLC7A10 is inhibited.

The reduced insulin-stimulated glucose uptake and the concomitant reduction in serine uptake upon SLC7A10 inhibition is consistent with a previous study showing that serine improves insulin sensitivity, and that lack of serine can cause cellular IR (Holm and Buschard, 2019). Thus, the SLC7A10 inhibitor-mediated reduction in serine uptake may, at least in part, explain the decreased insulin-dependent glucose uptake. The glucose uptake might also be reduced due to increased ROS and oxidative stress-mediated carbonylation of GLUT4 (Masschelin *et al.*, 2020), and to protect the cells against more glucose-fueling mitochondrial respiration which could generate even higher ROS levels (Stowe and Camara, 2009). A study in 3T3-L1 adipocytes found that exposure to transient ROS improved insulin sensitivity, while chronic ROS promoted IR (Ma *et al.*, 2018). Furthermore, if most of the available glucose has to be utilized in the pentose phosphate pathway to generate NADPH for lipogenesis (Mullarky and Cantley, 2015) in response to SLC7A10 inhibition, the amount of energy substrates available for glycolysis and subsequently oxidative phosphorylation might be reduced. This prioritization of substrates for TAG synthesis might, at least in part, contribute to explain the reduced mitochondrial respiration we observe in SLC7A10-inhibited adipocytes.

5.2 SLC7A10, 3-HIB and BCAAs in obesity and related diseases *in vivo*

5.2.1 Associations with traits of metabolic syndrome

Our human cohort data reveal inverse associations for adipocytic *SLC7A10* expression and plasma 3-HIB concentrations with multiple traits of metabolic syndrome. In line with our data for SLC7A10, a previous study in SC adipocytes points to an inverse relationship between *SLC7A10* mRNA expression in SC fat and BMI, HOMA-IR and circulating TAG levels (Small *et al.*, 2011). In this study, Small *et al*. showed that a T2D-risk SNP in the genetic locus encoding the TF KLF14 caused alter expression of several adipocytic genes in *trans*, where *SLC7A10* was the primary gene globally showing significantly reduced expression (Small *et al.*, 2011). In a follow-up study, the same group reported that the *KLF14* locus variants also associate with adipocyte

hypertrophy and body fat distribution specifically in females (Small *et al.*, 2018). These studies of the *KLF14* locus partly linked to expression of adipose *SLC7A10* mRNA indirectly support the associations we observe between *SLC7A10* mRNA and adipocyte hypertrophy. However, a recent study using machine learning based technics to investigate adipocyte phenotypes in 4 large cohorts (n=1288), found no association between the *KLF14* locus variants, or any other variants genome-wide, and adipocyte morphology phenotypes (Glastonbury *et al.*, 2020). The authors discuss that this might be due to the low cohort size (n<50) used in the initial study (Glastonbury *et al.*, 2020). To clarify the genetic influence on *SLC7A10* expression and function, future studies may use genome editing approaches to modulate candidate variants in human adipocytes *in vitro* to determine phenotypic effects on adipocyte biology, as demonstrated for the *FTO* locus (Claussnitzer *et al.*, 2015). Of note, although we here did not investigate sex-specific regulation of *SLC7A10*, and how this might relate to cellular functions, some of our cohorts (e.g., SibPair) show around twice as high *SLC7A10* mRNA in females compared to males with obesity (data not shown). It should also be noted that only one of the human primary cultures we used came from a man. Future studies should therefore investigate whether genetic variants influence *SLC7A10* mRNA expression and function differently in men and women, and also explore the biological basis for and phenotypic consequences of the apparent sex-specific *SLC7A10* expression.

Regardless of genetic and sex-specific effects, we confirm a link between *SLC7A10* and adipocyte hypertrophy in our human cohorts, consisting of both men and women, and our data from the overfeeding experiment in the Slc7a10b loss-of-function zebrafish indicates that the transporter is a causal factor regulating visceral fat cell size. A study in humans showed that overfeeding did not increase visceral fat cell size in subjects that were already insulin resistant, while people who were insulin sensitive developed significantly larger visceral fat cells (McLaughlin et al., 2016), which suggests that our mutant zebrafish were not insulin resistant before overfeeding. Thus, the negative effects of low adipocyte Slc7a10 levels on health might primarily occur when the nutrient supply is high. In people with obesity who are insulin sensitive, the expression levels of *SLC7A10* is high, while people with obesity who are insulin resistant have low expression, indicating that SLC7A10 might be important in regulating whole body

insulin sensitivity. However, more *in vivo* research on causality and the order of cellular events after SLC7A10 impairment is warranted, and the specific mechanisms regulating the relationship between SLC7A10 and IR must be further investigated.

There is some contradicting evidence in the obesity research field regarding the relationship between adipocyte hypertrophy and risk of obesity-related diseases, and this seems to be highly dependent on the adipose depot affected and on whether the subjects are insulin sensitive or resistant at the start of the study (McLaughlin *et al.*, 2016). Supporting the commonly accepted relationship, we observed a strong positive correlation between fat cell size and HOMA-IR in the cohort where we also found a marked inverse correlation between *SLC7A10* expression and SC adipocyte size (data not shown). A recent study found that SC hypertrophy was not related to HOMA-IR, while visceral adipocyte size significantly correlated positively with IR (Verboven *et al.*, 2018). This study by Verboven and coworkers however contained few participants, which might indicate that effects on visceral adipocyte size are more readily detected and hence stronger compared to SAT. Yet, a previous report showed a strong positive correlation between adipocyte size in SC and OM adipose tissue in the context of IR (Klöting *et al.*, 2010). In line with our results, this and other studies showed SC fat cell enlargement to be positively associated with both whole-body and SC adipocyte IR (Lundgren *et al.*, 2007), and to be defined as a predictor of T2D risk (Weyer *et al.*, 2001). In our human cohorts, we see highly consistent negative associations between *SLC7A10* expression in SAT, and both SC adipocyte cell size and HOMA-IR.

3-HIB levels in plasma have previously been linked to future risk of T2D (Mardinoglu *et al.*, 2018) and IR after gestational diabetes mellitus (Andersson-Hall *et al.*, 2018). In our study, we find a clear pattern showing high 3-HIB concentrations in plasma of people with T2D, lower in hyperglycemia and lowest in normoglycemia. Plasma 3-HIB levels were also strongly correlated with several metabolic risk factors in our human cohorts and were strongly reduced one year after bariatric surgery in association with major fat loss and improved insulin sensitivity. Interestingly, we also observed an increase in plasma 3-HIB concentration 1 week after the weight loss surgery, which may reflect transient adaptive changes in metabolism due to acutely reduced nutrient supply

and insulin sensitivity. Nonetheless, the data indicate that 3-HIB is a promising biomarker of future disease risk, at least under conditions of more stable nutrient supply. Moreover, we find an inverse association between *SLC7A10* mRNA expression in SAT and plasma 3-HIB and BCAA levels in the MyoGlu cohort. To assess causality, it would be valuable to generate an adipocyte-specific SLC7A10 knockout model and measure the effect on circulating 3-HIB and BCAA levels, along with changes in body fat distribution, adipocyte morphology, IR, glucose tolerance and other disease risk markers. In such a model, it would also be relevant to examine the metabolic fate of administered radiolabeled BCAAs, such as on circulating and tissue levels of radiolabeled valine-derived 3-HIB using the approaches in the recent study by Neinast *et al.* (Neinast *et al.*, 2019).

5.2.2 Processes related to obesity and insulin resistance

The levels of certain amino acids in plasma have been associated with IR and T2D (Yamada *et al.*, 2015). Serine and glycine levels are inversely linked to metabolic syndrome, while the BCAAs (as well as glutamate) are strongly associated with disease risk (Felig, Marliss and Cahill, 1969; Yamada *et al.*, 2015), consistent with our cohort data. In line with the reduced serine uptake and total GSH levels we observe when inhibiting SLC7A10 in adipocytes, previous studies in liver cells have found that inability to synthesize GSH is linked to increased IR (Guarino *et al.*, 2003; Guarino and Macedo, 2006) and GSH supplementation to decrease IR (El-Hafidi *et al.*, 2018). However, studies modulating GSH synthesis in adipocytes to investigate effects on thermogenesis and insulin sensitivity report mixed results (Kobayashi *et al.*, 2009; Lettieri Barbato *et al.*, 2015; Achari and Jain, 2017; Lu *et al.*, 2017).

We find increased FA uptake in both WAT and BAT after 3-HIB treatment, which is related to increased fat storage in adipocytes. However, a recent study reported that FA uptake into BAT is blunted in obesity (Saari *et al.*, 2020), suggesting that the FA uptake we see upon addition of 3-HIB might facilitate a transient increase to compensate for increased nutrient availability. Importantly, increased ROS in adipocytes has also been

found prior to IR, and is suggested to be a possible cause and not only consequence of metabolic disease (Maslov *et al.*, 2019), consistent with our data. In addition, reduced mitochondrial respiration in WAT has been described as a hallmark of obesity (mouse models) (Schöttl *et al.*, 2015). In light of these recent studies and our SLC7A10 overexpression data, increasing adipocyte SLC7A10 expression and function may be a possible treatment strategy in people with obesity or metabolic disease.

Although we found that SLC7A10 correlates inversely with obesity and IR, we cannot rule out that the increased lipid accumulation upon SLC7A10 inhibition could be metabolically beneficial in early phases, prior to fat overload (Lee, Mottillo and Granneman, 2014; Ghaben and Scherer, 2019). Consistent with a transient beneficial effect is the upregulation of *PPARγ* expression which we find when inhibiting SLC7A10 for 24 hours, together with increased expression of other adipogenic TFs. Genetic evidence supports that upregulation of *PPARγ* and a related increase in adipogenesis, at least in SC fat, supports metabolically beneficial functions in adipocytes that protect against IR and T2D (Claussnitzer *et al.*, 2014). Such temporally beneficial effects might contribute to explain the observed down-regulation of genes involved in biological processes such as immune response in the zebrafish overfeeding study, in a reciprocal relationship between adipocyte metabolism and immune cell function (Macdougall *et al.*, 2018). With a persistent increase in ROS, shown to promote adipocyte IR (Ma *et al.*, 2018), lipid accumulation may become a primary outlet for handling excess nutrients. While this may initially be protective, especially in SAT, over time the lipid storage may translate into excessive hypertrophy of individual adipocytes concomitant with persistent oxidative stress and chronic low-grade inflammation (Masschelin *et al.*, 2020).

6. Conclusions

In the present thesis, we provide new knowledge on SLC7A10 functions in adipocytes, and the role of this carrier and adipocyte amino acids metabolism in obesity and development of related diseases.

Across several human cohorts we find a consistent inverse correlation between *SLC7A10* mRNA and traits related to the metabolic syndrome. In experimental studies, we find that inhibition of SLC7A10 reduces serine uptake, manifesting in decreased production of the antioxidant GSH and increased ROS generation. Furthermore, SLC7A10 impairment decreases insulin-dependent glucose uptake and maximal mitochondrial respiratory capacity, in association with increased lipid accumulation *in vitro* and adipocyte hypertrophy in *in vivo* overfed Slc7a10b loss-of-function zebrafish. When persistent, these processes contribute to development of systemic IR and obesity-related diseases. The sufficiency of altered SLC7A10 to exert marked effects on adipocyte metabolism was further supported by decreased ROS generation and increased mitochondrial respiratory capacity after SLC7A10 overexpression in 3T3-L1 adipocytes.

In our multiple human cohorts, circulating BCAA and 3-HIB levels are positively associated with metabolic risk traits and T2D. BCAA uptake and catabolism and 3-HIB efflux increase during human and mouse adipocyte differentiation *in vitro*, reflecting and possibly promoting lipid storage. Causal effects are supported by knockdown of the free 3-HIB generating enzyme *Hibch*, which leads to a reduction in both lipid accumulation and 3-HIB release from murine white and brown adipocytes. Furthermore, 3-HIB treatment markedly modulates insulin-stimulated glucose uptake, FA uptake, mitochondrial respiration and ROS generation in both white and brown adipocytes, but for the two latter in the opposite direction between adipocyte subtypes, which may reflect as well as contribute to their different functional characteristics. Together, this indicates that 3-HIB may serve as an adipocyte-derived signaling molecule that contributes to communication between cells and/or tissues to modulate whole body glucose, fat and amino acid metabolism.

Our data indicate, that the increased lipid accumulation observed when SLC7A10 is inhibited might be fueled by a concomitant increase in adipocytic uptake of the amino acids aspartate, glutamate and BCAAs, associated with higher FA uptake, TCA cycle activity, glyceroneogenesis and lipogenesis. *SLC7A10* mRNA expression levels in SAT *in vivo* are inversely correlated with the 3-HIB level in plasma, in line with our *in vitro* data showing increased HIBCH protein expression and 3-HIB release when SLC7A10 function is impaired. This further strengthens the inverse association between SAT *SLC7A10* expression and traits of metabolic syndrome, IR and T2D.

Overall, we have identified SLC7A10 and 3-HIB as novel players in the regulation of adipocyte metabolism, and as potential therapeutic targets and biomarkers in the context of obesity, IR and related diseases.

7. Future perspectives

In the present study we have uncovered novel functions of SLC7A10 and 3-HIB in the regulation of adipocyte metabolism. However, the research has raised new questions which remain to be addressed.

Firstly, it would be interesting to investigate effects on adipocyte amino acid flux, GSH synthesis and metabolic processes when SLC7A10 is overexpressed, in combination with treatment with ROS-inducing compounds such as lipopolysaccharide (LPS), to explore to what extent SLC7A10 can protect against adipocyte dysfunction. Secondly, it would be valuable to delineate more details on the mechanisms by which SLC7A10 and 3-HIB mediate their effects on adipocyte function, such as measuring the effect of SLC7A10 overexpression or 3-HIB stimulation on mitochondrial biogenesis. This could help reveal whether the observed changes in mitochondrial respiration could be, at least in part, caused by an increase in the number of mitochondria, or if they are caused by altered substrate availability or substrate-channeling towards oxidative phosphorylation.

Importantly, we find a novel relationship between adipocytic SLC7A10, uptake of BCAAs and other amino acids not known to be transported by SLC7A10, and the release of 3-HIB from adipocytes. Here, the specific mechanistic pathways linking these features needs to be further explored, by for example performing radioactive or immunohistochemistry studies following the metabolic path of carbons from all the BCAAs when SLC7A10 is inhibited compared to control. In addition, future studies should investigate the effect of Hibch KD on the uptake of leucine and isoleucine, and other relevant amino acids.

Moreover, it would be of great interest to further explore the time-dependent metabolic effects of 3-HIB treatment in adipocytes. Also, the total contribution of adipocyte 3-HIB release to the total circulating concentration *in vivo* should be estimated, and examining whether this catabolite might participate in organ cross-talk, and how it might exert its communicative effects in both an autocrine, paracrine and endocrine manner, thereby influencing systemic IR and metabolic disease risk.

Furthermore, it appears relevant to investigate SLC7A10 in relation to aging, since serine has emerged as an important player in aging via epigenetic and metabolic mechanisms (Wu *et al.*, 2020). In addition, determining whether SLC7A10 has sex-specific effects would be valuable, in part because alleles close to *KLF14*, a "master regulator" of adipocyte gene expression including effects on SLC7A10, might influence T2D-risk through female-specific effects on fat distribution and adipocyte size (Small *et al.*, 2018). Because the relationship between BCAAs and metabolic risk traits are found to be affected by sex differences (Newbern *et al.*, 2014; Thalacker-Mercer *et al.*, 2014), this should be investigated in regard to adipocyte 3-HIB release as well.

It would also be of interest to identify SNPs within the human *SLC7A10* locus and investigate their possible impact on adipocyte metabolism as well as the mechanisms (e.g., enhancers and transcription factors) that control *SLC7A10* expression. Additionally, generating a stable KD of SLC7A10 or editing relevant SNPs in human immortalized adipocytes by genome editing *in vitro* would be valuable tools to further explore SLC7A10's role in adipocytes, obesity and IR.

In our overfeeding study, we utilized zebrafish with a global Slc7a10b loss-of-function mutation, and we collected RNA, DNA and proteins from VAT at the end of the study. Due to few zebrafish-specific antibodies available, we did not measure protein expression. It would be useful to develop antibodies for the zebrafish proteins of interest and perform proteomics, comparing WT and loss-of-function, and/or perform similar experiments in other animal models. Because Slc7a10 is also expressed in the brain, it would have been of great interest to conduct another overfeeding experiment in zebrafish or mice with an adipocyte-specific Slc7a10 knockout. Another possibility would be to inject the SLC7A10 inhibitor LU into mouse peripheral tissues, since it is not able to cross the blood brain barrier, to investigate effects on fat storage and whole-body insulin sensitivity.

While impairment of SLC7A10 exerts negative effects on adipocyte metabolism, overexpressing or amplifying the transporter functions *in vivo* may cause improvements in adipocyte metabolism, as supported by our findings in Paper 1. Identifying upstream

positive regulators of SLC7A10 could provide additional direct targets for modulating adipocyte dysfunction, with the view to develop new strategies to improve insulin sensitivity and reduce risk of both T2D and CVD in people with obesity.

8. References

Abel, E. D. *et al.* (2001) 'Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver', *Nature*. Nature, 409(6821), pp. 729–733. doi: 10.1038/35055575.

Achari, A. E. and Jain, S. K. (2017) 'l-Cysteine supplementation increases insulin sensitivity mediated by upregulation of GSH and adiponectin in high glucose treated 3T3-L1 adipocytes', *Archives of Biochemistry and Biophysics*. Academic Press, 630, pp. 54–65. doi: 10.1016/J.ABB.2017.07.016.

Adami, G. F. *et al.* (2019) 'Adipose Tissue Composition in Obesity and After Bariatric Surgery', *Obesity Surgery*. Springer New York LLC, pp. 3030–3038. doi: 10.1007/s11695-019-04030-z.

Adeva-Andany, M. M. *et al.* (2017) 'Enzymes involved in branched-chain amino acid metabolism in humans', *Amino Acids*. Springer-Verlag Wien, pp. 1005–1028. doi: 10.1007/s00726-017-2412-7.

Adipose Tissue and Adipokines in Health and Disease (2007). Humana Press. doi: 10.1007/978-1-59745-370-7.

Albrektsen, T. *et al.* (2002) 'Novel genes regulated by the insulin sensitizer rosiglitazone during adipocyte differentiation', *Diabetes*. American Diabetes Association Inc., 51(4), pp. 1042–1051. doi: 10.2337/diabetes.51.4.1042.

Amelio, I. *et al.* (2014) 'Serine and glycine metabolism in cancer', *Trends in Biochemical Sciences*, 39(4), pp. 191–198. doi: 10.1016/j.tibs.2014.02.004.

Amores, A. *et al.* (1998) 'Zebrafish hox clusters and vertebrate genome evolution', *Science*. American Association for the Advancement of Science, 282(5394), pp. 1711–1714. doi: 10.1126/science.282.5394.1711.

Andersen, E. *et al.* (2019) 'Preadipocytes from obese humans with type 2 diabetes are epigenetically reprogrammed at genes controlling adipose tissue function', *International Journal of Obesity*. Nature Publishing Group, 43(2), pp. 306–318. doi: 10.1038/s41366-018-0031-3.

Andersson-Hall, U. *et al.* (2018) 'Higher Concentrations of BCAAs and 3-HIB Are Associated with Insulin Resistance in the Transition from Gestational Diabetes to Type 2 Diabetes', *Journal of Diabetes Research*. Hindawi, 2018, pp. 1–12. doi: 10.1155/2018/4207067.

Arany, Z. and Neinast, M. (2018) 'Branched Chain Amino Acids in Metabolic Disease', *Current Diabetes Reports*. Springer US, 18(10), p. 76. doi: 10.1007/s11892-018-1048-7.

Armstrong, J. S. *et al.* (2004) 'The redox regulation of intermediary metabolism by a superoxide-aconitase rheostat', *BioEssays*. John Wiley & Sons, Ltd, 26(8), pp. 894–900. doi: 10.1002/bies.20071.

Arner, P. (1997) 'Regional adipocity in man', in *Journal of Endocrinology*. J Endocrinol, pp. 191–192. doi: 10.1677/joe.0.1550191.

Association, A. D. (2017) ‘Classification and diagnosis of diabetes’, *Diabetes Care*. American Diabetes Association Inc., 40(Supplement 1), pp. S11–S24. doi: 10.2337/dc17-S005.

Badoud, F. *et al.* (2014) ‘Serum and adipose tissue amino acid homeostasis in the metabolically healthy obese’, *Journal of Proteome Research*. American Chemical Society, 13(7), pp. 3455–3466. doi: 10.1021/pr500416v.

Ballard, F. J., Hanson, R. W. and Leveille, G. A. (1967) *Phosphoenolpyruvate Carboxykinase and the Synthesis of Glyceride-Glycerol from Pyruvate in Adipose Tissue**, *The Journal of biological chemistry*. Available at: http://www.jbc.org/.

Berg, J. M., Tymoczko, J. L. and Stryer, L. (2002) ‘Carbon Atoms of Degraded Amino Acids Emerge as Major Metabolic Intermediates’. W H Freeman. Available at: https://www.ncbi.nlm.nih.gov/books/NBK22453/.

Billard, J.-M. and Freret, T. (2018) ‘Asc-1 transporter activation: an alternative to rescue age-related alterations in functional plasticity at rat hippocampal CA3/CA1 synapses’, *Journal of Neurochemistry*. doi: 10.1111/jnc.14586.

Blanchard, P.-G. *et al.* (2018) ‘PPARγ is a major regulator of branched-chain amino acid blood levels and catabolism in white and brown adipose tissues’, *Metabolism*. W.B. Saunders, 89, pp. 27–38. doi: 10.1016/J.METABOL.2018.09.007.

Brown, J. M. *et al.* (2014) ‘In vitro Characterization of a small molecule inhibitor of the alanine serine cysteine transporter -1 (SLC7A10).’, *Journal of neurochemistry*, 129(2), pp. 275–83. doi: 10.1111/jnc.12618.

Burnet, P. *et al.* (2008) ‘Expression of D-serine and glycine transporters in the prefrontal cortex and cerebellum in schizophrenia’, *Schizophrenia Research*, 102(1–3), pp. 283–294. doi: 10.1016/j.schres.2008.02.009.

Burrill, J. S. *et al.* (2015) ‘Inflammation and ER stress regulate branched-chain amino acid uptake and metabolism in adipocytes.’, *Molecular endocrinology (Baltimore, Md.)*. The Endocrine Society, 29(3), pp. 411–20. doi: 10.1210/me.2014-1275.

Cersosimo, E. *et al.* (2015) ‘Pathogenesis of Type 2 Diabetes Mellitus ncbi’. MDText.com, Inc. Available at: http://www.ncbi.nlm.nih.gov/books/NBK279115/.

Chaneton, B. *et al.* (2012) ‘Serine is a natural ligand and allosteric activator of pyruvate kinase M2’, *Nature*. Nature Publishing Group, 491(7424), pp. 458–462. doi: 10.1038/nature11540.

Chatterjee, S., Khunti, K. and Davies, M. J. (2017) ‘Type 2 diabetes’, *The Lancet*. Lancet Publishing Group, pp. 2239–2251. doi: 10.1016/S0140-6736(17)30058-2.

Chen, B., Zheng, Y.-M. and Zhang, J.-P. (2018) ‘Comparative Study of Different Diets-Induced NAFLD Models of Zebrafish’, *Frontiers in Endocrinology*. Frontiers Media S.A., 9(JUL), p. 366. doi: 10.3389/fendo.2018.00366.

Chen, L. *et al.* (2015) ‘Effect of lifestyle intervention in patients with type 2 diabetes: A meta-analysis’, *Metabolism: Clinical and Experimental*. W.B. Saunders, 64(2), pp. 338–347. doi:

10.1016/j.metabol.2014.10.018.

Chen, P. *et al.* (2018) 'Abdominal subcutaneous adipose tissue: A favorable adipose depot for diabetes?', *Cardiovascular Diabetology*. BioMed Central Ltd., 17(1), p. 93. doi: 10.1186/s12933-018-0734-8.

Claussnitzer, M. *et al.* (2014) 'Leveraging Cross-Species Transcription Factor Binding Site Patterns: From Diabetes Risk Loci to Disease Mechanisms', *Cell*, 156(1–2), pp. 343–358. doi: 10.1016/j.cell.2013.10.058.

Claussnitzer, M. *et al.* (2015) '*FTO* Obesity Variant Circuitry and Adipocyte Browning in Humans', *New England Journal of Medicine*. Massachusetts Medical Society, 373(10), pp. 895–907. doi: 10.1056/NEJMoa1502214.

Cohena, P. and Spiegelmanb, B. M. (2016) 'Cell biology of fat storage', *Molecular Biology of the Cell*. American Society for Cell Biology, pp. 2523–2527. doi: 10.1091/mbc.E15-10-0749.

Cornier, M. A. *et al.* (2011) 'Assessing adiposity: A scientific statement from the american heart association', *Circulation*, 124(18), pp. 1996–2019. doi: 10.1161/CIR.0b013e318233bc6a.

Courcoulas, A. P. *et al.* (2014) 'Long-term outcomes of bariatric surgery: a National Institutes of Health symposium.', *JAMA surgery*. NIH Public Access, 149(12), pp. 1323–9. doi: 10.1001/jamasurg.2014.2440.

Courcoulas, A. P. *et al.* (2020) 'Bariatric Surgery vs Lifestyle Intervention for Diabetes Treatment: 5-Year Outcomes From a Randomized Trial', *Journal of Clinical Endocrinology and Metabolism*. Endocrine Society, 105(3). doi: 10.1210/clinem/dgaa006.

Crown, S. B., Marze, N. and Antoniewicz, M. R. (2015) 'Catabolism of Branched Chain Amino Acids Contributes Significantly to Synthesis of Odd-Chain and Even-Chain Fatty Acids in 3T3-L1 Adipocytes', *PLOS ONE*. Edited by G. P. Tochtrop, 10(12), p. e0145850. doi: 10.1371/journal.pone.0145850.

Cushmans, S. W. and Wardzalafj, L. J. (1980) *Potential Mechanism of Insulin Action on Glucose Transport in the Isolated Rat Adipose Cell*, *The Journal of biological chemistry*. Available at: https://pubmed.ncbi.nlm.nih.gov/6989818/.

Cypess, A. M. *et al.* (2009) 'Identification and Importance of Brown Adipose Tissue in Adult Humans', *New England Journal of Medicine*. Massachussetts Medical Society, 360(15), pp. 1509–1517. doi: 10.1056/NEJMoa0810780.

Cypess, A. M. *et al.* (2013) 'Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat.', *Nature medicine*. NIH Public Access, 19(5), pp. 635–9. doi: 10.1038/nm.3112.

Czech, M. P. (2020) 'Mechanisms of insulin resistance related to white, beige, and brown adipocytes', *Molecular Metabolism*. Elsevier GmbH, pp. 27–42. doi: 10.1016/j.molmet.2019.12.014.

Dankel, S. N. *et al.* (2014) 'Weight cycling promotes fat gain and altered clock gene expression in adipose tissue in C57BL/6J mice', *American Journal of Physiology-*

Endocrinology and Metabolism. American Physiological Society Bethesda, MD, 306(2), pp. E210–E224. doi: 10.1152/ajpendo.00188.2013.

Das, U. N. (2010) 'Obesity: Genes, brain, gut, and environment', *Nutrition*. Elsevier, pp. 459–473. doi: 10.1016/j.nut.2009.09.020.

Deng, Y. and Scherer, P. E. (2010) 'Adipokines as novel biomarkers and regulators of the metabolic syndrome.', *Annals of the New York Academy of Sciences*. NIH Public Access, p. E1. doi: 10.1111/j.1749-6632.2010.05875.x.

Doerr, A. (2009) 'Membrane protein structures', *Nature Methods*. Nature Publishing Group, 6(1), p. 35. doi: 10.1038/nmeth.f.240.

Drábková, P. *et al.* (2014) 'An Assay of Selected Serum Amino Acids in Patients with Type 2 Diabetes Mellitus.', *Advances in clinical and experimental medicine : official organ Wroclaw Medical University*, 24(3), pp. 447–51. doi: 10.17219/acem/29223.

Ducker, G. S. and Rabinowitz, J. D. (2017) 'One-Carbon Metabolism in Health and Disease', *Cell Metabolism*. Cell Press, 25(1), pp. 27–42. doi: 10.1016/J.CMET.2016.08.009.

Eckel, R. H., Grundy, S. M. and Zimmet, P. Z. (2005) 'The metabolic syndrome', in *Lancet*. Elsevier Limited, pp. 1415–1428. doi: 10.1016/S0140-6736(05)66378-7.

Eden, E. *et al.* (2007) 'Discovering Motifs in Ranked Lists of DNA Sequences', *PLoS Computational Biology*. Edited by E. Fraenkel. Public Library of Science, 3(3), p. e39. doi: 10.1371/journal.pcbi.0030039.

Eden, E. *et al.* (2009) 'GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists', *BMC Bioinformatics*, 10(1), p. 48. doi: 10.1186/1471-2105-10-48.

Ehmsen, J. T. *et al.* (2016) 'The astrocytic transporter SLC7A10 (Asc-1) mediates glycinergic inhibition of spinal cord motor neurons', *Scientific Reports*. Nature Publishing Group, 6(1), p. 35592. doi: 10.1038/srep35592.

El-Hafidi, M. *et al.* (2018) 'Glycine Increases Insulin Sensitivity and Glutathione Biosynthesis and Protects against Oxidative Stress in a Model of Sucrose-Induced Insulin Resistance', *Oxidative Medicine and Cellular Longevity*. Hindawi, 2018, pp. 1–12. doi: 10.1155/2018/2101562.

Engin, A. (2017) 'The pathogenesis of obesity-associated adipose tissue inflammation', in *Advances in Experimental Medicine and Biology*. Springer New York LLC, pp. 221–245. doi: 10.1007/978-3-319-48382-5_9.

Enzi, G. *et al.* (1986) 'Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography', *American Journal of Clinical Nutrition*. American Society for Nutrition, 44(6), pp. 739–746.

Fazakerley, D. J. *et al.* (2019) 'Muscle and adipose tissue insulin resistance: Malady without mechanism?', *Journal of Lipid Research*. American Society for Biochemistry and Molecular Biology Inc., pp. 1720–1732. doi: 10.1194/jlr.R087510.

Felig, P., Marliss, E. and Cahill, G. F. (1969) 'Plasma amino acid levels and insulin secretion

in obesity.', *The New England journal of medicine*, 281(15), pp. 811–6. doi: 10.1056/NEJM196910092811503.

Ferrannini, E. *et al.* (2018) 'Adipose tissue and skeletal muscle insulin-mediated glucose uptake in insulin resistance: Role of blood flow and diabetes', *American Journal of Clinical Nutrition*. Oxford University Press, 108(4), pp. 749–758. doi: 10.1093/ajcn/nqy162.

Fukasawa, Y. *et al.* (2000) 'Identification and characterization of a Na(+)-independent neutral amino acid transporter that associates with the 4F2 heavy chain and exhibits substrate selectivity for small neutral D- and L-amino acids.', *The Journal of biological chemistry*, 275(13), pp. 9690–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10734121.

Gao, X. *et al.* (2018) 'Serine Availability Influences Mitochondrial Dynamics and Function through Lipid Metabolism.', *Cell reports*. NIH Public Access, 22(13), pp. 3507–3520. doi: 10.1016/j.celrep.2018.03.017.

Gar, C. *et al.* (2018) 'Serum and plasma amino acids as markers of prediabetes, insulin resistance, and incident diabetes', *Critical Reviews in Clinical Laboratory Sciences*. Taylor and Francis Ltd, pp. 21–32. doi: 10.1080/10408363.2017.1414143.

Garvey, W. T. (2018) 'Clinical definition of overweight and obesity', in *Bariatric Endocrinology: Evaluation and Management of Adiposity, Adiposopathy and Related Diseases*. Springer International Publishing, pp. 121–143. doi: 10.1007/978-3-319-95655-8_7.

Gastaldelli, A. (2011) 'Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus', *Diabetes Research and Clinical Practice*. Elsevier, 93(SUPPL. 1), pp. S60–S65. doi: 10.1016/S0168-8227(11)70015-8.

Gesta, S., Tseng, Y.-H. and Kahn, C. R. (2007) 'Developmental Origin of Fat: Tracking Obesity to Its Source', *Cell*, 131(2), pp. 242–256. doi: 10.1016/j.cell.2007.10.004.

Ghaben, A. L. and Scherer, P. E. (2019) 'Adipogenesis and metabolic health', *Nature Reviews Molecular Cell Biology*. Nature Publishing Group, 20(4), pp. 242–258. doi: 10.1038/s41580-018-0093-z.

Glastonbury, C. A. *et al.* (2020) 'Machine Learning based histology phenotyping to investigate the epidemiologic and genetic basis of adipocyte morphology and cardiometabolic traits', *PLOS Computational Biology*. Edited by L. M. Iakoucheva. Public Library of Science (PLoS), 16(8), p. e1008044. doi: 10.1371/journal.pcbi.1008044.

Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration (BMI Mediated Effects) *et al.* (2014) 'Metabolic mediators of the effects of body-mass index, overweight, and obesity on coronary heart disease and stroke: a pooled analysis of 97 prospective cohorts with 1·8 million participants', *The Lancet*, 383(9921), pp. 970–983. doi: 10.1016/S0140-6736(13)61836-X.

Gonzalez-Hurtado, E. *et al.* (2018) 'Fatty acid oxidation is required for active and quiescent brown adipose tissue maintenance and thermogenic programing', *Molecular Metabolism*. Elsevier GmbH, 7, pp. 45–56. doi: 10.1016/j.molmet.2017.11.004.

Green, C. R. *et al.* (2015) 'Branched-chain amino acid catabolism fuels adipocyte

differentiation and lipogenesis', *Nature Chemical Biology*. Nature Research, 12(1), pp. 15–21. doi: 10.1038/nchembio.1961.

Guarino, M. P. *et al.* (2003) 'Hepatic glutathione and nitric oxide are critical for hepatic insulin-sensitizing substance action', *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 284(4), pp. G588–G594. doi: 10.1152/ajpgi.00423.2002.

Guarino, M. P. and Macedo, M. P. (2006) 'Co-administration of glutathione and nitric oxide enhances insulin sensitivity in Wistar rats', *British Journal of Pharmacology*, 147(8), pp. 959–965. doi: 10.1038/sj.bjp.0706691.

Gupta, R. K. *et al.* (2010) 'Transcriptional control of preadipocyte determination by Zfp423', *Nature*. NIH Public Access, 464(7288), pp. 619–623. doi: 10.1038/nature08816.

Halama, A. *et al.* (2016) 'Metabolic switch during adipogenesis: From branched chain amino acid catabolism to lipid synthesis', *Archives of Biochemistry and Biophysics*, 589, pp. 93–107. doi: 10.1016/j.abb.2015.09.013.

Halberg, N. *et al.* (2009) 'Hypoxia-Inducible Factor 1α Induces Fibrosis and Insulin Resistance in White Adipose Tissue', *Molecular and Cellular Biology*. American Society for Microbiology, 29(16), pp. 4467–4483. doi: 10.1128/mcb.00192-09.

Hall, K. D. *et al.* (2019) 'Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake', *Cell Metabolism*. Cell Press, 30(1), pp. 67-77.e3. doi: 10.1016/j.cmet.2019.05.008.

Hankir, M. K. and Klingenspor, M. (2018) 'Brown adipocyte glucose metabolism: a heated subject', *EMBO reports*. EMBO, 19(9). doi: 10.15252/embr.201846404.

Harris, L. A. L. S. *et al.* (2017) 'Alterations in 3-hydroxyisobutyrate and FGF21 metabolism are associated with protein ingestion-induced insulin resistance', *Diabetes*. American Diabetes Association Inc., 66(7), pp. 1871–1878. doi: 10.2337/db16-1475.

Haslam, D. W. and James, W. P. T. (2005) 'Obesity', *The Lancet*, 366(9492), pp. 1197–1209. doi: 10.1016/S0140-6736(05)67483-1.

Hauck, A. K. *et al.* (2019) 'Adipose oxidative stress and protein carbonylation.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 294(4), pp. 1083–1088. doi: 10.1074/jbc.R118.003214.

Haufe, S. *et al.* (2017) 'Branched-chain amino acid catabolism rather than amino acids plasma concentrations is associated with diet-induced changes in insulin resistance in overweight to obese individuals', *Nutrition, Metabolism and Cardiovascular Diseases*, 27(10), pp. 858–864. doi: 10.1016/j.numecd.2017.07.001.

Helboe, L. *et al.* (2003) 'Distribution and pharmacology of alanine-serine-cysteine transporter 1 (asc-1) in rodent brain.', *The European journal of neuroscience*, 18(8), pp. 2227–38. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14622183.

Hellman, B., Larsson, S. and Westman, S. (1963) 'Mast Cell Content and Fatty Acid Metabolism in the Epididymal Fat Pad of Obese Mice', *Acta Physiologica Scandinavica*. John Wiley & Sons, Ltd, 58(2–3), pp. 255–262. doi: 10.1111/j.1748-1716.1963.tb02647.x.

Heymsfield, S. B. and Wadden, T. A. (2017) 'Mechanisms, Pathophysiology, and Management of Obesity', *New England Journal of Medicine*. Edited by D. L. Longo. Massachussetts Medical Society, 376(3), pp. 254–266. doi: 10.1056/NEJMra1514009.

Holm, L. J. and Buschard, K. (2019) 'L-serine: a neglected amino acid with a potential therapeutic role in diabetes', *APMIS*, p. apm.12987. doi: 10.1111/apm.12987.

Hosios, A. M. *et al.* (2016) 'Amino Acids Rather than Glucose Account for the Majority of Cell Mass in Proliferating Mammalian Cells', *Developmental Cell*, 36, pp. 540–549. doi: 10.1016/j.devcel.2016.02.012.

Hosios, A. M. and Vander Heiden, M. G. (2018) 'The redox requirements of proliferating mammalian cells', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, p. jbc.TM117.000239. doi: 10.1074/jbc.TM117.000239.

Huang, H. *et al.* (2009) 'BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage', *Proceedings of the National Academy of Sciences of the United States of America*. Proc Natl Acad Sci U S A, 106(31), pp. 12670–12675. doi: 10.1073/pnas.0906266106.

HUNT4 - NTNU (2019). Available at: https://www.ntnu.no/hunt/hunt4 (Accessed: 7 October 2020).

Hunter, D. J. (2005) 'Gene-environment interactions in human diseases', *Nature Reviews Genetics*. Nature Publishing Group, pp. 287–298. doi: 10.1038/nrg1578.

Hutson, S. M., Sweatt, A. J. and LaNoue, K. F. (2005) 'Brached-chain amino acid metabolism: Implications for establishing safe intakes', in *Journal of Nutrition*. J Nutr. doi: 10.1093/jn/135.6.1557s.

Ibrahim, M. M. (2010) 'Subcutaneous and visceral adipose tissue: structural and functional differences', *Obesity Reviews*, 11(1), pp. 11–18. doi: 10.1111/j.1467-789X.2009.00623.x.

Ikeda, K., Maretich, P. and Kajimura, S. (2018) 'The Common and Distinct Features of Brown and Beige Adipocytes', *Trends in Endocrinology & Metabolism*. Elsevier Current Trends, 29(3), pp. 191–200. doi: 10.1016/J.TEM.2018.01.001.

Imrie, D. and Sadler, K. C. (2010) 'White adipose tissue development in zebrafish is regulated by both developmental time and fish size', *Developmental Dynamics*. John Wiley & Sons, Ltd, 239(11), pp. 3013–3023. doi: 10.1002/dvdy.22443.

Jackson, A. S. *et al.* (2002) 'The effect of sex, age and race on estimating percentage body fat from body mass index: The Heritage Family Study', *International Journal of Obesity*, 26(6), pp. 789–796. doi: 10.1038/sj.ijo.0802006.

Jang, C. *et al.* (2016) 'A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance', *Nature Medicine*, 22(4), pp. 421–426. doi: 10.1038/nm.4057.

Jankovic, A. *et al.* (2015) 'Redox implications in adipose tissue (dys)function--A new look at old acquaintances.', *Redox biology*. Elsevier, 6, pp. 19–32. doi: 10.1016/j.redox.2015.06.018.

Janković, D. *et al.* (2012) 'Prevalence of Endocrine Disorders in Morbidly Obese Patients and the Effects of Bariatric Surgery on Endocrine and Metabolic Parameters', *Obesity Surgery*, 22(1), pp. 62–69. doi: 10.1007/s11695-011-0545-4.

Jatllon, O. *et al.* (2004) 'Genome duplication in the teleost fish Tetraodon nigroviridis reveals the early vertebrate proto-karyotype', *Nature*. Nature Publishing Group, 431(7011), pp. 946–957. doi: 10.1038/nature03025.

Jeffery, E. *et al.* (2015) 'Rapid depot-specific activation of adipocyte precursor cells at the onset of obesity', *Nature Cell Biology*. Nature Publishing Group, 17(4), pp. 376–385. doi: 10.1038/ncb3122.

Jersin, R. Å. (2016) *Novel functions of the amino acid transporter SLC7A10 in adipocytes*. University of Bergen.

Jia, B. *et al.* (2012) 'Activation of protein kinase a and exchange protein directly activated by cAMP promotes adipocyte differentiation of human mesenchymal stem cells', *PLoS ONE*. Public Library of Science, 7(3). doi: 10.1371/journal.pone.0034114.

Jitrapakdee, S., Vidal-Puig, A. and Wallace, J. C. (2006) 'Anaplerotic roles of pyruvate carboxylase in mammalian tissues', *Cellular and Molecular Life Sciences*. Cell Mol Life Sci, pp. 843–854. doi: 10.1007/s00018-005-5410-y.

Jones IV, A. R. *et al.* (2016) 'Extracellular Redox Regulation of Intracellular Reactive Oxygen Generation, Mitochondrial Function and Lipid Turnover in Cultured Human Adipocytes', *PLOS ONE*. Edited by G. López Lluch, 11(10), p. e0164011. doi: 10.1371/journal.pone.0164011.

de Jong, J. M. A. *et al.* (2015) 'A stringent validation of mouse adipose tissue identity markers', *American Journal of Physiology - Endocrinology and Metabolism*. American Physiological Society, 308(12), pp. E1085–E1105. doi: 10.1152/ajpendo.00023.2015.

Justice, A. E. *et al.* (2017) 'Genome-wide meta-analysis of 241,258 adults accounting for smoking behaviour identifies novel loci for obesity traits', *Nature Communications*. Nature Publishing Group, 8(1), pp. 1–19. doi: 10.1038/ncomms14977.

Kahn, C. R., Wang, G. and Lee, K. Y. (2019) 'Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome', *Journal of Clinical Investigation*. American Society for Clinical Investigation, pp. 3990–4000. doi: 10.1172/JCI129187.

Kandasamy, P. *et al.* (2018) 'Amino acid transporters revisited: New views in health and disease', *Trends in Biochemical Sciences*. Elsevier Current Trends. doi: 10.1016/J.TIBS.2018.05.003.

Karastergiou, K. *et al.* (2012) 'Sex differences in human adipose tissues - The biology of pear shape', *Biology of Sex Differences*. BioMed Central, pp. 1–12. doi: 10.1186/2042-6410-3-13.

Karpe, F. and Pinnick, K. E. (2015) 'Biology of upper-body and lower-body adipose tissue - Link to whole-body phenotypes', *Nature Reviews Endocrinology*. Nature Publishing Group, pp. 90–100. doi: 10.1038/nrendo.2014.185.

Kedishvili, N. Y. *et al.* (1994) 'Coordinated expression of valine catabolic enzymes during

adipogenesis: Analysis of activity, mRNA, protein levels, and metabolic consequences', *Archives of Biochemistry and Biophysics*, 315(2), pp. 317–322. doi: 10.1006/abbi.1994.1506.

KEGG PATHWAY: Valine, leucine and isoleucine degradation - Reference pathway (2020). Available at: https://www.genome.jp/kegg-bin/show_pathway?map=map00280&show_description=show&fbclid=IwAR0DcECg81Xh8AnqN4p1fSvRQyPhFpIVy0E3XMwNGfZ302ZgKF9y3s57gQM (Accessed: 7 October 2020).

Kellar, D. and Craft, S. (2020) 'Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches', *The Lancet Neurology*. Elsevier BV, 19(9), pp. 758–766. doi: 10.1016/s1474-4422(20)30231-3.

Khan, T. *et al.* (2009) 'Metabolic Dysregulation and Adipose Tissue Fibrosis: Role of Collagen VI', *Molecular and Cellular Biology*. American Society for Microbiology, 29(6), pp. 1575–1591. doi: 10.1128/mcb.01300-08.

Kim, J. B. (2016) 'Dynamic cross talk between metabolic organs in obesity and metabolic diseases', *Experimental and Molecular Medicine*. Nature Publishing Group, pp. e214–e214. doi: 10.1038/emm.2015.119.

Kim, S. M. *et al.* (2014) 'Loss of white adipose hyperplastic potential is associated with enhanced susceptibility to insulin resistance', *Cell Metabolism*. Cell Press, 20(6), pp. 1049–1058. doi: 10.1016/j.cmet.2014.10.010.

Klingenspor, M. *et al.* (2008) 'An ancient look at UCP1', *Biochimica et Biophysica Acta - Bioenergetics*. Elsevier, pp. 637–641. doi: 10.1016/j.bbabio.2008.03.006.

Klöting, N. *et al.* (2010) 'Insulin-sensitive obesity', *American Journal of Physiology - Endocrinology and Metabolism*, 299(3).

Knight, K. (2018) 'The biology of fat', *Journal of Experimental Biology*. Company of Biologists Ltd, 121. doi: 10.1242/jeb.178020.

Kobayashi, H. *et al.* (2009) 'Dysregulated glutathione metabolism links to impaired insulin action in adipocytes', *American Journal of Physiology-Endocrinology and Metabolism*. American Physiological Society, 296(6), pp. E1326–E1334. doi: 10.1152/ajpendo.90921.2008.

Kolb, H. *et al.* (2018) 'Insulin translates unfavourable lifestyle into obesity', *BMC Medicine*. BioMed Central Ltd., 16(1), pp. 1–10. doi: 10.1186/s12916-018-1225-1.

Kratz, M. (2020) 'Long-Term Diabetes Remission Rates after Bariatric Surgery: Surprisingly Low in Spite of Sustained Weight Loss.', *The Journal of clinical endocrinology and metabolism*, 105(6). doi: 10.1210/clinem/dgaa179.

Krokstad, S. *et al.* (2013) 'Cohort Profile: The HUNT Study, Norway', *International Journal of Epidemiology*. Oxford University Press, 42(4), pp. 968–977. doi: 10.1093/ije/dys095.

Krycer, J. R. *et al.* (2017) 'Dynamic Metabolomics Reveals that Insulin Primes the Adipocyte for Glucose Metabolism', *Cell Reports*. Elsevier B.V., 21(12), pp. 3536–3547. doi: 10.1016/j.celrep.2017.11.085.

Krycer, J. R. *et al.* (2020) 'Mitochondrial oxidants, but not respiration, are sensitive to glucose in adipocytes', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology Inc., 295(1), pp. 99–110. doi: 10.1074/jbc.RA119.011695.

Kuri-Harcuch, W., Wise, L. S. and Green, H. (1978) 'Interruption of the adipose conversion of 3T3 cells by biotin deficiency: differentiation without triglyceride accumulation', *Cell*. Cell, 14(1), pp. 53–59. doi: 10.1016/0092-8674(78)90300-8.

Kutchukian, P. S. *et al.* (2017) 'Iterative Focused Screening with Biological Fingerprints Identifies Selective Asc-1 Inhibitors Distinct from Traditional High Throughput Screening', *ACS Chemical Biology*, 12(2), pp. 519–527. doi: 10.1021/acschembio.6b00913.

Labuschagne, C. F. *et al.* (2014) 'Serine, but Not Glycine, Supports One-Carbon Metabolism and Proliferation of Cancer Cells', *Cell Reports*. Cell Press, 7(4), pp. 1248–1258. doi: 10.1016/J.CELREP.2014.04.045.

Lackey, D. E. *et al.* (2013) 'Regulation of adipose branched-chain amino acid catabolism enzyme expression and cross-adipose amino acid flux in human obesity', *American Journal of Physiology-Endocrinology and Metabolism*, 304(11), pp. E1175–E1187. doi: 10.1152/ajpendo.00630.2012.

Ladeira, M. M. *et al.* (2016) 'Nutrigenomics and beef quality: A review about lipogenesis', *International Journal of Molecular Sciences*. MDPI AG. doi: 10.3390/ijms17060918.

Laforest, S. *et al.* (2015) 'Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction', *Critical Reviews in Clinical Laboratory Sciences*. Taylor and Francis Ltd, pp. 301–313. doi: 10.3109/10408363.2015.1041582.

Lam, B. C. C. *et al.* (2015) 'Comparison of Body Mass Index (BMI), Body Adiposity Index (BAI), Waist Circumference (WC), Waist-To-Hip Ratio (WHR) and Waist-To-Height Ratio (WHtR) as predictors of cardiovascular disease risk factors in an adult population in Singapore', *PLoS ONE*. Public Library of Science, 10(4). doi: 10.1371/journal.pone.0122985.

Laviola, L. *et al.* (2006) 'Insulin signalling in human adipose tissue', in *Archives of Physiology and Biochemistry*. Arch Physiol Biochem, pp. 82–88. doi: 10.1080/13813450600736174.

Lee, H. *et al.* (2009) 'Reactive oxygen species facilitate adipocyte differentiation by accelerating mitotic clonal expansion.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 284(16), pp. 10601–9. doi: 10.1074/jbc.M808742200.

Lee, J., Ellis, J. M. and Wolfgang, M. J. (2015) 'Adipose fatty acid oxidation is required for thermogenesis and potentiates oxidative stress-induced inflammation', *Cell Reports*. Elsevier B.V., 10(2), pp. 266–279. doi: 10.1016/j.celrep.2014.12.023.

Lee, K. Y. *et al.* (2019) 'Developmental and functional heterogeneity of white adipocytes within a single fat depot', *The EMBO Journal*. EMBO, 38(3). doi: 10.15252/embj.201899291.

Lee, M.-J. and Fried, S. K. (2014) 'Optimal Protocol for the Differentiation and Metabolic Analysis of Human Adipose Stromal Cells', in *Methods in enzymology*, pp. 49–65. doi: 10.1016/B978-0-12-800280-3.00004-9.

Lee, M.-J., Wu, Y. and Fried, S. K. (2013) 'Adipose tissue heterogeneity: Implication of depot differences in adipose tissue for obesity complications', *Molecular Aspects of Medicine*, 34(1), pp. 1–11. doi: 10.1016/j.mam.2012.10.001.

Lee, Y.-H., Mottillo, E. P. and Granneman, J. G. (2014) 'Adipose tissue plasticity from WAT to BAT and in between', *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1842(3), pp. 358–369. doi: 10.1016/j.bbadis.2013.05.011.

Lefterova, M. I. *et al.* (2008) 'PPARγ and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale', *Genes and Development*. Genes Dev, 22(21), pp. 2941–2952. doi: 10.1101/gad.1709008.

Lettieri Barbato, D. *et al.* (2015) 'Glutathione Decrement Drives Thermogenic Program In Adipose Cells.', *Scientific reports*, 5, p. 13091. doi: 10.1038/srep13091.

Letto, J., Brosnan, M. E. and Brosnan, J. T. (1986) 'Valine metabolism. Gluconeogenesis from 3-hydroxyisobutyrate', *Biochemical Journal*. Biochem J, 240(3), pp. 909–912. doi: 10.1042/bj2400909.

Letto, J, Brosnan, M. E. and Brosnan, J. T. (1986) 'Valine metabolism. Gluconeogenesis from 3-hydroxyisobutyrate', *Biochemical Journal*. Portland Press Ltd, 240(3), pp. 909–912. doi: 10.1042/bj2400909.

Liemburg-Apers, D. C. *et al.* (2015) 'Interactions between mitochondrial reactive oxygen species and cellular glucose metabolism.', *Archives of toxicology*. Springer, 89(8), pp. 1209–26. doi: 10.1007/s00204-015-1520-y.

Ló Pez-Sobaler, A. M. *et al.* (2016) 'General and Abdominal Obesity Is Related to Physical Activity, Smoking and Sleeping Behaviours and Mediated by the Educational Level: Findings from the ANIBES Study in Spain'. doi: 10.1371/journal.pone.0169027.

Locasale, J. W. (2013) 'Serine, glycine and one-carbon units: cancer metabolism in full circle', *Nature Reviews Cancer*, 13(8), pp. 572–583. doi: 10.1038/nrc3557.

Locke, A. E. *et al.* (2015) 'Genetic studies of body mass index yield new insights for obesity biology', *Nature*. Nature Research, 518(7538), pp. 197–206. doi: 10.1038/nature14177.

Longo, M. *et al.* (2019) 'Adipose tissue dysfunction as determinant of obesity-associated metabolic complications', *International Journal of Molecular Sciences*. MDPI AG, 20(9). doi: 10.3390/ijms20092358.

Lu, X. *et al.* (2017) 'The early metabolomic response of adipose tissue during acute cold exposure in mice', *Scientific Reports*. Nature Publishing Group, 7(1), p. 3455. doi: 10.1038/s41598-017-03108-x.

Lucas, S. *et al.* (2018) 'Serine catabolism is essential to maintain mitochondrial respiration in mammalian cells', *Life Science Alliance*, 1(2), p. e201800036. doi: 10.26508/lsa.201800036.

Lundgren, M. *et al.* (2007) 'Fat cell enlargement is an independent marker of insulin resistance and "hyperleptinaemia"', *Diabetologia*. Springer, 50(3), pp. 625–633. doi: 10.1007/s00125-006-0572-1.

Lusis, A. J., Attie, A. D. and Reue, K. (2008) 'Metabolic syndrome: from epidemiology to systems biology', *Nature Reviews Genetics*, 9(11), pp. 819–830. doi: 10.1038/nrg2468.

Lynch, C. J. and Adams, S. H. (2014) 'Branched-chain amino acids in metabolic signalling and insulin resistance.', *Nature reviews. Endocrinology*. NIH Public Access, 10(12), pp. 723–36. doi: 10.1038/nrendo.2014.171.

Lyon, E. S. *et al.* (2019) 'Actions of chronic physiological 3-hydroxyisobuterate treatment on mitochondrial metabolism and insulin signaling in myotubes', *Nutrition Research*. Elsevier Inc., 66, pp. 22–31. doi: 10.1016/j.nutres.2019.03.012.

Ma, M. *et al.* (2018) 'Bidirectional modulation of insulin action by reactive oxygen species in 3T3-L1 adipocytes', *Molecular Medicine Reports*. Spandidos Publications, 18(1), pp. 807–814. doi: 10.3892/mmr.2018.9016.

Macdougall, C. E. *et al.* (2018) 'Visceral Adipose Tissue Immune Homeostasis Is Regulated by the Crosstalk between Adipocytes and Dendritic Cell Subsets', *Cell Metabolism*, 27(3), pp. 588-601.e4. doi: 10.1016/j.cmet.2018.02.007.

Maddocks, O. D. K. *et al.* (2016) 'Serine Metabolism Supports the Methionine Cycle and DNA/RNA Methylation through De Novo ATP Synthesis in Cancer Cells', *Molecular Cell*. Elsevier, 61(2), pp. 210–221. doi: 10.1016/j.molcel.2015.12.014.

Maddocks, O. D. K. *et al.* (2017) 'Modulating the therapeutic response of tumours to dietary serine and glycine starvation', *Nature*. Nature Publishing Group, 544(7650), pp. 372–376. doi: 10.1038/nature22056.

Manna, P. and Jain, S. K. (2015) 'Obesity, Oxidative Stress, Adipose Tissue Dysfunction, and the Associated Health Risks: Causes and Therapeutic Strategies', *Metabolic Syndrome and Related Disorders*. Mary Ann Liebert Inc., pp. 423–444. doi: 10.1089/met.2015.0095.

Mardinoglu, A. *et al.* (2014) 'Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with non-alcoholic fatty liver disease', *Nature Communications*. Nature Publishing Group, 5(1), pp. 1–11. doi: 10.1038/ncomms4083.

Mardinoglu, A. *et al.* (2018) 'Elevated Plasma Levels of 3-Hydroxyisobutyric Acid Are Associated With Incident Type 2 Diabetes', *EBioMedicine*, 27, pp. 151–155. doi: 10.1016/j.ebiom.2017.12.008.

Mariman, E. C. M. and Wang, P. (2010) 'Adipocyte extracellular matrix composition, dynamics and role in obesity', *Cellular and Molecular Life Sciences*. Cell Mol Life Sci, pp. 1277–1292. doi: 10.1007/s00018-010-0263-4.

van Marken Lichtenbelt, W. D. *et al.* (2009) 'Cold-Activated Brown Adipose Tissue in Healthy Men', *New England Journal of Medicine*, 360(15), pp. 1500–1508. doi: 10.1056/NEJMoa0808718.

Martínez-Reyes, I. and Chandel, N. S. (2020) 'Mitochondrial TCA cycle metabolites control physiology and disease', *Nature Communications*. Nature Research. doi: 10.1038/s41467-019-13668-3.

Martínez, Y. *et al.* (2017) 'The role of methionine on metabolism, oxidative stress, and

diseases', *Amino Acids*. Springer-Verlag Wien, pp. 2091–2098. doi: 10.1007/s00726-017-2494-2.

Maslov, L. N. *et al.* (2019) 'Is oxidative stress of adipocytes a cause or a consequence of the metabolic syndrome?', *Journal of Clinical & Translational Endocrinology*. Elsevier, 15, pp. 1–5. doi: 10.1016/J.JCTE.2018.11.001.

Masschelin, P. M. *et al.* (2020) 'The Impact of Oxidative Stress on Adipose Tissue Energy Balance', *Frontiers in Physiology*. Frontiers Media S.A. doi: 10.3389/fphys.2019.01638.

Matsuo, H. *et al.* (2004) 'High affinity d- and l-serine transporter Asc-1: cloning and dendritic localization in the rat cerebral and cerebellar cortices', *Neuroscience Letters*. Elsevier, 358(2), pp. 123–126. doi: 10.1016/J.NEULET.2004.01.014.

Matthews, D. R. *et al.* (1985) 'Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man', *Diabetologia*. Springer-Verlag, 28(7), pp. 412–419. doi: 10.1007/BF00280883.

McCormack, S. E. *et al.* (2013) 'Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents.', *Pediatric obesity*. NIH Public Access, 8(1), pp. 52–61. doi: 10.1111/j.2047-6310.2012.00087.x.

McLaughlin, T. *et al.* (2016) 'Adipose cell size and regional fat deposition as predictors of metabolic response to overfeeding in insulin-resistant and insulin-sensitive humans', *Diabetes*. American Diabetes Association Inc., 65(5), pp. 1245–1254. doi: 10.2337/db15-1213.

Mehran, A. E. *et al.* (2012) 'Hyperinsulinemia drives diet-induced obesity independently of brain insulin production', *Cell Metabolism*. Cell Metab, 16(6), pp. 723–737. doi: 10.1016/j.cmet.2012.10.019.

Mesuret, G. *et al.* (2018) 'A neuronal role of the Alanine-Serine-Cysteine-1 transporter (SLC7A10, Asc-1) for glycine inhibitory transmission and respiratory pattern', *Scientific Reports*. Nature Publishing Group, 8(1), p. 8536. doi: 10.1038/s41598-018-26868-6.

Mikou, A. *et al.* (2020) 'Asc-1 Transporter (SLC7A10): Homology Models And Molecular Dynamics Insights Into The First Steps Of The Transport Mechanism', *Scientific Reports*. Nature Research, 10(1), pp. 1–12. doi: 10.1038/s41598-020-60617-y.

Minton, D. R. *et al.* (2018) 'Serine Catabolism by SHMT2 Is Required for Proper Mitochondrial Translation Initiation and Maintenance of Formylmethionyl-tRNAs', *Molecular Cell*. Cell Press, 69(4), pp. 610-621.e5. doi: 10.1016/J.MOLCEL.2018.01.024.

Molloy, A. M. *et al.* (2016) 'A Common Polymorphism in HIBCH Influences Methylmalonic Acid Concentrations in Blood Independently of Cobalamin', *American Journal of Human Genetics*. Cell Press, 98(5), pp. 869–882. doi: 10.1016/j.ajhg.2016.03.005.

Mullarky, E. and Cantley, L. C. (2015) 'Diverting Glycolysis to Combat Oxidative Stress'. Springer. doi: 10.1007/978-4-431-55651-0_1.

Nakauchi, J. *et al.* (2000) 'Cloning and characterization of a human brain Na(+)-independent transporter for small neutral amino acids that transports D-serine with high affinity.',

Neuroscience letters, 287(3), pp. 231–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10863037.

Naomi R. Wray, P. D. (2008) 'Estimating Trait Heritability | Learn Science at Scitable'. Available at: https://www.nature.com/scitable/topicpage/estimating-trait-heritability-46889/ (Accessed: 25 August 2020).

Neinast, M. D. *et al.* (2019) 'Quantitative Analysis of the Whole-Body Metabolic Fate of Branched-Chain Amino Acids', *Cell Metabolism*. Cell Press, 29(2), pp. 417-429.e4. doi: 10.1016/j.cmet.2018.10.013.

Neinast, M., Murashige, D. and Arany, Z. (2019) 'Branched Chain Amino Acids', *Annual Review of Physiology*. Annual Reviews Inc., pp. 139–164. doi: 10.1146/annurev-physiol-020518-114455.

Newbern, D. *et al.* (2014) 'Sex differences in biomarkers associated with insulin resistance in obese adolescents: Metabolomic profiling and principal components analysis', *Journal of Clinical Endocrinology and Metabolism*, 99(12), pp. 4730–4739. doi: 10.1210/jc.2014-2080.

Newman, A. C. and Maddocks, O. D. K. (2017a) 'One-carbon metabolism in cancer', *British Journal of Cancer*. Nature Publishing Group, pp. 1499–1504. doi: 10.1038/bjc.2017.118.

Newman, A. C. and Maddocks, O. D. K. (2017b) 'Serine and Functional Metabolites in Cancer', *Trends in Cell Biology*. Elsevier Current Trends, 27(9), pp. 645–657. doi: 10.1016/J.TCB.2017.05.001.

Nie, C. *et al.* (2018) 'Branched Chain Amino Acids: Beyond Nutrition Metabolism', *International Journal of Molecular Sciences*. MDPI AG, 19(4), p. 954. doi: 10.3390/ijms19040954.

Nilsen, M. S. *et al.* (2020) '3-Hydroxyisobutyrate, a Strong Marker of Insulin Resistance in Type 2 Diabetes and Obesity That Modulates White and Brown Adipocyte Metabolism', *Diabetes*. American Diabetes Association, p. db191174. doi: 10.2337/db19-1174.

Nishida, C. *et al.* (2004) 'Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies', *The Lancet*. Elsevier Limited, 363(9403), pp. 157–163. doi: 10.1016/S0140-6736(03)15268-3.

Nye, C. *et al.* (2008) 'Reassessing triglyceride synthesis in adipose tissue', *Trends in Endocrinology and Metabolism*. Elsevier Current Trends, pp. 356–361. doi: 10.1016/j.tem.2008.08.003.

Nye, C. K., Hanson, R. W. and Kalhan, S. C. (2008) 'Glyceroneogenesis is the dominant pathway for triglyceride glycerol synthesis in vivo in the rat', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 283(41), pp. 27565–27574. doi: 10.1074/jbc.M804393200.

Obregon, M.-J. (2008) 'Thyroid Hormone and Adipocyte Differentiation', *Thyroid*, 18(2), pp. 185–195. doi: 10.1089/thy.2007.0254.

Oka, T. *et al.* (2010) 'Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity', *BMC Physiology*. BioMed Central, 10(1), p. 21. doi:

10.1186/1472-6793-10-21.

Oliveira, M. de *et al.* (2013) 'Triiodothyronine Increases mRNA and Protein Leptin Levels in Short Time in 3T3-L1 Adipocytes by PI3K Pathway Activation', *PLoS ONE*. Edited by V. Sanchez-Margalet. Public Library of Science, 8(9), p. e74856. doi: 10.1371/journal.pone.0074856.

Perry, R. J. *et al.* (2015) 'Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes', *Cell*. Cell Press, 160(4), pp. 745–758. doi: 10.1016/j.cell.2015.01.012.

Petersen, R. K. *et al.* (2008) 'Cyclic AMP (cAMP)-Mediated Stimulation of Adipocyte Differentiation Requires the Synergistic Action of Epac- and cAMP-Dependent Protein Kinase-Dependent Processes', *Molecular and Cellular Biology*. American Society for Microbiology, 28(11), pp. 3804–3816. doi: 10.1128/mcb.00709-07.

Pineda, M. *et al.* (2004) 'The amino acid transporter asc-1 is not involved in cystinuria', *Kidney International*. Blackwell Publishing Inc., 66(4), pp. 1453–1464. doi: 10.1111/j.1523-1755.2004.00908.x.

Pond, C. M. (1992) 'An evolutionary and functional view of mammalian adipose tissue', *Proceedings of the Nutrition Society*. Cambridge University Press (CUP), 51(3), pp. 367–377. doi: 10.1079/pns19920050.

Qu, H.-Q. *et al.* (2011) 'The Definition of Insulin Resistance Using HOMA-IR for Americans of Mexican Descent Using Machine Learning', *PLoS ONE*. Edited by A. Vella. Public Library of Science, 6(6), p. e21041. doi: 10.1371/journal.pone.0021041.

Quach, J. M. *et al.* (2011) 'Zinc finger protein 467 is a novel regulator of osteoblast and adipocyte commitment', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 286(6), pp. 4186–4198. doi: 10.1074/jbc.M110.178251.

Quail, D. F. and Dannenberg, A. J. (2019) 'The obese adipose tissue microenvironment in cancer development and progression', *Nature Reviews Endocrinology*. Nature Publishing Group, pp. 139–154. doi: 10.1038/s41574-018-0126-x.

Quijano, C. *et al.* (2016) 'Interplay between oxidant species and energy metabolism.', *Redox biology*. Elsevier, 8, pp. 28–42. doi: 10.1016/j.redox.2015.11.010.

Ramirez, A. K. *et al.* (2020) 'Single-cell transcriptional networks in differentiating preadipocytes suggest drivers associated with tissue heterogeneity', *Nature Communications*. Nature Research, 11(1). doi: 10.1038/s41467-020-16019-9.

Ran, G. *et al.* (2017) 'Resveratrol ameliorates diet-induced dysregulation of lipid metabolism in zebrafish (Danio rerio)', *PLOS ONE*. Edited by K. Biswas. Public Library of Science, 12(7), p. e0180865. doi: 10.1371/journal.pone.0180865.

Rankinen, T. *et al.* (2015) 'Are There Genetic Paths Common to Obesity, Cardiovascular Disease Outcomes, and Cardiovascular Risk Factors?', *Circulation Research*, 116(5), pp. 909–922. doi: 10.1161/CIRCRESAHA.116.302888.

Reddy, A. *et al.* (2014) 'Reliability of nine programs of topological predictions and their

application to integral membrane channel and carrier proteins.', *Journal of molecular microbiology and biotechnology*. NIH Public Access, 24(3), pp. 161–90. doi: 10.1159/000363506.

Remedi, M. S. and Nichols, C. G. (2009) 'Hyperinsulinism and Diabetes: Genetic Dissection of β Cell Metabolism-Excitation Coupling in Mice', *Cell Metabolism*. NIH Public Access, pp. 442–453. doi: 10.1016/j.cmet.2009.10.011.

Reshef, L. *et al.* (2003) 'Glyceroneogenesis and the triglyceride/fatty acid cycle', *Journal of Biological Chemistry*. J Biol Chem, pp. 30413–30416. doi: 10.1074/jbc.R300017200.

Reshef, L., Hanson, $ R W and Ballard, F. J. (1969) *Glyceride-glycerol synthesis from pyruvate. Adaptive changes in phosphoenolpyruvate carboxykinase and pyruvate carboxylase in adipose tissue and liver*, *J Biol Chem*. Available at: https://www.jbc.org/content/244/8/1994.long.

Reshef, L., Niv, J. and Shapiro, B. (1967) 'Effect of propionate on pyruvate metabolism in adipose tissue', *Journal of Lipid Research*. American Society for Biochemistry and Molecular Biology, 8(6), pp. 688–691.

Reusch, J. E. B., Colton, L. A. and Klemm, D. J. (2000) 'CREB Activation Induces Adipogenesis in 3T3-L1 Cells', *Molecular and Cellular Biology*. American Society for Microbiology, 20(3), pp. 1008–1020. doi: 10.1128/mcb.20.3.1008-1020.2000.

Ribas, V., García-Ruiz, C. and Fernández-Checa, J. C. (2014) 'Glutathione and mitochondria.', *Frontiers in pharmacology*. Frontiers Media SA, 5, p. 151. doi: 10.3389/fphar.2014.00151.

Rohde, K. *et al.* (2019) 'Genetics and epigenetics in obesity', *Metabolism: Clinical and Experimental*. W.B. Saunders, pp. 37–50. doi: 10.1016/j.metabol.2018.10.007.

Rosen, E. D. *et al.* (1999) 'PPARγ is required for the differentiation of adipose tissue in vivo and in vitro', *Molecular Cell*. Cell Press, 4(4), pp. 611–617. doi: 10.1016/S1097-2765(00)80211-7.

Rosen, E. D. *et al.* (2014) 'What we talk about when we talk about fat.', *Cell*. Elsevier, 156(1–2), pp. 20–44. doi: 10.1016/j.cell.2013.12.012.

Rosen, E. D. and Spiegelman, B. M. (2014) 'What we talk about when we talk about fat.', *Cell*, 156(1–2), pp. 20–44. doi: 10.1016/j.cell.2013.12.012.

Rosenberg, D. *et al.* (2013) 'Neuronal d-Serine and Glycine Release Via the Asc-1 Transporter Regulates NMDA Receptor-Dependent Synaptic Activity', *Journal of Neuroscience*, 33(8).

Rutter, A. R. *et al.* (2007) 'Evidence from gene knockout studies implicates Asc-1 as the primary transporter mediating d-serine reuptake in the mouse CNS.', *The European journal of neuroscience*, 25(6), pp. 1757–66. doi: 10.1111/j.1460-9568.2007.05446.x.

Saari, T. J. *et al.* (2020) 'Basal and cold-induced fatty acid uptake of human brown adipose tissue is impaired in obesity', *Scientific Reports*. Nature Research, 10(1), p. 14373. doi: 10.1038/s41598-020-71197-2.

Safory, H. *et al.* (2015) 'The alanine-serine-cysteine-1 (Asc-1) transporter controls glycine levels in the brain and is required for glycinergic inhibitory transmission', *EMBO Rep*, 16(5), pp. 590–598. doi: 10.15252/embr.201439561.

Saha, A. *et al.* (2014) 'Akt Phosphorylation and Regulation of Transketolase Is a Nodal Point for Amino Acid Control of Purine Synthesis', *Molecular Cell*. Cell Press, 55(2), pp. 264–276. doi: 10.1016/J.MOLCEL.2014.05.028.

Sakimura, K. *et al.* (2016) 'A novel Na+-Independent alanine-serine-cysteine transporter 1 inhibitor inhibits both influx and efflux of D-Serine', *Journal of Neuroscience Research*, 94(10), pp. 888–895. doi: 10.1002/jnr.23772.

Salazar, A., Keusgen, M. and von Hagen, J. (2016) 'Amino acids in the cultivation of mammalian cells', *Amino Acids*. Springer, 48(5), pp. 1161–1171. doi: 10.1007/s00726-016-2181-8.

Salmi, T. M., Tan, V. W. T. and Cox, A. G. (2019) 'Dissecting metabolism using zebrafish models of disease', *Biochemical Society Transactions*. Portland Press Ltd, pp. 305–315. doi: 10.1042/BST20180335.

Sarwar, R., Pierce, N. and Koppe, S. (2018) 'Obesity and nonalcoholic fatty liver disease: Current perspectives', *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*. Dove Medical Press Ltd., pp. 533–542. doi: 10.2147/DMSO.S146339.

Sason, H. *et al.* (2016) 'Asc-1 Transporter Regulation of Synaptic Activity via the Tonic Release of d-Serine in the Forebrain.', *Cerebral cortex (New York, N.Y. : 1991)*, p. bhv350. doi: 10.1093/cercor/bhv350.

Scherer, P. E. *et al.* (1995) 'A novel serum protein similar to C1q, produced exclusively in adipocytes', *Journal of Biological Chemistry*, 270(45), pp. 26746–26749. doi: 10.1074/jbc.270.45.26746.

Schlegel, A. and Gut, P. (2015) 'Metabolic insights from zebrafish genetics, physiology, and chemical biology', *Cellular and Molecular Life Sciences*, 72(12), pp. 2249–2260. doi: 10.1007/s00018-014-1816-8.

Schoettl, T., Fischer, I. P. and Ussar, S. (2018) 'Heterogeneity of adipose tissue in development and metabolic function', *Journal of Experimental Biology*. Company of Biologists Ltd. doi: 10.1242/jeb.162958.

Schöttl, T. *et al.* (2015) 'Limited OXPHOS capacity in white adipocytes is a hallmark of obesity in laboratory mice irrespective of the glucose tolerance status.', *Molecular metabolism*. Elsevier, 4(9), pp. 631–42. doi: 10.1016/j.molmet.2015.07.001.

Schupp, M. and Lazar, M. A. (2010) 'Endogenous ligands for nuclear receptors: Digging deeper', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, pp. 40409–40415. doi: 10.1074/jbc.R110.182451.

Seth, A., Stemple, D. L. and Barroso, I. (2013) 'The emerging use of zebrafish to model metabolic disease.', *Disease models & mechanisms*. Company of Biologists, 6(5), pp. 1080–8. doi: 10.1242/dmm.011346.

Simpson, J. C. (2006) 'Functional Assays', in *Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine*. Springer Berlin Heidelberg, pp. 617–620. doi: 10.1007/3-540-29623-9_3700.

Sjöholm, K. *et al.* (2013) 'Evaluation of current eligibility criteria for bariatric surgery: Diabetes prevention and risk factor changes in the swedish obese subjects (SOS) study', *Diabetes Care*. American Diabetes Association, 36(5), pp. 1335–1340. doi: 10.2337/dc12-1395.

Sjöström, L. *et al.* (2007) 'Effects of Bariatric Surgery on Mortality in Swedish Obese Subjects', *New England Journal of Medicine*. Massachusetts Medical Society , 357(8), pp. 741–752. doi: 10.1056/NEJMoa066254.

Small, K. S. *et al.* (2011) 'Identification of an imprinted master trans regulator at the KLF14 locus related to multiple metabolic phenotypes', *Nature Genetics*, 43(6), pp. 561–564. doi: 10.1038/ng.833.

Small, K. S. *et al.* (2018) 'Regulatory variants at KLF14 influence type 2 diabetes risk via a female-specific effect on adipocyte size and body composition', *Nature Genetics 2018 50:4*. Nature Publishing Group, 50(4), p. 572. doi: 10.1038/s41588-018-0088-x.

Spalding, K. L. *et al.* (2008) 'Dynamics of fat cell turnover in humans', *Nature*. Nature Publishing Group, 453(7196), pp. 783–787. doi: 10.1038/nature06902.

Stamboulian, M. *et al.* (2020) 'The ortholog conjecture revisited: the value of orthologs and paralogs in function prediction', *Bioinformatics (Oxford, England)*. NLM (Medline), 36(1), pp. i219–i226. doi: 10.1093/bioinformatics/btaa468.

Stenson, B. M. *et al.* (2011) 'Liver X Receptor (LXR) regulates human adipocyte lipolysis', *Journal of Biological Chemistry*. J Biol Chem, 286(1), pp. 370–379. doi: 10.1074/jbc.M110.179499.

Stowe, D. F. and Camara, A. K. S. (2009) 'Mitochondrial reactive oxygen species production in excitable cells: Modulators of mitochondrial and cell function', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc., pp. 1373–1414. doi: 10.1089/ars.2008.2331.

Stunkard, A. J. *et al.* (1990) 'The Body-Mass Index of Twins Who Have Been Reared Apart', *New England Journal of Medicine*, 322(21), pp. 1483–1487. doi: 10.1056/NEJM199005243222102.

Stunkard, A. J., Foch, T. T. and Hrubec, Z. (1986) 'A twin study of human obesity.', *JAMA*, 256(1), pp. 51–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3712713.

Svensson, P.-A. *et al.* (2014) 'Characterization of brown adipose tissue in the human perirenal depot.', *Obesity (Silver Spring, Md.)*, 22, pp. 1830–7. doi: 10.1002/oby.20765.

Swinburn, B. A. *et al.* (2011) 'The global obesity pandemic: Shaped by global drivers and local environments', *The Lancet*. Lancet Publishing Group, pp. 804–814. doi: 10.1016/S0140-6736(11)60813-1.

Tam, C. S. *et al.* (2012) 'Defining insulin resistance from hyperinsulinemic-euglycemic clamps', *Diabetes Care*. American Diabetes Association, 35(7), pp. 1605–1610. doi:

10.2337/dc11-2339.

Taniguchi, K. *et al.* (1996) 'The valine catabolic pathway in human liver: Effect of cirrhosis on enzyme activities', *Hepatology*. Wiley, 24(6), pp. 1395–1398. doi: 10.1002/hep.510240614.

Taylor, R. (2012) 'Insulin resistance and type 2 diabetes', *Diabetes*. American Diabetes Association, pp. 778–779. doi: 10.2337/db12-0073.

Tchoukalova, Y. D. *et al.* (2010) 'Regional differences in cellular mechanisms of adipose tissue gain with overfeeding', *Proceedings of the National Academy of Sciences of the United States of America*, 107(42), pp. 18226–18231. doi: 10.1073/pnas.1005259107.

Tedeschi, P. M. *et al.* (2013) 'Contribution of serine, folate and glycine metabolism to the ATP, NADPH and purine requirements of cancer cells.', *Cell death & disease*. Nature Publishing Group, 4(10), p. e877. doi: 10.1038/cddis.2013.393.

Thalacker-Mercer, A. E. *et al.* (2014) 'BMI, RQ, diabetes, and sex affect the relationships between amino acids and clamp measures of insulin action in humans', *Diabetes*, 63(2), pp. 791–800. doi: 10.2337/db13-0396.

Thomas, P. D. *et al.* (2003) 'PANTHER: A library of protein families and subfamilies indexed by function', *Genome Research*. Cold Spring Harbor Laboratory Press, 13(9), pp. 2129–2141. doi: 10.1101/gr.772403.

Tormos, K. V. *et al.* (2011) 'Mitochondrial Complex III ROS Regulate Adipocyte Differentiation', *Cell Metabolism*, 14(4), pp. 537–544. doi: 10.1016/j.cmet.2011.08.007.

Torrecillas, I. R. *et al.* (2019) 'Inhibition of the Alanine-Serine-Cysteine-1 Transporter by BMS-466442', *ACS Chemical Neuroscience*. American Chemical Society, p. acschemneuro.9b00019. doi: 10.1021/acschemneuro.9b00019.

Unick, J. L. *et al.* (2013) 'The long-term effectiveness of a lifestyle intervention in severely obese individuals', *American Journal of Medicine*. Elsevier Inc., 126(3), pp. 236-242.e2. doi: 10.1016/j.amjmed.2012.10.010.

Ussar, S. *et al.* (2014) 'ASC-1, PAT2, and P2RX5 are cell surface markers for white, beige, and brown adipocytes', *Science Translational Medicine*, 6(247).

Vattikuti, S., Guo, J. and Chow, C. C. (2012) 'Heritability and genetic correlations explained by common SNPs for metabolic syndrome traits.', *PLoS genetics*. Public Library of Science, 8(3), p. e1002637. doi: 10.1371/journal.pgen.1002637.

Vazquez, A., Markert, E. K. and Oltvai, Z. N. (2011) 'Serine Biosynthesis with One Carbon Catabolism and the Glycine Cleavage System Represents a Novel Pathway for ATP Generation', *PLoS ONE*. Edited by Y. Moreno. Public Library of Science, 6(11), p. e25881. doi: 10.1371/journal.pone.0025881.

Verboven, K. *et al.* (2018) 'Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans', *Scientific Reports*. Nature Publishing Group, 8(1), p. 4677. doi: 10.1038/s41598-018-22962-x.

Verrey, F. *et al.* (2004) 'CATs and HATs: the SLC7 family of amino acid transporters', *Pflugers Archiv European Journal of Physiology*. Springer-Verlag, 447(5), pp. 532–542. doi: 10.1007/s00424-003-1086-z.

Veum, V. L. *et al.* (2012) 'The nuclear receptors NUR77, NURR1 and NOR1 in obesity and during fat loss', *International Journal of Obesity*. Nature Publishing Group, 36(9), pp. 1195–1202. doi: 10.1038/ijo.2011.240.

Vijay, J. *et al.* (2020) 'Single-cell analysis of human adipose tissue identifies depot- and disease-specific cell types', *Nature Metabolism*. Nature Research, 2(1), pp. 97–109. doi: 10.1038/s42255-019-0152-6.

Virtanen, K. A. *et al.* (2009) 'Functional Brown Adipose Tissue in Healthy Adults', *New England Journal of Medicine*. Massachussetts Medical Society, 360(15), pp. 1518–1525. doi: 10.1056/NEJMoa0808949.

Voight, B. F. *et al.* (2010) 'Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis.', *Nature genetics*, 42(7), pp. 579–89. doi: 10.1038/ng.609.

Voldoire, E. *et al.* (2017) 'Expansion by whole genome duplication and evolution of the sox gene family in teleost fish', *PLOS ONE*. Edited by H. Vaudry. Public Library of Science, 12(7), p. e0180936. doi: 10.1371/journal.pone.0180936.

Wajchenberg, B. L. (2000) 'Subcutaneous and visceral adipose tissue: Their relation to the metabolic syndrome', *Endocrine Reviews*. Endocrine Society, pp. 697–738. doi: 10.1210/edrv.21.6.0415.

Wang, J. *et al.* (2019) 'BCAA Catabolic Defect Alters Glucose Metabolism in Lean Mice', *Frontiers in Physiology*. Frontiers Media S.A., 10, p. 1140. doi: 10.3389/fphys.2019.01140.

Wang, Q. A. *et al.* (2013) 'Tracking adipogenesis during white adipose tissue development, expansion and regeneration', *Nature Medicine*. Nature Publishing Group, 19(10), pp. 1338–1344. doi: 10.1038/nm.3324.

Wang, T. *et al.* (2003) 'Metabolic Partitioning of Endogenous Fatty Acid in Adipocytes', *Obesity Research*. North American Assoc. for the Study of Obesity, 11(7), pp. 880–887. doi: 10.1038/oby.2003.121.

Wang, T. *et al.* (2010) 'Respiration in Adipocytes is Inhibited by Reactive Oxygen Species', *Obesity*, 18(8), pp. 1493–1502. doi: 10.1038/oby.2009.456.

Wang, W. *et al.* (2015) 'Mitochondrial Reactive Oxygen Species Regulate Adipocyte Differentiation of Mesenchymal Stem Cells in Hematopoietic Stress Induced by Arabinosylcytosine', *PLOS ONE*. Edited by G. Saretzki, 10(3), p. e0120629. doi: 10.1371/journal.pone.0120629.

Wang, X. Y. *et al.* (2018) 'Evaluation and optimization of differentiation conditions for human primary brown adipocytes', *Scientific Reports*. Nature Publishing Group, 8(1). doi: 10.1038/s41598-018-23700-z.

Weyer, C. *et al.* (2001) 'Subcutaneous abdominal adipocyte size, a predictor of type 2 diabetes, is linked to chromosome 1q21-q23 and is associated with a common polymorphism

in LMNA in Pima Indians', *Molecular Genetics and Metabolism*, 72(3), pp. 231–238. doi: 10.1006/mgme.2001.3147.

WHO (2017) 'WHO | Obesity and overweight', *WHO*. World Health Organization. Available at: http://www.who.int/mediacentre/factsheets/fs311/en/ (Accessed: 13 November 2017).

Wing, R. R. *et al.* (1998) 'Lifestyle intervention in overweight individuals with a family history of diabetes', *Diabetes Care*. American Diabetes Association, 21(3), pp. 350–359. doi: 10.2337/diacare.21.3.350.

Wu, G. (2010) 'Functional Amino Acids in Growth, Reproduction, and Health', *Advances in Nutrition*. Oxford Academic, 1(1), pp. 31–37. doi: 10.3945/an.110.1008.

Wu, G. (2013) *Amino acids: Biochemistry and nutrition*, *Amino Acids: Biochemistry and Nutrition*. CRC Press. doi: 10.1201/b14661.

Wu, Q. *et al.* (2020) 'Serine and Metabolism Regulation: A Novel Mechanism in Antitumor Immunity and Senescence', *Aging and disease*, p. 0. doi: 10.14336/AD.2020.0314.

Wulaningsih, W. *et al.* (2017) 'Investigating nutrition and lifestyle factors as determinants of abdominal obesity: An environment-wide study', *International Journal of Obesity*. Nature Publishing Group, 41(2), pp. 340–347. doi: 10.1038/ijo.2016.203.

Xie, X. *et al.* (2005) 'Lack of the alanine-serine-cysteine transporter 1 causes tremors, seizures, and early postnatal death in mice.', *Brain research*, 1052(2), pp. 212–21. doi: 10.1016/j.brainres.2005.06.039.

Yamada, C. *et al.* (2015) 'Association between insulin resistance and plasma amino acid profile in non-diabetic Japanese subjects', *Journal of Diabetes Investigation*. Blackwell Publishing, 6(4), pp. 408–415. doi: 10.1111/jdi.12323.

Yang, M. and Vousden, K. H. (2016) 'Serine and one-carbon metabolism in cancer', *Nature Reviews Cancer*, 16(10), pp. 650–662. doi: 10.1038/nrc.2016.81.

Ye, J. *et al.* (2012) 'Pyruvate kinase M2 promotes de novo serine synthesis to sustain mTORC1 activity and cell proliferation', *Proceedings of the National Academy of Sciences of the United States of America*. Proc Natl Acad Sci U S A, 109(18), pp. 6904–6909. doi: 10.1073/pnas.1204176109.

Ye, Z. *et al.* (2020) 'Coordinated Modulation of Energy Metabolism and Inflammation by Branched-Chain Amino Acids and Fatty Acids', *Frontiers in Endocrinology*. Frontiers Media S.A., p. 617. doi: 10.3389/fendo.2020.00617.

Yoneshiro, T. *et al.* (2019) 'BCAA catabolism in brown fat controls energy homeostasis through SLC25A44', *Nature*. Nature Publishing Group, 572(7771), pp. 614–619. doi: 10.1038/s41586-019-1503-x.

Yu, Y.-H. and Ginsberg, H. N. (2005) 'Adipocyte Signaling and Lipid Homeostasis', *Circulation Research*. Lippincott Williams & Wilkins, 96(10), pp. 1042–1052. doi: 10.1161/01.RES.0000165803.47776.38.

Zang, L., Maddison, L. A. and Chen, W. (2018) 'Zebrafish as a Model for Obesity and

Diabetes', *Frontiers in Cell and Developmental Biology*. Frontiers, 6, p. 91. doi: 10.3389/fcell.2018.00091.

Zang, L., Shimada, Y. and Nishimura, N. (2017) 'Development of a Novel Zebrafish Model for Type 2 Diabetes Mellitus', *Scientific Reports*, 7(1), p. 1461. doi: 10.1038/s41598-017-01432-w.

Zechner, R. *et al.* (2012) 'FAT SIGNALS - Lipases and lipolysis in lipid metabolism and signaling', *Cell Metabolism*. Cell Metab, pp. 279–291. doi: 10.1016/j.cmet.2011.12.018.

Zhang, Y. *et al.* (1994) 'Positional cloning of the mouse obese gene and its human homologue', *Nature*. Nature Publishing Group, 372(6505), pp. 425–432. doi: 10.1038/372425a0.

Zhou, M. *et al.* (2019) 'Targeting BCAA Catabolism to Treat Obesity-Associated Insulin Resistance', *Diabetes*. American Diabetes Association, 68(9), pp. 1730–1746. doi: 10.2337/DB18-0927.

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