SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Deactivation or maintenance of anti-obesity heat-generating processes in human beige adipocytes

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The PhD Defense takes place at the Lecture Hall of Bldg. A Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 11:00, on 7th of October, 2024.

1. Introduction

In recent decades, the prevalence of obesity has increased worldwide, reaching epidemic levels. Over the past half century, body mass index (BMI) has increased globally among both women and men. Weight gain is due to chronic positive energy balance, when energy expenditure is less than energy intake. Although several studies suggest that the main cause of obesity is related to abnormal food intake, certain recent behavioral changes (eg, a large decrease in physical activity-related actions) may provide an additional explanation for the continued global weight gain. In addition, the development of obesity is strongly related to socioeconomic status and environmental factors that may determine epigenetic modifications through gene-environment interactions. Adipose tissue dysfunction in obese individuals causes abnormal inflammation with an imbalance in adipocytokine production, which is associated with the development of cardiovascular disease and several site-specific tumors. Based on the descriptions above, it can be seen that we have information about obesity, but it is important to gain a more precise understanding of the molecular mechanisms involved in its development in order to create new therapeutic approaches.

Several experiments have already been carried out in rodent models, which investigated the metabolic pathways behind excessive fat storage in adipose tissue, the central tissue of obesity. In one of these publications, it was reported that selective mitochondria removal (mitophagy) has an important role in the inactivation of heat-producing fat cells, which leads to fat storage in these cells. In another study, the causative single-nucleotide polymorphism (SNP) (rs1421085) was identified, the presence of which triggers adipogenesis towards fat- storing white adipocyte differentiation, thus increasing the storage of excess energy in the form of fatty acids. In my doctoral thesis, I examined the aforementioned mitophagy and the Fat mass and obesity associated (FTO) rs1421085 SNP in human primary adipocytes, and my results can bring us closer to the metabolism of fat cells, and through this to a better understanding of obesity, which may lead to the development of new therapeutic options in the future.

2. Theoretical background

2.1. Metabolic syndrome (MetS)

MetS is a set of major metabolic disorders that include abdominal adiposity, insulin resistance, dyslipidemia, hypertension, and systemic inflammation. Several health organizations have established different criteria for defining MetS.

MetS has been associated with several risk factors as well as several putative pathophysiological mechanisms. In recent decades, the complex pathological characteristics of MetS, which includes many risk factors, have been revealed. The components of MetS are pathophysiologically related and determined partly by genetic factors as well as environmental variables.

Obesity, as one of the risk factors, is itself related to the most important symptoms of MetS, such as blood glucose concentration. Glucose intolerance, insulin resistance, and dyslipidemia are the focus of many studies in connection with obesity.

2.2. Obesity

Over the past few decades, the prevalence of obesity has increased dramatically worldwide. Chronic obesity can lead to various cancers, type 2 diabetes mellitus (DM), and cardiovascular diseases. Therefore, obesity has been identified as a leading cause of death and disability worldwide, accounting for 10-13% of deaths in different regions of the world. The most common method of determining obesity is the calculation of the Body Mass Index (BMI), which is the ratio of body weight in kilograms to the square of body height in meters. An imbalance of energy homeostasis, when energy intake is significantly greater than energy expenditure, has been identified as the main pathophysiological cause of obesity. However, obesity is a multifactorial disease that may result in from other components, including social, lifestyle, behavioral networks, and the genetic background of individuals.

Adipose tissue, along with several other organs, such as the liver, pancreas, or skeletal muscle, has been shown to play a role in obesity and obesity-related metabolic disorders. Nevertheless, the fact is that even a small amount of weight loss (5%) can actually increase insulin sensitivity and reduce the plasma levels of parameters indicating certain risk factors (e.g. circulating glucose, triglyceride (TG), and alanine transaminase levels); due to metabolic disorders, it is assumed that adipose tissue is the central organ that can regulate the balance of metabolism.

2.3. Adipose tissue and adipocyte types

In rodents, adipocytes are classified into three types. Energy-storing white adipocytes, located in white adipose tissue (WAT), have a large unilocular lipid droplet and low mitochondrial density. Brown adipocytes in brown adipose tissue (BAT) are active thermogenic cells with high mitochondrial content, fragmentation, and uncoupling protein (UCP) 1 expression and numerous small lipid droplets in the cytoplasm. "Brown-like-in-white" or beige cells have cold-inducible thermogenic potential and multilocular lipid droplets. Beige adipocytes are scattered throughout WAT. Under normal conditions, their gene expression pattern is similar to that of white adipocytes, but upon long-term stimuli (cold exposure, β-adrenergic stimulation, peroxisome proliferator-activated receptor (PPAR) γ agonist treatment) they show a brown-like phenotype, which develops during a process called browning in the cells. Inguinal WAT was discovered as the most characteristic depot of beige adipocytes, in which adipocytes had a multilocular morphology and a thermogenic gene expression profile in response to thermogenic stimuli.

Initially, BAT was considered in humans as a tissue present only in infants and located in hard-to-reach anatomical locations. Several studies using positron emission tomography (PET) proved that adults also have a significant amount of BAT, most often the depot above the neck-clavicle (cervical-supraclavicular) was characterized by high glucose uptake, especially after cold exposure. Using the elegant approach of the PET computed tomography (CT) method, tannable adipose tissue was found in several anatomical regions, such as the cervical, supraclavicular, axillary, mediastinal, paravertebral, and abdominal regions. However, in contrast to rodents, the molecular features of human BAT remain uncharted. Several studies have reported that human BAT isolated from the cervical-supraclavicular depot and primary adipocytes from fetal interscapular adipose tissue have characteristics of classical brown adipocytes characterized by high expression of zinc finger protein-1 (ZIC1). Other studies using human brown adipocytes of clonal origin isolated from the supraclavicular depot reported that the population of UCP1-positive cells rather contained beige adipocytes. Another study using total RNA isolated from fat biopsies from different anatomical sites, including subcutaneous (SC) supraclavicular, posterior mediastinal, retroperitoneal, intra-abdominal, or mesenteric depots, reported that beige-selective markers, such as Homeobox C (*HOXC*) 8, *HOXC9*, and *CITED1* were highly expressed in human BAT, while classical brown markers were not detected.

2.4. The role of brown and beige adipocytes in thermogenesis

BAT, which contains thermogenic brown and beige adipocytes, is able to dissipate energy through heat generation, which is mainly mediated by UCP1-dependent proton leak, which uncouples oxidative phosphorylation from ATP synthesis in mitochondria. Then heat is released, and ATP production stops. In this case, during the terminal oxidation, the H^+s forming the proton gradient will flow back into the mitochondrial matrix through the channel of the UCP1 protein (thermogenin) instead of the ATP synthase. Thus, heat is generated as a result of gradient equalization.

A creatine-dependent ATP-using substrate cycle has been described to promote energy uncoupling in brown and beige adipocytes. This cycle was initially identified in mitochondria isolated from the beige fat of cold-exposed animals in the presence of limited ADP, and was later confirmed to exist in both murine and human brown adipocytes. Previous studies have shown that creatine stimulates substrate cycling and enhances ADP-dependent respiration in beige adipocyte mitochondria when ADP is limited. In the absence of UCP1, genes involved in creatine metabolism, including creatine kinase B (CKB), are upregulated by thermogenic cAMP signaling. Additionally, depletion of creatine in thermogenic adipocytes of mice by knocking out the rate-limiting enzyme of creatine biosynthesis, glycine amidinotransferase, reduces energy expenditure due to inhibited thermogenesis and causes diet-induced obesity. The existence of this cycle in BAT supports the idea that increasing mitochondrial ATP synthesis can be used as an approach to promote energy uncoupling.

Activation of UCP1 for heat production in brown/beige adipocytes results in increased uptake of nutrients, such as glucose and fatty acids, which sustains the formation of tricarboxylic acid (TCA) cycle intermediates. As a result, NADH and $FADH_2$ are produced, which enter the electron transport chain. Active brown/beige adipocytes also take up large amounts of TCA cycle intermediates, e.g. succinate, to enhance their proton leak respiration. A recent study reported that labeled glucose used by mouse BAT was converted to pyruvate during cold exposure, which was further oxidized to acetyl-CoA in a reaction catalyzed by pyruvate dehydrogenase. In addition to glucose and fatty acids, active brown/beige adipocytes also catabolize branched-chain amino acids to meet their high energy needs. Our previous study also emphasized the importance of serine, cysteine, and glycine uptake mediated by alanine-serine-cysteine transporter 1 (ASC-1) during the effective thermogenic response of deep cervical adipocytes to adrenergic stimulation. The transporter encoded by the *SLC7A10* gene facilitates sodium-independent bidirectional transport of the above-mentioned small, neutral amino acids.

We observed that the gene showed higher expression in thermogenic adipocytes of deep cervical origin, especially during brown differentiation. ASC-1 is a major regulator of extracellular D-serine levels in synaptic systems. The ASC-1 transporter was previously known as a specific cell surface marker for white adipocytes. ASC-1 mRNA expression was recently reported to be high and induced in adipocytes freshly isolated from abdominal SC and omental WAT, and to be strongly negatively correlated with visceral obesity and insulin resistance in humans, and to promote mitochondrial respiration and insulin-stimulated glucose uptake. The ability of active brown/beige fat cells to absorb nutrients can contribute to the reduction of sugar and lipid levels in the blood, which indirectly improves glucose tolerance and insulin sensitivity.

BAT plays a key role in maintaining constant core body temperature through non-shivering thermogenesis and may open promising therapeutic opportunities in the fight against obesity. BAT is found in six anatomical regions (cervical, supraclavicular, axillary, mediastinal, paraspinal, and abdominal) in adult humans, accounting for 4.3% of total fat and 1.5% of total body weight. Based on mathematical modeling, BAT can oxidize approximately 4 kg of fat per year in adult humans, and its thermogenic activity can contribute up to 5% to the basal metabolism. Active brown/beige adipocytes increase energy expenditure through high metabolic substrate utilization, thus promoting weight loss and becoming a potential pharmacological target for the treatment of obesity and related metabolic diseases.

2.5. Origin of brown and beige adipocytes

Similar to skeletal muscle cells, brown adipocytes originate from embryonic mesoderm precursors. These precursor cells transiently express myogenic factor-5 (Myf5), engrailed 1 (En1), and paired box 7 (Pax7), which indicate the mesodermal origin of the myogenic cells of the skeletal system. This muscle-like gene expression pattern, as well as the mitochondrial proteome similar to muscle cells, suggests that there is a cellular ontological relationship between classical brown adipocytes and muscle cells. In a mouse model, it was described that the primitive BAT depots, formed during embryogenesis, were generated by hyperproliferative fibroblast-like cells, which remained lipid-poor until the postpartum period. Early "fate-mapping" studies of embryonic precursors showed that brown adipocytes originated from the paraxial mesoderm of early somites (primary segments) within the dermomyotome. The main regulator of brown adipocyte formation is PRD1-BF-1-RIZ1 homologous domain-containing protein-16 (Prdm16). Prdm16 acts as a molecular switch that determines whether Myf5-expressing precursor cells differentiate into brown adipocytes or myocytes. In committed BAT,

precursors express En1, Myf5, and Pax7 transcription factors. While early B cell factor 2 (EBF2) is expressed in brown adipocyte precursors, myoblast determination protein (MyoD) and Myf5 production downstream takes place in somites. MyoD, expressed in somites, prevents the precursors from turning into brown adipocytes.

In contrast to classical brown adipocytes, the embryonic origin of beige adipocytes has not been fully clarified until now. These two cells are probably derived from different cell lineages, as Myf5+ cells are not found in SC fat depots. Using the "pulse-chase fate-mapping" technique, the formation of beige adipocytes from a previously uncharacterized precursor cell was observed using cold and β3-adrenergic agonists in the SC lumbar region. Lineage-tracing studies have described that beige adipocytes in mice originate from progenitors that express Myosin heavy chain (Myh) 11, Spinal muscular atrophy/ Survival motor neuron protein (SMA), Platelet derived growth factor receptor (PDGFR) α, or PDGFRβ. Roscovitine, which is a pharmacological inhibitor of cyclin-dependent kinase (CDK) 5, promotes the development of UCP1-positive adipocytes, which show a different molecular pattern compared to classical brown adipocytes.

Based on the above, beige adipocytes are highly plastic, they respond to environmental stimuli, so they can be converted between the beige and white fat cell states. Furthermore, beige adipocytes appear to be metabolically heterogeneous and exhibit substrate specificity.

2.6. Activation of heat production in adipocytes

In most laboratory studies of beige thermogenesis, thermogenic activity is induced by prolonged cold exposure or pharmacological activation of β3-adrenergic receptors (β3-AR) in mice. Both mechanisms require transient and continuous stimuli. Cold-activated brown adipocytes increase metabolic activity and thermogenesis, the process of which includes the upregulation of GLUT1 and GLUT4 glucose transporters and the increased expression of glycolytic enzymes such as hexokinase, lactate dehydrogenase, and phosphofructokinase. Thermogenic activation in most adipose tissues can occur to varying degrees in different regions, such as inguinal, lumbar, retroperitoneal, and SC WAT (sWAT), as well as interscapular, cervical, supraclavicular, paravertebral, SC, and perirenal BAT. Several stimuli such as cold exposure, β-adrenergic agonist administration, exercise, or natural plant extracts have been shown to be effective in activating adipocytes to acquire high thermogenic capacity while simultaneously increasing their metabolic activity. The most frequently used and most effective stimulation in research is the previously mentioned cold exposure and β-adrenergic

stimulation, during which β-adrenergic signaling is activated. In rodents, β3-ARs have been shown to activate brown adipocytes and adipose tissue browning, leading to increased UCP1 protein levels in various adipose depots and decreased free fatty acid (FFA) storage in WAT, resulting in higher FFA oxidation and utilization in BAT. Although recent evidence suggests that β3-AR may not be essential and that tanning may occur without the presence of β3-AR, the fact that β3-AR is involved in the activation cascade of adrenergic stimulation has been widely confirmed. However, in humans, β2-AR, and not β3-AR, was identified as the key receptor for activation of BAT thermogenesis and was the predominant β-AR (up to 91%) expressed at the mRNA level in WAT, suggesting that humans and rodents differ in response to β-AR stimulation.

The binding of norepinephrine to β-ARs activates the production of cyclic adenosine monophosphate (cAMP) and the activity of protein kinase A (PKA). PKA phosphorylates cAMP response element-binding protein (CREB) and p38 mitogen-activated protein kinase (p38-MAPK), both of which regulate the transcription of PPARγ coactivator (PGC) 1α and increase the expression of UCP1 proteins. PKA also phosphorylates the mammalian target of rapamycin (mTOR), which is a key regulator of cell metabolism and responds to nutrients in the cellular microenvironment, and is necessary for the formation of brown morphology in adipose tissue. The role of PKA in BAT stimulation and metabolic modulation has been shown to correlate with the transcription factor PGC-1α, which is a key regulator of mitochondrial metabolism required for adaptive thermogenesis. The role of PKA in browning is site-specific and depends on the individual physiological state, because only stimulation of SC white adipocytes increased lipolysis through mitochondrial uncoupling, and obesity reduced the cell's thermogenic capacity. In addition to activation via β-AR, a recent study revealed a non-canonical pathway of UCP1 regulation and thermogenic activation via fibroblast growth factor 6 and 9 (FGF). In this study, the authors found that FGF6 and FGF9 were induced during exercise and cold exposure, which activates FGFR3 receptors. This promotes biosynthesis of the mediator prostaglandin E2, which regulates UCP1 expression through the binding of FLII transcriptional coactivators and estrogen-related receptor alpha (ERRα) to the *UCP1* enhancer, suggesting a novel mode of activation of thermogenesis.

Thermogenic genes can also be regulated by activation of thyroid receptor α and β (TRα and β), which are expressed in both WAT and BAT. The specific isoforms of TR in BAT can vary widely among different species. Thyroid hormone (TH) has two main isoforms. The less active thyroxine isoform (T4) can be transformed into its active form, 3,3′,5-triiodothyronine (T3),

catalyzed by type II deiodinase (DIO2). TH plays an important role in the regulation of metabolism in various tissues during growth and development. In interscapular BAT (iBAT), T3 was found to activate TRα, which promoted the differentiation of the adipocyte progenitor cell (APC) population and the transition of APCs from an elongated morphology to an adipogenic commitment, during which iBAT in its depots, Myc-mediated glycolysis was activated. Another study revealed that T3 induced forkhead box O1 (FOXO1), a target of sirtuin 1 (SIRT1) downstream, whose enzymatic activity depended on cellular NAD^+. Activation of SIRT1 has been described to improve BAT metabolism and function, and improves glucose homeostasis. In addition, SIRT1 induced inhibition of mTOR, leading to increased autophagic and lysosomal gene expression in brown adipocytes, resulting in increased mitochondrial turnover, biogenesis, and improved thermogenesis in BAT. T3 also increased the expression of another thermogenin protein isoform, UCP3, which was involved in thermogenesis and correlated with UCP1 expression in BAT during cold exposure. The role of UCP3 in thermogenesis has been demonstrated in many tissues, including skeletal and cardiac muscle. Although UCP3 may be involved in BAT thermogenesis and is essential for non-shivering heat production in hamsters, it may only be complementary to the thermogenic function of UCP1 in mouse BAT, suggesting that UCP1 is always the major thermogenin isoform in BAT during cold exposure.

Hyperthyroidism also leads to increased TH levels in the hypothalamus, and increased TH in the ventromedial nucleus of the hypothalamus at both 4°C and 23°C, upregulates UCP1 expression in BAT, resulting in weight loss, suggesting that TH has an indirect central effect on thermogenesis, systemic metabolism, and energy expenditure. Hypothalamic regulation of thermogenesis and energy expenditure can also be achieved by the secretion of the adipokine leptin, which regulates BAT thermogenesis, probably by increasing the activity of sympathetic nerves to BAT and involving the BAT circuit in sympathetic regulatory regions, which increases the expression of UCP1 in mouse models when exposed to a high-fat diet. In a lipodystrophy mouse model, leptin increases the expression of Pgc-1a, Cell Death Inducing DFFA-Like Effector A (Cidea), and Ucp1 in BAT, as well as increases body temperature, despite the fact that the beneficial effects of this process on metabolism have not been proven so far. The molecular effect of leptin on BAT thermogenesis and metabolism has been investigated to a limited extent, however, the effect of leptin increasing energy consumption can be related to the regulation of hypothalamic TH. These data suggest a systemic interaction whereby BAT is involved in the centrally controlled regulation of thermogenesis and energy

expenditure. It has not yet been clarified in detail how the different physiological states of the individual affect the activity of BAT and its influence on the systemic metabolism. Aspects related to the specific physiological and metabolic state of each individual should also be taken into account when planning future studies.

2.7. The beige-to-white adipocyte transition

The thermogenic phenotype of beige adipocytes becomes reversible after withdrawal of external stimuli. Upon cessation of adrenergic stimulation (e.g. at warm temperatures), beige adipocytes gradually transform into cells with unilocular lipid droplets and lose their beige properties while increasing their white features (e.g. decreased mitochondrial number and thermogenesis). This beige-to-white transition is accompanied by reduced sympathetic innervation, vascularization, and UCP1 expression, as well as increased neural chemorepellent (semaphorin III) secretion and leptin expression. The phenotypic and morphological transformation observed in beige fat depots after withdrawal of stimuli is not observed in classical brown fat cells. In 2013, Christian Wolfrum's research group used cell lineage tracing to follow the beige-white interconversion. They showed that cold-induced beige fat was transformed within 5 weeks of the onset of warm temperatures, however, almost 75% of beige adipocytes with a white-like phenotype could become beige again upon cold re-induction. Interestingly, after the second cold exposure, half of the beige adipocytes were formed from the previously whitened beige adipocytes, and the other half of the newly formed beige adipocytes were originated from a different source. Although beige adipocytes lost their brown-like phenotype and acquired a white-like phenotype upon increasing temperature, they retained their epigenetic memory of cold exposure, allowing them to reactivate browning genes once exposed to cold temperatures. Interestingly, beige adipocyte apoptosis and death were not found to be the cause of the loss of the beige phenotype. In contrast, BAT whitening has been shown to increase cell death by increasing adipose tissue inflammation, suggesting a lack of plasticity in BAT. In 2015, Kozak and his research group reported much greater dynamics of UCP1 and mitochondrial trafficking in beige fat compared to BAT. Recent publications have reported that autophagy plays a role in decreasing beige adipocyte thermogenesis. In rodents, parkin-dependent selective mitochondrial removal (mitophagy) initiates the formation of inactive beige adipocytes, which are morphologically white but can be reactivated. Mitophagy activity increased upon withdrawal of adrenergic stimuli and was shown to be mediated by the recruitment of parkin (PARK2) to mitochondria. Inhibition of autophagy by deletion of autophagy-associated protein (ATG) 5, ATG12, and PARK2 preserved the beige phenotype

even after the stimuli were removed. In single cell experiments, it was observed that the same process caused direct transdifferentiation (beige-to-white transition), which did not involve an intermediate step. In recent years, our research group described that cAMP-induced thermogenic activation in differentiated human primary SC and Simpson–Golabi–Behmel syndrome (SGBS) adipocytes downregulated mitophagy, thus blocking the beige to white transition. Preventing entry into this remodeling process may be a potential way to maintain increased thermogenesis for the fight against obesity.

2.8. Mitophagy and its role in the regulation of thermogenic beige adipocytes

Mitochondria are crucial for cellular homeostasis. They perform vital functions such as bioenergetics, biosynthesis, and cellular signaling. They also play an important role in the thermogenic and metabolic function of classical brown and beige adipocytes. Proper maintenance of these processes is key for preventing diseases and ensuring optimal cellular function. Mitochondria can change their morphology through fission and fusion. During fusion, a large network of elongated mitochondria is created, while during fission smaller fragmented units are formed. Damaged or unwanted mitochondria can be removed by selective autophagy, called mitophagy, which is considered as a key mechanism of mitochondrial quality control.

Autophagy is a well-described intracellular catabolic process in which protein aggregates or damaged organelles are delivered to lysosomes by double-membrane-bound structures called autophagosomes for degradation and then their components are recycled. During the formation of the autophagosome, the molecular signal of mTOR complex 1 (mTORC1) triggers the activation of the unc-51–like autophagy activating kinase (ULK) 1 complex, which consists of ULK1, ATG13, and the focal adhesion kinase family interacting protein of 200 kDa (FIP200), which initiates the formation of the isolation membrane from existing membrane sources such as the endoplasmic reticulum (ER) or the Golgi. The membrane continues to stretch to form a fully enclosed, double-membrane-bounded vesicle known as an autophagosome. The formation of the autophagosome is controlled by several central proteins linked to autophagy. A key step in autophagosome formation is the conjugation of phosphatidylethanolamine (PE) and microtubule-associated protein light chain 3 (LC3)-I — an ATG8 homologue — to generate the lipidated form of LC3, LC3-II. This conjugation is partially mediated by ATG7 and the ATG5-ATG12-ATG16L1 complex. LC3 is a widely accepted autophagosomal marker, and LC3-II content is an indicator of the degree of autophagosome formation. Detection of the conversion of LC3-I to LC3-II by western blotting is a commonly used method to monitor autophagy activity, as the amount of LC3-II correlates with the number of autophagosomes.

Once the autophagosome has matured, it fuses with the lysosome to form an autolysosome (a membrane-bound acidic vesicle) where lysosomal hydrolytic enzymes, such as cathepsins, degrade its contents. Lysosome biogenesis is an important component of the autophagy machinery and it is regulated by the microphthalmia/transcription factor E (MiT/TFE) transcription factor family, which includes microphthalmia-associated transcription factor (MITF), EB transcription factor (TFEB), and the IGHM enhancer 3-binding transcription factor (TFE3).

In the case of mitophagy, selectivity is provided by specific proteins that physically connect mitochondria to the autophagosomal protein LC3. These receptors interact with the autophagosome through the LC3-interacting region (LIR). Mitochondrial damage is a major physiological trigger for selective mitochondrial removal. Damage-induced mitophagy can occur through two different mechanisms: adapter-mediated ubiquitin-dependent mitophagy and direct ubiquitin-independent mitophagy. Adapter-mediated mitophagy, mediated by phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin, requires target ubiquitination. Damage to mitochondria leads to reduced mitochondrial membrane potential, stabilization of PINK1 on the outer mitochondrial membrane, and subsequent recruitment of Parkin, which ubiquitinates outer mitochondrial proteins. Ubiquitinated substrates are then recognized by autophagy adapter proteins. These include p62, optineurin (OPTN), nuclear dot protein 52 kDa (NDP52/ CALCOCO2), and neighbor of Brca1 gene 1 (NBR1), which link ubiquitinated targets to LC3. Adapter proteins contain two defining domains: a ubiquitin-binding domain (UBD) for target recognition and a LIR domain that interacts with LC3 to promote autophagosome formation. Damage-induced mitophagy can also occur through a direct interaction between mitochondria-localized proteins and LC3, independent of ubiquitination. For example, BCL2/E1B 19 kDa–interacting protein 3 (BNIP3), BNIP3 Like/NIP3-Like Protein X (BNIP3L/NIX), and FUN14 domain-containing protein 1 (FUNDC1) interact directly with LC3 to promote mitophagy, in response to hypoxia-induced mitochondrial damage. The mammalian homologue BCL2-like protein 13 (BCL2L13) mitophagy receptor Atg32 directly interacts with LC3 through the LIR domain, however, the mechanism by which it activates BCL2L13 is still unclear. We have considerable knowledge about selective mitophagy; however, many questions remain unanswered, such as how mitochondrial removal is regulated in a cell type- or tissue-specific manner.

2.9. The *FTO* gene

In order to better understand the biological mechanisms underlying polygenic diseases, genome-wide association studies (GWAS) aim to better understand the genetic variants of the human genome. These first GWAS showed that *FTO* has a predisposing effect on obesity, and discovered two single-nucleotide polymorphisms (SNPs) located in the first intron of the gene (rs9930506, rs9939609). These studies eventually led to the investigation of the effect of *FTO* on obesity.

FTO is a gene consisting of 9 exons and 8 introns located on chromosome 16 in humans, which encodes a protein belonging to the AlkB DNA repair dioxygenase enzyme family. The enzyme catalyzes 2-oxoglutarate and Fe(II)-dependent demethylation in single-stranded DNAs and RNAs. During demethylation, it can catalyze the transformation of several nucleotides (e.g. 3-methyluracil (m^3U), 3-methylthymine (m^3T)), but its main substrate is N^6-methyladenosine (m^6A). FTO can bind to a variety of RNAs, including mRNA, snRNA, tRNA, and demethylate m^6A and N^6,2'-O-dimethyladenosine (m^6Am) in mRNA, m^6A in U6RNA, m^6Am in snRNAs and N^1-methyladenosine (m^1A) in tRNA.

The methylation modification of m^6A on the sixth nitrogen atom of adenine is the most common mRNA methylation, which is enriched in the 3'-untranslated region (3'-UTR) between the stop and start codons. Modifications of m^6A are subject to reversible and dynamic regulation, including writers (METTL3, METTL14, and WTAP), removers (FTO and ALKBH5) and readers (YTH domain family and IGF2BP). Based on dynamic regulation, it can be seen that m^6A plays an important role in regulatory processes following transcription, such as RNA splicing, nuclear production, degradation, and translation. In addition to DNA repair and post-translational regulation, the FTO enzyme is also involved in fatty acid metabolism.

FTO is widely expressed in adipose tissue and skeletal muscle, with the highest expression in the hypothalamus in the energy balance regulatory region (nucleus arcuatus), indicating that it may play a critical role in the regulation of appetite and energy metabolism. Mutations in the exon of the *FTO* gene can cause severe polymalformation syndrome and growth retardation. In FTO KO mice, complete FTO deficiency has been reported to be associated with postnatal growth retardation, significant reduction in adipose tissue, and lean body mass, but no developmental abnormalities in the central nervous system or cardiovascular system have been reported. In contrast, FTO overexpression in rodents significantly increased fat mass in abdominal WAT under both normal and high-calorie diets.

Genetic variants appearing in the first intron of the *FTO* gene predispose to obesity. Individuals homozygous for the risk allele of the previously mentioned rs9939609 polymorphism weigh 3 kg more on average and have a 1.7 times greater chance of obesity than homozygous for the risk-free allele. A significant decrease in body weight, fat mass, and WAT volume was observed in FTO-deficient mice compared to wild-type or heterozygous mice. The ratio of BAT to body weight was increased, but not significantly. FTO is not an essential enzyme for embryonic development, however, postnatal lethality was more frequent in mice homozygous for the risk allele.

With the development of techniques used in GWAS, it was possible to find the causal SNP, the presence of which disrupts a conserved motif within the *FTO* locus. In the early stage of adipocyte differentiation, changes in the rs1421085 risk allele (T→C transition) lead to increased dual expression of the Iroquois homeobox transcription factor 3 and 5 (IRX3/5), as binding of the AT-rich interaction domain 5b (ARID5B) repressor protein is inhibited. As a result, progenitor cells committed to the beige program are more likely to differentiate into white adipocytes, which reduces mitochondrial thermogenesis five times more. After it became possible to demonstrate which part of the *FTO* locus predisposes to obesity, the next task was to find out which tissue regulates the function of the SNP. In the human body, most genetic variants affecting body weight exert their effects in the hypothalamic region. This area is responsible for regulating food intake and energy consumption. In a mouse model, it was described that partial inhibition of hypothalamic Irx3 expression decreased Ucp1 expression, which aggravated obesity. In the first intron of *FTO*, the SNPs are located within a so-called super-enhancer region, which are most active in cells of mesenchymal origin, such as mesenchymal cells of adipocyte origin. Increased IRX3/5 expression induces the expression of genes that drive adipocyte precursors towards white instead of beige adipocyte differentiation.

3. Aim of the study

We distinguish three adipocyte cell types: the energy-storing white adipocytes, which have a unilocular lipid droplet, and the brown and beige adipocytes, whose cytoplasm contain multilocular lipid droplets, express UCP1, and promote energy dissipation. The latter two cell types play an important role in maintaining core body temperature after cold exposure through non-shivering heat production.

It was previously described in mouse models that in the absence of external thermogenic stimulus, beige adipocytes are inactivated with the help of mitophagy, thus these cells enter a white-like state. According on this, we have set the following goals:

- Investigating whether mitophagy is activated cell-autonomously during the beige to white transition of primary human abdominal SC adipocytes

- Study of the molecular mechanism underlying the activation of mitophagy during the inactivation of human beige adipocytes

In 2015, the causative SNP (rs1421085) was identified, the presence of which disrupts the conserved motif within the *FTO* locus, which regulates mesenchymal adipocyte precursors to white adipocyte differentiation. During my studies, we have set the following goals:

- Characterization of white, active beige, and inactive beige adipocytes derived from SC fat using a system biology approach

- Examination of the effect of the beige to white transition on primary human SC adipocytes (by gene expression analysis)

- Clarification of how the *FTO* rs1421085 SNP affects SC adipocyte differentiation, nutrient utilization, and beige to white transition

4. Materials and methods

4.1. Materials

All chemicals were obtained from Sigma Aldrich (Munich, Germany) unless stated otherwise.

4.2. Origin of the fat samples and ethical statement

For our studies, we used SC fat tissue from abdominal and inguinal plastic surgery. After adequate information, the donor patients gave their written consent to the sampling. Our experiments on these human-derived cells were carried out in accordance with the guidelines contained in the Declaration of Helsinki. The research plan was accepted by the Medical Research Council of Hungary (No. 20571-2/2017/EKU) and the Regional and Institutional Research Ethics Committee of the University of Debrecen (No. 3186-2010/DEOEC RKEB/IKEB; No. 4734-2017 /DE RKEB/IKEB).

4.3. Isolation and maintenance of human adipose-derived stromal cells (hASCs, preadipocytes)

After multiple washings of adipose tissue samples from liposuction with sterile PBS, the connective tissue was digested with 120 U/ml collagenase treatment in sterile PBS for 3-4 hours while shaking the samples at 37°C. After incubation, the samples were purified through a filter with a pore diameter of 140 μm, followed by washing with culture media and centrifugation at 1300 rpm for 10 minutes. Cells were suspended in growth medium consisting of: Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham (DMEM-F12) + 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltman, MA, USA) + 33 μM biotin + 17 μM pantothenic acid + 1% penicillin/streptomycin. The cells were then plated in T-75 flasks, and the day after isolation, they were washed with sterile PBS, and then the medium was changed every 3 days. The flasks were incubated at 37°C with a 5% CO_2 content until the appropriate confluence was reached. To verify the absence of Mycoplasma contamination, a polymerase chain reaction (PCR)-based analysis was performed with the PCR Mycoplasma Test Kit I/C (PromoKine, PromoCell, Heidelberg, Germany).

4.4. Differentiation of hASCs

hASCs were seeded in 6-well plates, which were kept in the growth medium and conditions described in chapter 4.3 until the appropriate confluence was reached.

White adipogenic differentiation was induced for 3 days with serum-free DMEM-F12 medium supplemented with 17 μM pantothenic acid, 33 μM biotin, 100 U/mL penicillin/streptomycin, 100 nM cortisol, 10 μg/mL human apo-transferrin, 200 pM triiodothyronine, 20 nM human insulin, 2 μM rosiglitazone (Cayman Chemicals, Ann Arbor, MI, USA), 25 nM dexamethasone, and 500 μM 3-isobutyl-l-methylxantine. After the third day, rosiglitazone, dexamethasone, and 3-isobutyl-l-methylxantine were removed from the medium during the remaining 25 days of the differentiation. The medium was exchanged in every third day. The active beige differentiation was induced for 3 days with serum-free DMEM-F12 medium supplemented with 17 μM pantothenic acid, 33 μM biotin, 100 U/mL penicillin/streptomycin, 10 μg/mL human apo-transferrin, 200 pM triiodothyronine, 20 nM human insulin, 2 μM rosiglitazone, 25 nM dexamethasone, and 500 μM 3-isobutyl-l-methylxantine. After the third day, dexamethasone and 3-isobutyl-l-methylxantine were removed and 500 nM rosigliazone was added to the medium for the remaining 25 days of differentiation. On the 14th day, a 4 h long 500 μM dibutyryl-cAMP treatment was carried out to mimic the *in vivo* cold-induced thermogenesis. After the treatment, the aforementioned beige differentiation medium was applied until the end of the differentiation. The inactive beige differentiation was induced in the first 14 days as in the case of the active beige adipocytes, but after the dibutyryl-cAMP treatment, the medium was replaced to the white cocktail without rosiglitazone, dexamethasone, and 3-isobutyl-l-methylxantine for additional 14 days. The medium of active and inactive beige adipocytes was exchanged in every third day.

To investigate mitophagy, we modeled white and beige differentiation and beige-white transformation. hASCs were differentiated in 6-well plates (Costar, Corning, NY, USA) for 14, 21, and 28 days using white or beige adipogenic differentiation protocols. For white differentiation, see the section indicated above. Beige differentiation was the same as active beige differentiation, however, on day 14, 500 μM dibutyryl-cAMP treatment did not occur. The transformation was initiated by adding 100 nM cortisol and removing 500 nM rosiglitazone. The concentration of human insulin was reduced by 42.5 times when the transition was induced. The cells were incubated in the presence of 5% CO_2 and at 37°C, and the medium was changed every three days.

4.5. RNA isolation using TRIzol reagent

At the end of the differentiation, after removing the differentiation medium, 1000 μl TRIzol reagent (Thermo Fisher Scientific) was added to the cells, and then the samples were thoroughly vortexed. The prepared lysates were left at room temperature for 10 minutes. Then 200 μl of

chloroform was added to them, vortexed and then incubated for 5 minutes at room temperature. The samples were centrifuged for 15 minutes at 2000 g at 4°C, during which three phases were separated. The upper, colorless phase contained the RNA, which was transferred to an Eppendorf tube. Genomic DNA isolation was performed from the lower, magenta-colored phases. 500 µl of isopropanol was added to the colorless phase, then incubated for 10 minutes at room temperature, followed by 10 minutes of centrifugation at 4°C at 2000 g. The supernatant was then removed from the pellets, then washed with 1000 µl of 70% ethanol, and centrifuged again for 5 minutes at 4°C at 2000 g. The supernatant was removed from the pellets and then dried at room temperature. Afterwards, the pellets were redissolved in nuclease-free water at 65°C for 10 minutes. The quality and concentration of the RNA samples were checked with a Nanodrop (Thermo Fisher Scientific) device, and then they were diluted to a concentration of 50 ng/µl.

4.6. Isolation of genomic DNA using TRIzol reagent

DNA was precipitated from the magenta phases obtained in chapter 4.5 with 300 µl of 100% ethanol, then incubated for 2-3 minutes at room temperature, and then centrifuged for 5 minutes at 4°C at 2000 g. After careful removal of the supernatant, the DNA pellet was washed with 1.5 ml of 0.1 M sodium citrate for 1 hour, with 20 minutes rotations, and then centrifuged again for 5 minutes at 4°C at 2000 g. After removing the washing liquid, the samples were washed with 1.5 ml of 70% ethanol, then left to stand for 15 minutes at room temperature, and then centrifuged for 5 minutes at 4°C at 2000 g. The supernatant was removed from the pellets, which were then dried at room temperature. Finally, the DNA pellet was redissolved in nuclease-free water at 65°C for 10 minutes. The quality and concentration of the DNA samples was checked with a Nanodrop (Thermo Fisher Scientific) device, and then they were diluted to a concentration of 50 ng/µl.

4.7. Genotyping by real-time quantitative PCR (qPCR)

For the analysis of the rs1421085 SNP, we performed quantitative PCR, for which we used TaqMan® Genotyping Master Mix and TaqMan® Genotyping Assay (Thermo Fisher Scientific). Genotyping Master Mix consisted of the following components: ultrapure DNA polymerase (Thermo Fisher Scientific), dNTP mix, ROX passive reference (for reporter signal normalization), and PCR buffer. The assay contained the sequence-specific primers we aimed to investigate, as well as two TaqMan® MGB probes for allele detection. qPCR was performed on an Applied Biosystems QuantStudio 12K Flex instrument. The amplification protocol was

as follows: 10 min incubation at 95°C, followed by 15 s at 95°C and 1 min at 60°C (for 50 cycles). We worked with two parallels per sample. The evaluation was performed by Applied Biosystems QuantStudio 12K Flex Software v1.2.2. software.

4.8. Reverse transcription, qPCR and RNA-Sequencing analysis (RNA-Seq)

To generate cDNA, we used TaqMan® Reverse Transcription Reagents kit (Applied Biosystems), which was used according to the manufacturer's instructions with primers and probes designed by Applied Biosystems. qPCR analysis was performed on a LightCycler 480 (Roche, Basel, Switzerland) using the following protocol: 10 s at 94°C, 12 s at 94°C, and 30 s at 60°C (for 40 cycles). Each sample was tested in three technical replicates. The gene expression was normalized to the human *GAPDH* gene or to the average of the Ct values of the *GAPDH* and *ACTB* genes, which were calculated using the ΔCt method.

High-throughput mRNA sequencing analysis was performed on the MGI DNBSEQ G400 sequencing platform. Total RNA sample quality was checked on the Agilent BioAnalyzer using the Eukaryotic Total RNA Nano Kit according to the manufacturer's protocol. Samples with an RNA integrity number (RIN) greater than 7 were used in the library construction process. Libraries were prepared from total RNA using MGIEasy RNA Library Prep Set V3.0 (MGI, Shenzhen, China) according to the manufacturer's protocol. Briefly, poly-A RNAs were captured with oligo-dT conjugated magnetic beads, then the RNAs were eluted and fragmented at 94°C. First-strand cDNA was generated by random primer reverse transcription, and double-stranded cDNA was generated after second-strand synthesis. After end repair, A-tail and adapter ligation steps, the adapter-ligated fragments were amplified by enrichment PCR and finally sequencing libraries were generated. After sequencing, reads were aligned to the GRCh38 reference genome with EnsEMBL 95 annotation using Star aligner (version 2.7.0a). We quantified our reads for genes using FeatureCounts. Significantly differentially expressed genes (DEGs) were defined based on corrected $p<0.05$ and log2-fold change threshold >0.85. Heatmaps were created using GraphPad 8.0 (GraphPad Software, San Diego, CA, USA), and interactome maps were created using Gephi 0.9 based on the STRING interaction (http//string-db.org/). Pathway analysis was performed by subjecting the list of DEGs to STRING analysis, and overrepresented KEGG pathways were selected based on a false discovery rate (FDR) of less than 0.05. The brown fat cell content and browning capacity of the samples were calculated using BATLAS and ProFat open access applications by examining the transcriptomic data of both marker groups.

4.9. Mitochondrial DNA (mtDNA) isolation and quantification by PCR

Whole cell DNA was isolated as described in chapter 4.6. The qPCR was performed in triplicate with diluted DNA 10 μM using primers (mtDNA-specific PCR, forward primer 5′-CTATGTCGCAGTATCTGTCTTTG-3′, reverse primer 5′-GTTATGATGTCTGTGTGGAAAG-3′, and nuclear specific PCR, forward primer 5′-CTTTGTGTGCTATAGATGATATGGTAAATTG-3′, reverse primer 5′-GATTAAACAGTGTACAAAAGTAG-3′) and Maxima Sybr Green/Rox qPCR MasterMix (Thermo Scientific). The reaction was run on a LightCycler 480 with the following program: 20 minutes at 95°C, followed by 50 cycles of 15 seconds at 95°C, 20 seconds at 58°C, and 20 seconds at 72°C. Single-product amplification was verified by melting curve analysis after an integrated run. The results were calculated from the difference between the Ct values of mtDNA and core-specific amplification. Data are expressed as mitochondrial genome normalized to diploid nuclei.

4.10. Antibodies and immunoblotting

The investigated proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then blotted onto a polyvinylidene fluoride (PVDF) membrane, which was blocked with a 5% skimmed milk powder solution. Primary antibodies were used in the following dilutions: anti-β-actin (1:5000, A2066), anti-UCP1 (1:750, R&D Systems, Minneapolis, MN, USA, MAB6158), anti-OXPHOS (1:1000, Abcam , Cambridge, MA, USA, ab110411), anti-SLC7A10 (1:500, Abnova, Taipei City, Taiwan, H00056301-B01P), anti-PGC1α (1:1000, Novus Biologicals, Centennial, CO, USA, NBP1-04676), anti-glutamate-pyruvate transaminase (GPT) 2 (1:2000, Thermo Fisher Scientific, PA5-62426), anti-serine hydroxymethyltransferase (SHMT) 1 (1:2000, Thermo Fisher Scientific, PA5-88581), anti-p62 (1:5000, Novus Biologicals, NBP1-49956), anti-LC3 (1:2000, Novus Biologicals, NB100-2220), anti-Parkin (1:750, Santa Cruz Biotechnology, Dallas, TX, USA, sc- 32282), and anti-NBR1 (1:1000, Novus Biologicals, NBP1-71703). Secondary antibodies corresponding to the following species were used: HRP-conjugated goat anti-mouse (1:5000, Advansta, San Jose, CA, USA, R-05071-500) or anti-rabbit (1:5000, Advansta, R-05072- 500) IgG. The expression of the visualized immunoreactive proteins was quantified by densitometry using FIJI ImageJ software (National Institutes of Health, Bethesda, MD, USA).

4.11. Immunostaining and image analysis

hASCs were seeded and differentiated in eight-well Ibidi μ-chambers (Ibidi GmbH, Gräfelfing, Germany) in the manner and for the time detailed in the 4.4. chapter. The cells were washed once with PBS and fixed with 4% paraformaldehyde, then permeabilized with 0.1% saponin, and then blocked with 5% skimmed milk powder solution. Primary antibody incubation was performed overnight with anti-TOM20 (1:75, WH0009804M1) and anti-LC3 (1:200 Novus Biologicals, NB10-2220). Secondary antibody incubation was performed with Alexa Fluor 647 goat anti-mouse IgG (1:1000, Thermo Fisher Scientific, A21236) and Alexa Fluor 488 goat anti-rabbit IgG (1:1000, Thermo Fisher Scientific, A11034) for 3 hours. Propidium iodide (PI, 1.5 μg/ml, 1 hour) was used to label the nuclei. Images were captured using an Olympus FluoView 1000 (Olympus Scientific Solutions, Tokyo, Japan) confocal microscope and FluoView10-ASW (Olympus Scientific Solutions) software (version 3.0). The 488 nm wavelength of an argon laser was used to excite Alexa Fluor 488, while a 633 nm He-Ne laser was used for Alexa Fluor 647, and a 543 nm He-Ne laser was used for PI. The fluorescence emission of Alexa Fluor 488 and Alexa Fluor 647 was detected through a band filter of 500-530 nm and 655-755 nm, respectively, while the fluorescence of PI was detected through a band filter of 555-625 nm. Images were captured in sequential mode to minimize crosstalk between channels. Images are approximately 1 μm thick optical sections, each containing 512 x 512 pixels (pixel size: ~137 nm) and captured with a 60 x UPLSAPO oil immersion objective (NA 1.35).

The LC3 and outer mitochondrial membrane translocase 20 (TOM20) immunostaining images were converted into binary form and then analyzed with FIJI. The number of LC3 puncta was determined based on size ($pixel^2$), with 50-infinity arbitrary unit (AU) and circular 0-1 AU. Fragmented mitochondria were analyzed from binary TOM20 immunostaining images with 0–100 AU size ($pixel^2$) and circular 0–1 AU. The optimal size of LC3 puncta and fragmented mitochondria was determined based on analysis of all immunostaining images and manual verification of counting accuracy by checking the outlines of the counts. Both LC3 punctate and fragmented mitochondria content were normalized to nuclei for each image. The colocalization of LC3 and TOM20 was evaluated by calculating the Pearson correlation coefficient (PCC).

4.12. Determination of oxygen consumption and extracellular acidification

Real-time oxygen consumption and extracellular acidification were measured using a Seahorse XF96 oximeter (Seahorse Biosciences, North Billerica, MA, USA). hASCs were seeded in 96-well XF96 cell culture plates. We kept these cells in growth medium for 24 hours, and then started the differentiation process detailed in 4.4. chapter. After baseline oxygen consumption was recorded, cells were treated with 500 µM dibutyryl-cAMP to model adrenergic stimulation. Stimulated oxygen consumption was then measured every 30 minutes. The final reading was taken 6 hours after the treatment. Differentiated adipocytes were treated with 50 µM etomixir (ETO, a mitochondrial fatty acid transport inhibitor) to block β-oxidation of fatty acids. ETO-resistant (ETO-R) respiration is proportional to the cells' use of glucose and amino acids. In addition, proton leak was measured with oligomycin (Enzo, USA) at a concentration of 2 µM, which blocks ATP synthase. In order to correct the baseline, cells received a single 10 µM Antimycin A treatment, with which non-mitochondrial respiration was measured. After the measurements, the amount of oxygen consumption was normalized to the protein content per well - determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific).

4.13. Quantification of amino acid fluxes in adipocytes

The cell supernatant was collected at the end of the differentiation process. These conditioned media were filtered using a 3 kDa filter (Pall Corporation, Port Washington, NY, USA) and 10 µl of this filtrate was derivatized with the AccQ Tag Ultra Derivatization Kit (Waters, Milford, MA, USA). Chromatographic separation was performed on an H-class UPLC (Waters) using an AccQ·Tag Ultra Column (2.1 x 100 mm), AccQ·Tag A and B eluents, and the gradient was provided by the AccQ·Tag Ultra Chemistry Kit (Waters). The amino acid derivatives were detected at 260 nm with the UPLC PDA detector. Amino acid concentrations were calculated with the Empower software (Waters) using a 7-point calibration curve. The inflow or outflow of amino acids into adipocytes was calculated by comparing the concentration differences measured in the conditioned medium with the use of unconditioned control medium. The flux of amino acids was normalized for the number of cells.

4.14. Statistical analysis and figure preparation

All measured values were expressed as mean ± SD. The normality of the distribution of data was determined using the Kolmogorov-Smirnov (n=5 or more) or Shapiro-Wilk (n=4) tests. One-way ANOVA with Tukey's post hoc test was used to compare multiple groups when the data followed a normal distribution. Friedman and Dunn's multiple comparison tests were used to compare multiple groups when data did not follow a normal distribution. Figures and statistical analyzes were prepared with Graphpad Prism 8.

5. Results

5.1. Mitophagy mediates the beige to white transition of human primary subcutaneous adipocytes *ex vivo*

5.1.1. Thermogenic competency of human abdominal subcutaneous derived adipocytes is induced following continuous peroxisome proliferator-activated receptor (PPAR) γ stimulation and subsides as a result of beige to white transition

To study the adipogenic potential of primary hASCs and the thermogenic competency of differentiated adipocytes, our research group optimized previously published white and brown/beige adipogenic differentiation protocols. These regimens contain diverse compositions of hormones, in which the PPARγ agonist rosiglitazone is the key driver of browning. As expected, abdominal SC hASCs expressed the major functional marker gene and protein of thermogenesis, UCP1, at the limit of detection. Moderate UCP1 expression was found in adipocytes that were differentiated up to 28 days to white *ex vivo*. Consistent with previous results, continuous PPARγ stimulation resulted in a marked increase in gene and protein expression of UCP1 in adipocytes differentiated to beige compared with white ones. UCP1 was further upregulated when beige differentiation was carried out for three or four weeks. When the beige cocktail was replaced by the white and rosiglitazone was omitted at the fourteenth day of differentiation, UCP1 gene and protein expression tended to elevate in the following week, similarly to those adipocytes that were continuously exposed to the beige regimen. After two weeks of rosiglitazone withdrawal, UCP1 gene and protein expression was significantly decreased as compared to beige adipocytes and showed a gene expression level that was comparable to white adipocytes. The decline in UCP1 expression was slower at the protein level than at the mRNA level. The expression of another thermogenic marker gene, *CIDEA* followed the pattern of *UCP1* mRNA expression. In contrast, the *LEP* gene, which encodes the white adipokine, leptin, was expressed at a low level in both preadipocytes and beige adipocytes; however, it was strongly upregulated when adipocytes were constantly differentiated in the presence of the white cocktail or when the beige regimen was replaced by the white.

As expected, white adipocytes gained a few large lipid droplets in a time-dependent manner. By contrast, beige differentiation resulted in numerous smaller droplets. However, the droplets merged and enlarged when the beige protocol was replaced by the white. The quantification of this phenomenon was performed by texture sum variance, which separated the white and beige

adipocyte populations well in several previous cellular models. To investigate the mitochondrial morphology, we performed immunostaining for TOM20. Consistent with the literature, the number of fragmented mitochondria that reflect thermogenic potential was higher during beige compared to white differentiation. However, the number of fragmented mitochondria was decreased to the same as that of the white adipocytes within a week when the beige regimen was discontinued, followed by the white. The changes in UCP1 content and morphological features of the adipocytes suggest that they strongly increase their thermogenic competency, for up to four weeks, as a result of PPARγ agonist; however, they could undergo beige to white transition in response to the removal of the browning inducer.

5.1.2. Elevated mitochondrial content, respiration, and extracellular acidification of beige adipocytes disappear after their transition to the white phenotype

As a next step, we investigated the mitochondrial content and function during differentiation and transition. As expected, the mtDNA number was higher in beige compared to white differentiated adipocytes and subsequently decreased as a result of beige to white transition. White and beige differentiation resulted in the same expression level of the mitochondrial biogenesis master regulator, PGC1α. In response to transition, *PGC1α* tended to be downregulated; however, this effect did not reach the level of statistical significance. Undifferentiated progenitors expressed *PGC1α* only at the limit of detection.

Then we carried out an extracellular flux analysis to reveal the functional parameters of differentiated adipocytes. In agreement with previously reported data, the basal oxygen consumption rate (OCR) of beige adipocytes was higher than that of white ones. Adipocytes undergoing two weeks of transition had significantly higher basal OCR than the white, but lower than the beige adipocytes, which were differentiated for the same period of time. As expected, the cell-permeable cAMP analogue, which mimics adrenergic stimulus-driven activation of thermogenesis, promptly increased the OCR of each type of adipocytes. Proton leak respiration of cAMP-stimulated adipocytes, which positively correlates with UCP1 activity, could be assessed after the inhibition of the ATP synthase complex by oligomycin. Beige adipocytes had elevated stimulated and proton leak OCR compared to the white ones. After the transition, these parameters remained comparable to those observed in beige adipocytes. Consistent with previous results, the basal extracellular acidification rate (ECAR) was increased in beige compared to white adipocytes, while the transition had a significant suppressing effect on this parameter. Although cAMP stimulated the ECAR of each adipocyte type, fully differentiated and converted beige adipocytes showed a greater response to the

thermogenic cue than white ones. In summary, our data suggest that adipocytes that undergo the beige to white transition appear as a cell population with distinct features as compared to fully differentiated white or beige cells.

5.1.3. Autophagy is increased at beige to white transition

In rodents, it was proven that the withdrawal of cold or β3-adrenergic stimuli activates mitophagy and mediates the beige to white transition *in vivo*. Primarily, we investigated the expression of *ATG* genes, which orchestrate autophagosome formation. In response to transition, *ATG5* tended to be upregulated in the second week; however, this difference did not reach statistical significance. The mRNA expression of *ATG7* and *ATG12* was significantly increased in the first week of transition compared to beige adipocytes. The *ATG* genes investigated were expressed less in undifferentiated preadipocytes and at a greater rate, but to the same extent, in white and beige adipocytes.

To assess the ongoing autophagy, we examined the specific autophagy marker, the conversion of LC3-I to LC3-II, in adipocytes that were fully differentiated to white or beige or underwent transition. Quantification of this process by immunoblotting is a widely accepted method to monitor autophagy rate. We found a continuous elevation of the LC3-II/LC3-I ratio in white adipocytes differentiated up to 28 days, which indicated high autophagy activity. In beige adipocytes, the activity remained at a moderate level, significantly lower than with the white cells, after four weeks of differentiation. When the beige protocol was replaced by white, the autophagy level was significantly increased after two weeks, as compared to the fully differentiated beige adipocytes. This was confirmed when the subcellular distribution of LC3 was visualized by immunostaining. As expected, white adipocytes, which were differentiated after three or four weeks, contained more LC3 punctae per cell than beige ones. In addition, the beige to white transition significantly increased the number of LC3 punctae, as compared to the beige adipocytes. Our data demonstrated that general autophagy was induced in a cell-autonomous manner during the *ex vivo* beige to white transition of human SC adipocytes.

In addition to what was described above, we found that, in accordance with the literature, the number of fragmented mitochondria reflecting thermogenic potential was higher during beige than white differentiation. However, the number of fragmented mitochondria decreased to the same level as that of white adipocytes within a week when the beige treatment was stopped and white was applied. The changes in the UCP1 content and morphological characteristics of fat cells indicate that under the influence of the PPARγ agonist, their thermogenic ability is

strongly increased for up to four weeks; however, they transform from beige to white in response to the removal of the tan-inducing agent. To monitor the formation of autophagosomes and the entry of mitochondria into the site of degradation, we used the immunostaining detailed above, during which we quantified the colocalization of the autophagosome and the mitochondrial marker with the PCC values. In line with the increased autophagy activity, we found increased PCC values during white adipogenesis compared to beige adipocytes for four weeks. The colocalization was stronger in transformed adipocytes than in adipocytes differentiated throughout the beige direction.

5.1.4. Beige differentiation represses, while transition to white increases, mitophagy involving selective autophagy adapters

Parkin, an E3 ubiquitin ligase encoded by the *PARK2* gene, is one of the key regulators of mitophagy. Parkin was expressed at a low extent in preadipocytes at both the mRNA and protein levels. The applied adipogenic protocols similarly upregulated its expression. The transition did not alter the expression of parkin at the gene or protein levels. Next, we investigated the abundance of selective autophagy adapter proteins that are consumed, building a molecular link between the target organelles and LC3-II of the autophagosomes during ongoing mitophagy. The NBR1 protein content of white adipocytes was comparable to that of the undifferentiated progenitors and showed a decreasing trend after up to 28 days of differentiation. The p62 protein was detectable at constant, moderate levels in preadipocytes and white adipocytes. In beige adipocytes, significantly more of the aforementioned adapters could be detected. This further supports the low activity of selective autophagy in beige adipocytes. After the removal of the browning inducer from differentiation media, the NBR1 and p62 amount declined significantly after one or two weeks, respectively. Our data suggest that the selective autophagic degradation of mitochondria is enhanced during the beige to white transition.

Next, we assessed the expression of other marker genes related to the adapter and parkin-dependent mitophagy pathway, *OPTN* and *CALCOCO2/NDP52*. Both genes were expressed at a low level in preadipocytes. *OPTN* was expressed to the same extent in white and beige adipocytes and tended to be upregulated in a time-dependent manner, but the transition did not influence the mRNA level. *CALCOCO2/NDP52* expression was increased during white adipogenesis and transition compared with fully differentiated beige adipocytes; this suggests the possibility of enhanced removal of the mitochondrial mass by the NDP52-dependent pathway.

5.1.5. Parkin-independent mitophagy-related genes are induced during transition

Finally, to study whether parkin-independent mitophagy contributes to beige to white transition, we investigated the expression of several genes that are involved in this pathway, *FUNDC1, BNIP3, BNIP3L/NIX*, FKBP Prolyl Isomerase 8 (*FKBP8*), and *BCL2L13*. The aforementioned markers were expressed to a low extent in undifferentiated progenitors. *BNIP3L/NIX, FKBP8,* and *BCL2L13* expression tended to increase during white adipogenesis, in a time-dependent manner. Similar expression levels of *FUNDC1* and *BNIP3* were found during white or beige differentiation. After four weeks of differentiation, the expression of *BNIP3L/NIX, FKBP8,* and *BCL2L13* was repressed in beige compared to white adipocytes. Two weeks following the replacement of a beige protocol with white, the investigated parkin-independent mitophagy markers were significantly upregulated. This suggests that the parkin-independent pathway plays an important role during the beige to white transition of human SC adipocytes.

5.2. Human abdominal subcutaneous-derived active beige adipocytes carrying *FTO* rs1421085 obesity-risk alleles exert lower thermogenic capacity

To investigate the effect of the *FTO* rs1421085 SNP on adipocyte differentiation and thermogenic function, we further optimized the beige differentiation protocol used for our mitophagy experiments, which was supplemented with a 4-hour 500 μM dibutyryl-cAMP treatment on the 14th day of differentiation. With this modification, we intended to increase the thermogenic capacity of beige adipocytes. Adipocytes differentiated by this way will be called active beige adipocytes in the thesis.

5.2.1. Active beige adipocytes derived from abdominal SC exert high browning capacity

Primarily, we aimed to investigate the global gene expression patterns of the three types of differentiated SC adipocytes, white, active, and inactive beige by performing RNA-seq analysis. We found that general adipocyte markers, such as *SLC2A4, FABP4, LPL, ADIPOQ, AGPAT2, PLIN1, LEP,* and *LEPR* were not expressed differentially among the three types of adipocytes suggesting that their differentiation rate was similar. The thermogenic markers, such as *UCP1, ELOVL3, PGC1a, CIDEA, CITED1, AQP3, GK, CKMT1a/b,* and *PM20D1* had higher expression in active beige as compared to white or inactive beige adipocytes. Next, we subjected our transcriptomic data from RNA-seq analysis to open source webtools to estimate brown adipocyte content by BATLAS and browning capacity by ProFAT based on the expression levels of well-defined marker genes. We did not find significant differences in brown adipocyte content, however, active beige adipocytes showed higher browning capacity

score as compared to white or inactive beige ones. According to our RNA-seq analysis data, we found that 211 and 147 genes had higher expression in the comparison of active beige vs. white and active beige vs. inactive beige, respectively; out of those, 100 genes were common. Among the commonly highly expressed 100 genes, thermogenic markers, such as *GK, PM20D1, PLIN5, CITED1*, and *AQP3* were found. Interestingly, *SLC7A10*, encoding ASC-1, which was described as an important transporter during thermogenic activation was also commonly upregulated in both comparisons. 248 and 226 genes had lower expression in the comparison of active beige vs. white and active beige vs. inactive beige, respectively, and 164 genes had commonly lower expression. We did not find any DEGs when we compared the gene expression profile of white and inactive beige adipocytes.

We also analyzed the mitophagy rate and mitochondrial morphology by co-immunostaining of LC3 and TOM20. Confocal images were used to quantify the co-localization of LC3 and TOM20 by measuring the correlation between pixel intensities of two detection channels. We observed lower LC3 punctae counts per cell and PCC values in active beige as compared to white or inactive beige adipocytes. We also found that active beige adipocytes had higher amounts of fragmented mitochondria, which were shown to support uncoupled respiration and enhanced energy expenditure, as compared to white or inactive beige cells. Altogether, these data suggests that thermogenesis-related genes were upregulated, the mitophagy rate was lowered, and mitochondria were more fragmented when human abdominal SC adipocytes were activated for thermogenesis.

5.2.2. Active beige adipocytes carrying *FTO* rs1421085 obesity-risk alleles had lower brown adipocyte content and expressed lower level of genes involved in metabolic pathways

Next, we intended to investigate whether *FTO* rs1421085 SNP affected the browning capacity of human abdominal SC adipocytes, which were differentiated into white, active, or inactive beige. Therefore, we genotyped the hASCs for *FTO* rs1421085 SNP by using SNP genotyping assay and obtained the allelic discrimination plot. Then, we selected samples from 4 individuals with homozygous TT (risk-free) and from 4 individuals with homozygous CC (obesity-risk) genotypes for further analysis. We found that active beige adipocytes carrying *FTO* obesity-risk alleles exerted lower brown adipocyte content estimated by BATLAS, but no effect of the allelic distribution was observed in case of white or inactive beige adipocytes. We also found that the risk-free genotype carrier active beige adipocytes had higher tendency of BATLAS and significantly higher ProFAT scores as compared to white ones that carried the same TT

genotype. Interestingly, active beige adipocytes carrying *FTO* obesity-risk genotype showed similar estimated brown adipocyte content and browning capacity as compared to white adipocytes suggesting that active beige differentiation could not overcome the browning inhibitory effect of the CC alleles.

A total of 175 genes including thermogenic markers (*UCP2, CKMT2,* and *CITED1*) and 5 BATLAS markers (*PPARGC1B, ACO2, ACSF2, NNAT,* and *DMRT2*) were expressed less in active beige adipocytes carrying *FTO* obesity-risk variant as compared to risk-free carriers. Only 10 genes (7 of them were common in both comparisons) were expressed at a lower extent in CC as compared to TT carrier inactive beige adipocytes. We found only 14 and 46 genes (8 of them were common in both comparisons) which were expressed more in active or inactive beige adipocytes, respectively, carrying the *FTO* obesity-risk as compared to the risk-free variant. In white adipocytes, we did not find any DEGs which was affected by the *FTO* rs1421085 SNP. Next, we investigated the gene expression pathways affected by the *FTO* rs1421085 SNP and found that genes, which were less expressed in active beige adipocytes that carried the obesity-risk genotype, were overrepresented in several pathways, such as metabolic, PPAR signaling, lipolysis, fatty acid metabolism, or TCA cycle. More expressed genes in active beige adipocytes with CC alleles were not significantly overrepresented in any of the pathways. We did not find any overrepresented pathway with respect to the DEGs found in inactive beige adipocytes either. These results suggest that *FTO* rs1421085 SNP only affects the gene expression profile, particularly that of the thermogenesis-related genes, in active beige but not in white or inactive beige adipocytes. In addition, the applied differentiation protocols resulted in more pronounced differences in the gene expression patterns of adipocytes with *FTO* risk-free alleles which suggest their significant browning potential when thermogenic cues are constantly present.

5.2.3. Thermogenic marker genes were less expressed in active beige adipocytes carrying *FTO* obesity-risk alleles

Since we observed that the allelic discrimination at *FTO* rs1421085 SNP influences the expression of thermogenic and BATLAS markers, we went further to investigate the expression of thermogenic genes at mRNA and protein levels in abdominal SC adipocytes. Our results showed that the mRNA expression of *UCP1* was higher in active beige as compared to white or inactive beige adipocytes with *FTO* risk-free alleles, however, this difference was not observed in obesity-risk carriers. At the protein level, we observed that active beige adipocytes expressed more UCP1 as compared to white or inactive beige adipocytes regardless the *FTO*

rs1421085 genotypes, however, less UCP1 protein amount was detected in obesity-risk than in risk-free allele carrier active beige adipocytes. Other thermogenic genes, such as *UCP2, PM20D1, CIDEA, CITED1, CKMT1* and *2, CPT2,* and *PLIN1* were also expressed higher in active beige adipocytes carrying risk-free alleles as compared to white or inactive beige adipocytes with the same TT variant, however, we did not observe these differences in *FTO* obesity-risk carrier samples. As compared to risk-free carriers, active beige adipocytes with *FTO* obesity-risk genotype had lower expression of these thermogenic genes and also that of the neurotrophic factor S100b, which was postulated to stimulate sympathetic axon growth and to play an important role in BAT innervation. These results are in accordance with our RNA-seq data suggesting the critical importance of *FTO* rs1421085 SNP in active beige adipocytes and the compromised effect of active beige differentiation protocol in the presence of obesity-risk alleles.

5.2.4. Active beige adipocytes with *FTO* obesity-risk genotype expressed less amount of mitochondrial complex subunits and had lower proton leak respiration

Having observed that *FTO* rs1421085 SNP affected the expression of thermogenic genes, our next aim was to investigate whether the expression of mitochondrial complex subunits and cellular respiration were also suppressed in adipocytes with obesity-risk alleles. We found that active beige adipocytes carrying *FTO* risk-free genotype had higher amounts of mitochondrial complex subunits I, II, and IV as compared to white or inactive beige adipocytes carrying the same TT genotype. However, no difference was found between the three types of differentiation programs when adipocytes carried the obesity-risk variant. Active beige adipocytes with *FTO* obesity-risk alleles had lower expression of mitochondrial complex subunits I, II, and IV as compared to the risk-free carriers. We observed a similar but statistically not significant trend in the case of mitochondrial complex subunit III, while the expression of complex V subunit was similar for all types of adipocytes regardless the *FTO* rs1421085 genotype.

Next, we measured the cellular respiration of the three types of adipocytes carrying *FTO* risk-free or obesity-risk alleles. In accordance with the mitochondrial complex subunit expression, we found that active beige adipocytes carrying risk-free genotype had higher respiration (at both basal and maximal stimulated conditions), stimulated proton leak respiration, and extracellular acidification as compared to white or inactive beige adipocytes, but this difference was not pronounced when the adipocytes carried the risk variant. Stimulated ATP-dependent respiration was not affected significantly by either the applied differentiation protocols or the *FTO* rs1421085 genotype. Active beige adipocytes with *FTO* obesity-risk variant showed lower

cellular respiration, especially stimulated proton leak respiration which reflects UCP1-dependent heat production, and extracellular acidification, which associates with glycolytic activity, as compared to risk-free carriers. Intriguingly, the effect of *FTO* obesity-risk alleles on cellular respiration was observed in active beige but not in white or inactive beige adipocytes highlighting its exclusive effect in human abdominal SC adipocytes only when they are activated for thermogenesis.

5.2.5. Adipocytes carrying *FTO* obesity-risk genotype consume lower amounts of neutral amino acids when activated for thermogenesis

Active thermogenic adipocytes utilize metabolic substrates, such as carbohydrates, fatty acids, or amino acids to generate heat. Therefore, we aimed to investigate the fuel utilization by abdominal SC adipocytes with distinct *FTO* genotypes in three types of differentiation programs. Fatty acids are primarily released from lipid droplets via lipolysis. We found that the regulation of lipolysis in adipocytes and fatty acid metabolism gene expression pathways were downregulated in active beige adipocytes with *FTO* CC as compared to TT allele carriers. Although murine brown adipocytes can compensate the lack of lipid droplets-derived fatty acids, our *ex vivo* model did not include fatty acids in the applied differentiation media. Furthermore, stimulated ETO-sensitive oxygen consumption, which correlates with the activity of fatty acid oxidation, was not affected significantly by either the differentiation programs or the FTO genotypes. ASC-1, which is encoded by *SLC7A10*, plays an important role in mediating alanine, serine, cysteine, and glycine consumption in human adipocytes derived from abdominal SC and deep neck regions. To evaluate the preferable energy sources during thermogenic activation, we monitored the oxygen consumption of adipocytes upon ETO (inhibitor of carnitine palmitoyltransferase-1) administration.

ETO-R respiration, which reflects the activity of carbohydrate and amino acid oxidation, was higher in active beige adipocytes with *FTO* risk-free genotype than in white or inactive beige adipocytes carrying the same TT genotype. Active beige adipocytes with *FTO* obesity-risk alleles had lower level of ETO-R oxygen consumption as compared to risk-free carriers. These observations suggest less pronounced carbohydrate and/or amino acid utilization in active beige adipocytes of CC carriers at *FTO* rs1421085.

Because we found *SLC7A10* (encoding alanine-serine-cysteine transporter, ASC-1) as a DEG among the most abundantly expressed genes in active beige adipocytes with *FTO* risk-free genotype, we decided to investigate the effect of the applied differentiation protocols and *FTO* rs1421085 SNP on the expression of ASC-1 and the consumption of ASC-1 cargos by the adipocytes. We found that active beige adipocytes with risk-free alleles expressed higher mRNA level of *SLC7A10* as compared to white or inactive beige ones that carried the same TT variant, which could be confirmed at ASC-1 protein level. The presence of the *FTO* rs1421085 SNP resulted in lower expression of *SLC7A10* in active beige adipocytes; this effect was statistically significant at protein level but not at mRNA level. Next, we measured the consumption of amino acids in the conditioned media obtained from the three types of

differentiated adipocytes with CC or TT alleles, respectively. The applied differentiation programs did not affect the release of alanine regardless of the *FTO* allele status. In the case of adipocytes with *FTO* risk-free genotype, we found that active beige ones consumed higher amounts of serine and cysteine and released more glycine as compared to white or inactive beige adipocytes. In accordance with the aforementioned gene expression and ETO-R oxygen consumption results, we did not observe any differences in the fluxes of these amino acids between the three types of differentiation protocols in adipocytes with obesity-risk alleles. Active beige adipocytes with obesity-risk genotype consumed lower amount of cysteine as compared to risk-free carriers, but significant effect of the SNP was not observed on serine consumption suggesting that other amino acid transporters might compensate for the reduced expression of ASC-1.

Our RNA-seq data showed that serine hydroxymethyltransferase (SHMT) 1, which catalyzes the conversions of L-serine and tetrahydrofolate (THF) to glycine and 5,10-methylene-THF (5,10-CH_2-THF), was expressed lower in active beige adipocytes with FTO obesity-risk as compared to risk-free carriers. We validated our RNA-seq data of SHMT1 expression by RT-qPCR and immunoblotting. We also found that active beige adipocytes had higher protein content of SHMT1 as compared to white or inactive beige adipocytes in the presence of the FTO risk-free variant, but this difference was not observed in obesity-risk carrier samples.

Our metabolomic analysis and RNA-seq data allowed us to infer the effect of the risk-free allele on amino acid metabolism, including GPT2 expression. According to the RNA-seq data, active beige adipocytes carrying obesity-risk genotype expressed lower mRNA level of glutamic pyruvic transaminase (GPT) 2 as compared to risk-free allele carriers. In the case of adipocytes with *FTO* risk-free alleles, active beige cells expressed more *GPT2* both at mRNA and protein level as compared to white ones. We also observed lower expression of GPT2 in active and inactive beige adipocytes with *FTO* obesity-risk genotype as compared to risk-free allele carriers. Active beige adipocytes carrying risk-free genotypes also consumed higher amount of glutamine as compared to white or inactive beige adipocytes carrying the same TT genotype, but no difference was observed among the three types of differentiation protocols in the case of the samples with obesity-risk alleles. On the contrary to risk-free carrier cells, adipocytes with *FTO* obesity-risk genotype did not consume glutamine irrespective to the applied differentiation protocols. In contrast, we found that active beige adipocytes released higher amount of glutamate as compared to inactive ones, while white adipocytes rather consumed that amino acid. The consumption of glutamate did not depend on the allelic discrimination at *FTO*

rs1421085 locus. These results suggest that active beige adipocytes with *FTO* TT alleles utilize higher amounts of glutamine, which can be converted to glutamate, presumably to generate more of the TCA cycle intermediate, α-ketoglutarate which may contribute to elevated heat generation.

6. Discussion

6.1. Parkin-dependent and -independent mitophagy mediates the beige-to-white transition in human abdominal adipocytes *ex vivo*

BAT plays a central role in the energy homeostasis of mammals that are constantly exposed to cold challenge. Following the detection of active BAT depots by nuclear imaging approaches in adult humans, a strong negative correlation between obesity and the amount of active BAT was revealed. Independent studies have suggested that BAT depots in adult humans are predominantly composed of beige cells. Transplants of human beige adipocytes improved diet-induced obesity and systemic metabolism in mice, which highlights the possibility of the therapeutic application of beige cell implantation in the treatment of obesity and metabolic syndrome. This inspired researchers to characterize beige adipogenesis and thermogenic activation in distinct human cellular models. To our knowledge, however, the majority of these *ex vivo* studies have covered the differentiation period for a maximum of two weeks. Of note, the potential application of beige adipocyte activation or transplantation assumes that the applied cells maintain their energy expenditure for a significant period of time. Although abdominal SC WAT of human adults is not highly enriched in thermogenic adipocytes, it contains progenitors that can give rise to beige cells. Due to its relative accessibility, hASCs isolated from stromal–vascular fractions of abdominal SC fat biopsies or aspirations are frequently used for research and in regenerative medicine.

In this study, we followed the white and beige adipocyte differentiation of primary abdominal SC-derived hASCs for four weeks. The extension of the PPARγ-driven beige differentiation resulted in further upregulation of UCP1, both at the mRNA and protein level, and the CIDEA gene, while in white adipocytes they were expressed constantly at a moderate level. This phenomenon was reproduced by our research group in SGBS adipocytes, a cell line that is an accepted and widely used model of human white and beige adipogenesis. Consistent with our previous findings, the size and locularity of lipid droplets were different in the two cell populations during the entire differentiation.

In beige and brown adipocytes, mitochondria are critical for thermogenesis and energy metabolism. Mitochondria are fragmented in response to an adrenergic cue in rodent brown adipocytes, contributing to support uncoupled respiration and enhanced energy expenditure. UCP1-positive human adipocytes contain mitochondria mostly with a fragmented morphology. Recently, we found that cAMP-driven thermogenic stimulation resulted in increased

mitochondrial fragmentation in human masked and mature beige adipocytes, which were differentiated from the same progenitor populations for two instead of four weeks. When we sustained the beige differentiation, more mitochondria were fragmented in contrast to white adipocytes, in which these dynamic organelles remained elongated.

The regulation of mitochondrial biogenesis and clearance is important for energy homeostasis and maintaining the optimal number of mitochondria. Mitochondrial biogenesis is controlled by several nuclear-coded transcriptional regulators, such as PGC1α. Although the expression of PGC1α at mRNA tended to be elevated in the early phase of beige differentiation, it did not differ between white and beige adipocytes to a statistically significant degree. However, the amount of mtDNA showed an increasing tendency during the long-term beige adipogenesis. The functional extracellular flux assay detected high basal, cAMP-stimulated, and proton leak OCR and more prominent extracellular acidification, both in basal and activated conditions, in the case of beige adipocytes that were differentiated in the presence of rosiglitazone for four weeks. Respiration and extracellular acidification were significantly repressed in white adipocytes; however, they could be effectively stimulated in response to the cell-permeable cAMP analogue, suggesting that some of the adipocytes that were differentiated in the presence of the white cocktail are masked beige cells.

The activation of beige cells, for example by cold exposure or β-AR agonists, is required for maintaining high UCP1 expression. Cold exposure induces norepinephrine release from the sympathetic nervous system, which binds the β3-AR. The β-adrenergic signaling cascade is mediated through adenylate cyclase activation by Gs proteins, which leads to the production of cAMP for PKA activation. This promotes lipolysis, the breakdown of triglycerides, resulting in FFA release that activates UCP1. PKA has several downstream targets, including CREB, members from MAP kinase pathways (Erk1/2, p38, JNK), and hormone-sensitive lipase (HSL), which facilitate the enhancement of thermogenesis by upregulating thermogenic gene expression and/or mobilizing substrates to fuel thermogenesis.

PPARγ is well known as a master regulator of both white and brown adipocyte differentiation. Some synthetic PPARγ agonists, such as rosiglitazone, function as inducers of the beige fat gene transcription program in white adipocytes, which is mediated by SIRT1, PRDM16, C/EBPa, and PGC1α-dependent mechanisms. PPARγ directly regulates the expression of many genes, which are involved in the central functions of adipocytes, such as lipid transport, lipid metabolism, insulin signaling, and adipokine production. PPARγ target genes can control processes such as lipid transport (FABP4), fatty acid uptake (LPL, FATP/SLC27A1, OLR1),

the recycling of intracellular fatty acids (PEPCK/PCK1, GK, AQP7), and lipolysis (GPR81). In addition, PPARγ regulates insulin sensitivity via the expression of adipokines.

During my work, I followed the differentiation of primary abdominal SC-derived hASCs into white and beige adipocytes for four weeks. The temporal extension of PPARγ-driven beige differentiation resulted in further upregulation of UCP1 both at the mRNA and protein level, and at the level of the *CIDEA* gene, while these markers were continuously expressed at a moderate level in white adipocytes. This phenomenon was reproduced by our research group with adipocytes derived from the SGBS cell line, which is an accepted and widely used model of human white and beige adipogenesis.

Elevated mitochondrial fragmentation, mtDNA, and OCR raise the possibility of suppressed mitophagy in beige adipocytes. When the same progenitors were differentiated for two weeks, irrespective of the applied protocol, a few hours of cAMP treatment not only upregulated thermogenesis-related genes but also quickly downregulated mitophagy via PKA, resulting in more mitochondria and increased UCP1 levels. In a long-term differentiation setting, sustained rosiglitazone administration also resulted in a moderate suppression of mitophagy, shown by attenuated LC3-I to LC3-II conversion, the appearance of LC3-positive punctae, colocalization of punctae and mitochondria, and the degradation of adapter proteins. More research is needed to explore the underlying molecular mechanisms of how mitophagy is kept at a moderately low level in beige adipocytes.

Beigeing of WAT was induced in male mice by intraperitoneal administration of the β3-AR agonist, CL 316,243 for seven consecutive days. Low autophagy activity was observed in newly differentiated beige adipocytes, which is consistent with the *ex vivo* results presented in the thesis. After the withdrawal of this stimulus, they found that beige adipocytes lost their morphological and thermogenic characteristics and were converted to “white-like” adipocytes, triggered by mitochondrial clearance via mitophagy. During the beige to white adipocyte transition, the expression of autophagy-related *ATG5* and *ATG12* genes was upregulated, the number of Green Fluorescent Protein (GFP)-LC3 punctae and the colocalization of GFP-LC3 and TOM20 were significantly increased, the protein level of LC3-II was elevated, and in parallel the selective autophagy adapter proteins, NBR1 and p62, were degraded, compared to in mice that were chronically treated with the β3-adrenergic agonist during the entire experimental period.

In our experiments, we applied the human primary SC abdominal derived adipocyte *ex vivo* model to characterize the beige to white transition process in the context of mitophagy. UCP1, *CIDEA*, and *LEP* expression, the morphology of the lipid droplets, mitochondrial fragmentation, and the basal respiration of the adipocytes were significantly altered as a result of the transition; therefore, these cells gained several features characteristic of white adipocytes. A similar phenomenon was observed recently when we carried out a 28-day differentiation, in parallel with the replacement of beige protocol to white on the 14th day in the SGBS preadipocyte line. In contrast to our previous observations in SGBS adipocytes and the data presented here, Guennoun et al. observed a temporarily high UCP1 content of 14-day-differentiated SGBS adipocytes, even in response to the white differentiation protocol in the absence of any browning inducers. Interestingly, when the white differentiation of SGBS cells was extended for two additional weeks, the expression of UCP1 significantly decreased. The contribution of autophagy to this surprising finding has not been investigated so far.

Our data are consistent with the results of the *in vivo* study by Kajimura's group from 2016 and suggest that the autophagy pathway is activated for the clearance of beige adipocyte mitochondria during the adaptive transition induced by the removal of the browning inducer, thereby regulating the entry of beige adipocytes into a thermogenically inactive dormant state. However, beige adipocytes that underwent transition *ex vivo* responded more effectively to the adrenergic stimulus mimicking dibutyril-cAMP by the activation of OCR, proton leak respiration, and ECAR than the white ones. Of note, a significant amount of UCP1 protein remained expressed after two weeks of transition, which could underlie why converted adipocytes had higher stimulated and proton leak OCR compared to white ones. The observed functional differences suggest that converted beige and white adipocytes can be classified into two distinct cell populations. Systematic studies are needed to further explore the molecular signatures of the thermogenically active beige, converted beige, and white adipocytes in humans.

As a final step, we sought to clarify how the selective removal of mitochondria is mediated during the transition process. In the literature, contradictory data have been published about the involvement of parkin in the maintenance of murine beige adipocytes. During the adipogenesis of 3T3-L1 adipocytes, increased parkin expression was observed, whereas its expression decreased as a result of rosiglitazone treatment. It was found that parkin expression was induced during mouse beige adipocyte differentiation; moreover, the retention of mitochondria-rich beige adipocytes was demonstrated even after the elimination of adrenergic stimuli in PARK2

knockout mice. In contrast to these findings, Corsa and colleagues found that parkin deletion in mouse adipocytes did not affect adipogenesis, beige to white transition, and the maintenance of beige adipocytes. Recently, we reported that parkin-dependent and -independent mitophagy-associated genes were expressed in human masked and mature beige adipocytes, and the cAMP-driven thermogenic stimulus resulted in decreased expression of parkin-dependent mitophagy-related genes. In the current study, we have found that the gene and protein expression of parkin was not affected during beige to white transition. However, the level of selective adapter proteins, NBR1 and p62 significantly decreased and the expression of *CALCOCO2/NDP52* and the investigated parkin-independent mitophagy-related genes was significantly elevated during transition, compared to fully differentiated beige adipocytes. In summary, our data suggest that both parkin-dependent and -independent mitophagy pathways are involved in the regulation of mitochondrial elimination during beige to white adipocyte transition.

p62 is a multifunctional protein involved in several signaling pathways affecting various cellular processes, such as inflammation, cell death, tumorigenesis, and metabolism. It has been reported that whole-body p62 knockout mice show an obese phenotype due to increased adiposity and reduced energy expenditure. Furthermore, the mitochondrial function of BAT in adipocyte-specific $p62^{-/-}$ mice was impaired, resulting in BAT becoming unresponsive to β3-adrenergic stimuli. This suggests that p62 plays a significant role in the regulation of thermogenesis in BAT. A recent paper has demonstrated that NBR1 is required for the repression of adaptive thermogenesis via decreasing the activity of PPARγ in BAT of p62-deficient mice; thereby, the inhibitory role of NBR1 in thermogenesis in the presence of p62 inactivation was identified. Based on these studies, further investigations may reveal the role of p62 and NBR1 in the thermogenesis of human brown and beige adipocytes.

Individuals with obesity possess less active BAT but more “brownable” fat than lean ones. These “brownable” depots might contain a large number of beige adipocytes undergoing transition, in which autophagy and mitophagy are highly active. This is supported by the fact that *ATG* and autophagosome-related genes are highly expressed in the visceral and SC WAT of patients with obesity. In the future, well-established molecular markers and histological or cell sorting methods are necessary to discriminate between white, active beige, and dormant beige adipocytes in distinct anatomical areas. This might allow researchers to analyze gene expression changes during conversion in individual cells, which might reveal the novel molecular targets that control this process. More investigations are needed to use the inhibition of autophagy to cure diseases but there are feasible findings that show practicable perspectives.

For example, it was summarized that targeting autophagy in the early stage of SARS-CoV-2 infections can be a potential therapeutic strategy against viral replication and in the regulation of the exacerbated inflammatory response. A better understanding of the key molecular events that determine the entry into beige to white transition may offer new opportunities for specifically preventing this process in order to maintain active heat-producing adipocytes; they may be pharmacologically activated or transplanted, for instance, in humans for improving energy metabolism and combatting obesity.

6.2. The presence of the obesity risk *FTO* rs1421085 allele in human abdominal subcutaneous adipocytes causes a disturbance of energy release

Abdominal WAT is classified into SC and intraabdominal fat, which is mainly composed of visceral or intraperitoneal WAT. Several studies reported that accumulation of visceral WAT is strongly associated with the risk of metabolic disorders, whereas other studies claimed that abdominal SC WAT may possess a protective role. A more recent study using an elegant PET/CT technique reported that human BAT or brownable adipose tissue can be found in cervical, supraclavicular, axillary, mediastinal, paraspinal, and abdominal depots. In this study, we performed RNA-seq on human abdominal SC derived adipocytes with *FTO* rs1421085 risk-free or obesity-risk genotypes, which were differentiated by applying three types of protocols: white, active, or inactive beige. Irrespective to the *FTO* genotypes, we found that active beige adipocytes exerted greater thermogenic potential, marked by higher expression of thermogenic genes and browning capacity quantified by ProFAT, as compared to white or inactive beige cells. Our results suggest that human abdominal SC adipocytes have a significant thermogenic potential when they are activated using active beige differentiation protocol driven by a PPARγ agonist and adrenergic stimulation. However, this potential subsides when adipocytes are inactivated through beige to white transition. The comparison of active beige and white or inactive beige adipocytes found 100 and 164 genes, which were commonly more and less expressed, respectively, in active beige adipocytes. Notably, several well-known thermogenesis markers, such as *CITED1, PM20D1, PLIN5, GK*, and *AQP3* were commonly upregulated in active beige as compared to white or inactive beige adipocytes. No DEGs were found in the comparison of white and inactive beige adipocytes indicating a high similarity of the gene expression profiles in these two differentiation programs.

Dibutyryl-cAMP is extensively used to mimic *in vivo* thermogenesis due to its ability to penetrate the cell membrane. In contrast to cAMP, which can be hydrolyzed by phosphodiesterase (PDE), dibutyryl-cAMP is resistant to degradation by PDE. cAMP activates

PKA, which phosphorylates various proteins and initiate consecutive cascades of additional protein kinases. More recently, a role has emerged for PKA in the regulation of gene transcription. When we administered dibutyryl-cAMP in the middle of active and inactive beige differentiation programs (at day 14 for 4 h) we found that the effect of the compound on the thermogenic gene expression was sustained until the end of the differentiation of active beige, but not in inactive beige (undergoing beige to white transition) adipocytes. This suggests that the effect of dibutyryl-cAMP can be maintained for a long period of time in beige adipocytes.

We also evaluated the effect of rs1421085 T-to-C SNP of the *FTO* gene, which interrupts a conserved motif for ARID5B repressor, resulting in elevated expression of IRX3 and IRX5 during the early stage of adipocyte differentiation. As the consequence, the commitment of the cells diverts from beige towards the white program and lipid storage increases. When the gene expression profiles of the three types of adipocytes were analyzed by segregating the *FTO* rs1421085 risk-free (TT) and obesity-risk (CC) allele carrier samples, intriguingly, we found that the SNP affected the gene expression profile, in particular the expression of thermogenic markers (*CITED1, CIDEC, PLIN1, LIPE, CKMT2*, and *S100b*), in active beige adipocytes, but not in white or inactive beige adipocytes. CIDEC, PLIN1, and LIPE are lipid droplet-associated proteins, which regulate triglyceride accumulation and lipolysis. Decreased expression of these genes in active beige adipocytes with FTO obesity-risk alleles may contribute to the downregulation of lipolysis in the SC adipose tissue of affected individuals. CKMT1a/b and CKMT2 mitochondrial creatine kinases phosphorylate creatine generating phosphocreatine and contribute to UCP1-independent heat generation via creatine futile cycle. S100b protein was proposed to play a role in the sympathetic innervation of thermogenic adipose tissue by stimulating neurite outgrowth from sympathetic neurons through calsyntenin (CLSTN) 3β axis. Reduced expression or loss of function of S100b resulted in disrupted sympathetic innervation leading to reduced thermogenesis in brown or beige adipocytes. Of note, recently, it was shown that adipose tissue specific CLSTN3$\beta^{-/-}$ mice did not have defects in the sympathetic innervation and adrenergic signaling of BAT. Decreased expression of S100b in abdominal SC adipocytes with FTO obesity-risk carriers, however, might partially contribute to lower thermogenic capacity in abdominal SC WAT even when the adipocytes are activated for heat production. Importantly, genes overrepresented in metabolic, especially in energy metabolism-related pathways, such as TCA cycle, lipolysis, pyruvate metabolism, and PPAR signaling were downregulated in active beige adipocytes with obesity-risk genotypes as compared to risk-free allele carriers, indicating lower energy dissipation in active beige adipocytes with CC alleles.

Our findings suggest that C alleles at *FTO* rs1421085 suppress the thermogenic activation of human abdominal SC adipocytes; even long-term rosiglitazone treatment could not compensate for the effect of the obesity-risk genotype. In addition, we also observed that active beige adipocytes carrying *FTO* obesity-risk alleles exerted similar transcriptomic profiles as white or inactive beige adipocytes. This is in association with our previous study, which reported that the thermogenically prone human neck derived adipocytes carrying *FTO* obesity-risk genotype had lower expression of thermogenic genes, such as *CKMT1A/B, CITED1, PPARGC1A/B*, and *CPT1B* and genes involved in respiratory electron transport, fatty acid metabolism, and the signaling by retinoic acid pathways.

Active heat producing adipocytes utilize higher amounts of nutrients, such as glucose, fatty acids, and amino acids to provide sufficient fuel for thermogenesis and solute carrier (SLC) transporters play a crucial role in mediating the transport of these molecules. Our data showed that active beige adipocytes carrying risk-free genotype had higher ETO-R oxygen consumption that reflects carbohydrate and amino acid utilization and expressed higher level of the neutral amino acid transporter, ASC-1 (encoded by *SLC7A10*) as compared to white or inactive beige adipocytes with the same TT genotypes, whereas there was no difference when the three types of adipocytes with *FTO* obesity-risk alleles were compared. ASC-1 has been recently identified as a novel regulator of energy metabolism in human SC adipose tissue elevating mitochondrial respiration and preventing development of adipocyte hypertrophy and insulin resistance. Our previous study reported that ASC-1-mediated uptake of serine, cysteine, and glycine is essential for efficient thermogenic response upon adrenergic stimulation in human neck derived adipocytes. The role of ASC-1 in adipose tissue has been comprehensively reviewed highlighting its beneficial effects in enhancing mitochondrial activity and lowering reactive oxygen species production in white adipocytes. We also found that the consumption of serine and cysteine was higher in active beige as compared to white or inactive beige adipocytes with *FTO* risk-free genotype. Lower consumption of these amino acids was observed in active beige adipocytes with *FTO* obesity-risk genotype as compared to risk-free allele carriers. In addition, we observed release of less glycine by active beige adipocytes carrying obesity-risk genotypes as compared to those with risk-free alleles. Serine is an important metabolic source to generate one-carbon units in mammalian cells, which are produced by both isoforms of SHMT enzymes, SHMT1 (cytosolic) and SHMT2 (mitochondrial), resulting in glycine. Our data showed that active beige adipocytes carrying *FTO* obesity-risk genotype expressed lower level of SHMT1 as compared to risk-free allele carrier ones suggesting lower generation of one-

carbon units in thermogenic adipocytes with *FTO* obesity-risk genotype. We also revealed that one-carbon metabolism pathway, in which SHMT1 and GPT2 participate, was less expressed in active beige adipocytes with *FTO* obesity-risk alleles. One-carbon unit metabolism forms a functional interaction with mitochondrial oxidative phosphorylation that is crucial for ATP or heat generation in mammalian cells. Lower serine influx that can result in the decrease of one-carbon unit levels may lead to lower amounts of mitochondrial complex subunits I, II, and IV in active beige adipocytes carrying *FTO* obesity-risk genotype. As a consequence, stimulated maximal and proton leak respiration, which positively correlates with UCP1 activity, and extracellular acidification were suppressed in active beige adipocytes originated from *FTO* obesity-risk genotype carriers.

Active beige adipocytes with risk-free genotype also consumed higher amounts of glutamine as compared to white or inactive beige adipocytes carrying the same alleles. The *FTO* obesity-risk carrier adipocytes did not consume glutamine irrespective of the applied differentiation protocol. Glutamine is the most abundant free amino acid in the circulation and in the used DMEM-F12 cell culture medium. It is one of the main fuel resources for cells supplying carbon atoms to drive the TCA cycle and generate ATP (or heat). Lower expression of GPT2 and glutamine consumption may contribute to the downregulation of pyruvate metabolism and TCA cycle pathway in active beige adipocytes with obesity-risk alleles. In addition, sodium-dependent neutral amino acid transporter type 2 (ASCT2)/SLC1A5-mediated glutamine uptake is important for histone acetylation and methylation in murine WAT. Downregulation of ASCT2 as the consequence of disrupted PPAR-γ expression in WAT of obese mice led to reduced glutamine uptake and correlated with decreased H3K27ac and H3K4me3 at the *Bmal1* promoter. A recent publication reported that disrupted function of adipocyte ASC-1 led to the elevation of lipid storage and diverted adipocytes from releasing to consuming glutamate and aspartate. Our data showed that active beige adipocytes with risk-free *FTO* genotype, which expressed the highest level of ASC-1, consumed less aspartate as compared to white or inactive beige adipocytes. In contrast to white adipocytes that consumed glutamate, active beige adipocytes released it into the extracellular space. Altogether, our findings suggest that adipocytes derived from abdominal SC tissues of *FTO* obesity-risk carriers exert lower uptake of several amino acids as substrates of cellular metabolic processes contributing to compromised energy dissipation.

The positive correlation between *FTO* rs1421085 SNP and obesity or increased body mass index has been reported in several populations such as Estonian children, Chinese children,

Iranian adults, Arabic, Pakistani, Balinese, and Mexican Mayan girls. Through genome-wide association meta-analyses of more than 100000 individuals of European ancestry without diabetes, *FTO* rs1421085 SNP was found to be significantly associated with fasting insulin levels. A recently published study showed that an engineered deletion of the rs1421085 conserved cis-regulatory module in mice prevented high fat diet-induced obesity, decreased whole-body fat mass, and elevated the number of mitochondria in WAT. Our presented data highlight the critical effect of *FTO* rs1421085 SNP on human abdominal SC adipocytes only when they are activated for thermogenesis. Leitner et al. (2017) reported that large amounts of brownable adipocytes can be found in abdominal SC fat whose *in vivo* relevance in humans is still unrevealed. Furthermore, the activation of these adipocytes in humans to reduce adiposity remains challenging. Although these cells can be potentially activated, our previous and present results have pointed to a strong effect of obesity-risk genotype at *FTO* rs1421085 SNP, which has a high prevalence in the European population and Mexican children (12.93%–18.67%) that must be overcome to enable efficient thermogenesis and weight loss. Our findings further support the importance of genetic background not only in the pathogenesis of obesity but also in the potential effectivity of novel therapeutic approaches which target thermogenesis-related energy dissipation.

7. Summary

It has previously been shown in rodent models that when the browning stimulus is removed, parkin-dependent mitophagy is activated and inactive beige adipocytes are generated. However, in humans the molecular mechanisms of this beige to white transition have not yet been investigated in detail. In our experiments, human primary SC abdominal preadipocytes were differentiated into beige adipocytes for 14 days, then either the beige culture medium was used for another 14 days, or it was replaced with the white differentiation medium. The control white adipocytes were differentiated with a specific hormonal cocktail for 28 days. PPARγ-driven beige differentiation resulted in increased mitochondrial biogenesis, UCP1 expression, fragmentation, and respiration compared to white. Cellular morphology, UCP1 content, mitochondrial fragmentation, basal respiration, and mitophagy induction of the transistioned adipocytes were similar to those of control white adipocytes. However, adipocytes undergoing beige to white transition responded more robustly to dibutyryl-cAMP - which mimics the adrenergic stimulus - than control white ones. Gene expression changes indicated that mitochondrial removal in transitioning adipocytes may involve both parkin-dependent and -independent pathways. The avoidance of the inactive state of beige adipocytes can maintain continuous high thermogenesis and energy release.

Next, RNA-sequencing was performed to examine the gene expression pattern of adipocytes carrying different *FTO* alleles. In our experiments, we supplemented the previously used beige differentiation protocol with a 4-hour 500 μM dibutyryl-cAMP treatment, which resulted in active beige adipocytes with higher thermogenic potential. We observed that active beige adipocytes carrying risk-free TT alleles have a higher brown adipocyte content and browning capacity than the white or inactive beige adipocytes, however, no similar significant difference was observed in the case of obesity-risk CC genotype carriers. Active beige adipocytes carrying *FTO* CC alleles had lower thermogenic gene expression (e.g. *UCP1, PM20D1, CIDEA*) and thermogenesis measured by proton leak respiration than TT carriers. In addition, active beige adipocytes with CC alleles showed lower ASC-1 (encoded by *SLC7A10*) neutral amino acid transporter expression and less consumption of alanine, serine, cysteine, and glycine compared to risk-free alelle carriers. We did not observe an effect of the *FTO* rs1421085 SNP on white and inactive beige adipocytes, highlighting its exclusive and critical effect on adipocyte thermogenesis activation.

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Registry number: DEENK/495/2023.PL
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List of publications related to the dissertation

1. **Vámos, A.**, Arianti, R., Vinnai, B. Á., Alrifai, R., Shaw, A., Póliska, S., Guba, A., Csősz, É., Csomós, I., Mocsár, G., Lányi, C., Balajthy, Z., Fésüs, L., Kristóf, E.: Human Abdominal Subcutaneous-Derived Active Beige Adipocytes Carrying FTO rs1421085 Obesity-Risk Alleles Exert Lower Thermogenic Capacity.
Front. Cell. Dev. Biol. 11, 1-18, 2023.
DOI: http://dx.doi.org/10.3389/fcell.2023.1155673
IF: 5.5 (2022)

2. **Vámos, A.**, Shaw, A., Varga, K., Csomós, I., Mocsár, G., Balajthy, Z., Lányi, C., Bacsó, Z., Szatmári-Tóth, M., Kristóf, E.: Mitophagy Mediates the Beige to White Transition of Human Primary Subcutaneous Adipocytes Ex Vivo.
Pharmaceuticals (Basel). 15 (3), 1-21, 2022.
DOI: http://dx.doi.org/10.3390/ph15030363
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List of other publications

3. Arianti, R., Vinnai, B. Á., Bartáné Tóth, B., Shaw, A., Csősz, É., **Vámos, A.**, Győry, F., Fischer-Posovszky, P., Wabitsch, M., Kristóf, E., Fésüs, L.: ASC-1 transporter-dependent amino acid uptake is required for the efficient thermogenic response of human adipocytes to adrenergic stimulation.
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6. Bartáné Tóth, B., Arianti, R., Shaw, A., **Vámos, A.**, Veréb, Z., Póliska, S., Győry, F., Bacsó, Z., Fésüs, L., Kristóf, E.: FTO intronic SNP strongly influences human neck adipocyte browning determined by tissue and PPARγ specific regulation: a transcriptome analysis.
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