

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

Uncovering the role of thiamine transporters in the thermogenic activity of human adipocytes

by

Boglárka Ágnes Vinnai

Supervisor: Dr. Endre Károly Kristóf



UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF MOLECULAR CELLULAR AND IMMUNE BIOLOGY

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By Boglárka Ágnes Vinnai, MSc

Supervisor: Dr. Endre Károly Kristóf

Doctoral School of Molecular Cellular and Immune Biology, University of Debrecen

Head of the **Defense Committee**: Prof. Dr. Gábor Szabó, PhD, DSc

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Dr. Dávid Kovács, PhD

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Dr. Virág Vas, PhD

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1. INTRODUCTION

1.1. Different types of adipocytes and their functional characteristics

Human adipose tissue is a complex and functionally diverse system, consisting of various types of adipocytes with distinct morphological and metabolic characteristics. The most abundant form of adipose tissue in the body is white adipose tissue (WAT), which plays a crucial role in regulating energy homeostasis, primarily by storing excess energy in the form of triacylglycerols (TAGs). Anatomically, WAT can be divided into two main categories: subcutaneous (SuC) and visceral depots. SuC WAT is located beneath the skin, mainly in the abdominal and gluteofemoral regions, and can account for more than 80% of the total body fat. In contrast, visceral WAT is found within the abdominal cavity, in areas such as the omental and mesenteric depots. While in rodents, the secretions of gonadal WAT enter the systemic circulation, in humans, the molecules secreted by visceral WAT are directed into the portal circulation, which represents a significant metabolic difference. The adipocytes in WAT are large, spherical cells, primarily composed of a single large lipid droplet. These cells have a low mitochondrial content, and their main function is energy storage and its release according to the body's needs. WAT is also known as an endocrine organ, as it secretes various adipokines that participate in regulating insulin sensitivity (e.g., leptin, adiponectin), insulin resistance (e.g., resistin, RBP4), and inflammatory processes (e.g., TNF- α , IL-6, IL-1 β , IL-8).

In contrast to WAT, brown adipose tissue (BAT) and beige adipocytes are primarily known for their thermogenic function. Although these cells are present in smaller amounts relative to the total body fat, their metabolic impact is significant. In an activated state, they can increase the body's energy expenditure by 40–80%. Brown and beige adipocytes contain multiple small lipid droplets, have a high mitochondrial density, and express the uncoupling protein 1 (UCP1), which plays a key role in thermogenesis. The development of BAT begins during embryogenesis, and it is found in newborns in regions such as the neck, axillary, perirenal, and periadrenal areas. These depots regress during later life; however, PET/CT imaging studies have shown that active BAT can still be present in adults, primarily in the supraclavicular and cervical regions. Cells in these areas morphologically resemble classical brown adipocytes and are capable of enhanced glucose uptake. Beige adipocytes are scattered within WAT and primarily differentiate in response to external stimuli, such as cold exposure, increased physical activity, the presence of PPAR γ agonists, or tissue damage. These cells are capable of adapting to various environmental changes. In the absence of stimuli, they activate mitophagy, leading to a reversion to a white morphology with low UCP1 expression and a single large lipid droplet.

From a developmental perspective, brown adipocytes originate from myogenic precursors that express transcription factors such as Myf5, Pax7, and PRDM16. In contrast, beige adipocytes arise from a different progenitor population (e.g., SMA⁺, PDGFR α ⁺, PDGFR β ⁺ cells), and their differentiation process is mainly determined by environmental factors.

1.2. Mechanisms of thermogenic adipocytes

Thermogenic adipocytes, including those found in BAT, and beige adipocytes, play a key role in non-shivering thermogenesis. These cells are capable of producing heat through mitochondrial uncoupling mechanisms, which can occur either UCP1-dependent or UCP1-independent pathways. UCP1, located in the inner mitochondrial membrane, is a pivotal protein in thermogenic adipocytes. It allows for the uncoupling of nutrient oxidation from ATP synthesis, resulting in heat production instead. Activation of UCP1 is driven by sympathetic nervous system stimulation, where norepinephrine binds to adrenergic receptors, promoting lipolysis and enhancing thermogenic gene expression. Alongside UCP1, various metabolic substrates such as glucose, fatty acids, and amino acids contribute to the maintenance of the tricarboxylic acid (TCA) cycle, which in turn supports NADH and FADH₂ production for the electron transport chain. During cold exposure, BAT also utilizes branched-chain amino acids (BCAAs), which further stimulate NADH generation and promote heat production.

Beyond UCP1, multiple UCP1-independent thermogenic pathways have been identified, including calcium cycling, creatine-driven substrate cycling, and TAG cycling. These mechanisms enable thermogenesis even in the absence of UCP1. Creatine-mediated thermogenesis occurs through a futile creatine cycle, where creatine undergoes repeated phosphorylation and dephosphorylation, driven by ATP consumption. This process requires two key mitochondrial proteins: creatine kinase B (CKB) and tissue-nonspecific alkaline phosphatase (TNAP). These enzymes are critical for maintaining thermogenic capacity, and their absence impairs norepinephrine-stimulated heat production. Calcium cycling also plays a central role, especially in beige adipocytes. In response to cold, sympathetic nerves activate α 1-adrenergic (AR) and β 3-AR receptors, leading to increased intracellular calcium levels. This calcium is transported into the mitochondria, where it enhances ATP synthesis and promotes heat production in a UCP1-independent manner. The cycling of calcium itself, which involves the sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA) and ryanodine receptors (RyRs), creates an energy-consuming loop that contributes to thermogenesis. Another major heat-producing mechanism is the continuous hydrolysis and re-esterification of TAGs in WAT. This cycle, which involves the breakdown of TAGs into fatty acids and glycerol (or mono- and

diacylglycerols), followed by their reassembly into TAGs, requires ATP investment. The energy demand of this process leads to heat release, contributing to total thermogenic output. Finally, the *PM20D1* gene encodes an enzyme primarily secreted by brown and beige adipocytes, which significantly supports thermogenesis. This enzyme catalyzes the conjugation of fatty acids (e.g., arachidonic and oleic acid) with amino acids, primarily phenylalanine, resulting in the formation of N-acyl amino acids. These metabolites directly influence mitochondrial activity by acting as endogenous uncouplers that dissipate energy as heat. Elevated PM20D1 levels improve glucose metabolism and energy expenditure, while its deficiency is associated with glucose intolerance and insulin resistance. Enhanced expression of this enzyme has been shown to support energy balance and protect against obesity. Overall, mitochondrial uncoupling mechanisms are estimated to account for approximately 20% of total mitochondrial respiration, underlining the critical role of thermogenesis in whole-body energy expenditure.

1.3. Activation of thermogenic adipocytes

In recent years, extensive research has focused on stimulating thermogenic fat cells, particularly brown and beige adipocytes. The most well-known activating factor is cold exposure, which enhances UCP1 expression and heat production via sympathetic nervous system signaling and norepinephrine release. Even short-term cold exposure can significantly boost the expansion of BAT, increase glucose uptake, and enhance oxidative metabolism. The effect of regular physical exercise on BAT in humans is less clear than in animal studies. However, muscle-derived signaling molecules, such as IL-6 and irisin, can promote mitochondrial biogenesis and the activation of thermogenic genes, mainly in WAT. These adaptations may increase energy expenditure and improve metabolic health. Recent findings also suggest that BAT may communicate via hormones with the heart, providing protective effects in conditions like cardiac injury.

Certain natural compounds – including capsinoids, omega-3 fatty acids, and caffeine – are known to stimulate thermogenesis. Capsaicin and its analogues activate TRPV1 receptors in the gut, boosting energy expenditure, fat oxidation, and BAT vascularization. Omega-3 fatty acids, particularly eicosapentaenoic acid, enhance mitochondrial function, reduce inflammation, and support UCP1-dependent thermogenesis. Caffeine, even at low doses, can activate thermogenic pathways in both BAT and WAT. Among pharmacological interventions, β 3-AR agonists, such as mirabegron, have drawn considerable interest due to their ability to increase BAT activity, raise basal metabolic rate, and improve glucose utilization. Additional

agents, such as GLP-1 receptor agonists and mineralocorticoid receptor antagonists, have also been shown to promote thermogenic activity in adipose tissue and support overall metabolic regulation.

1.4. Simpson-Golabi-Behmel Syndrome (SGBS) cells

In vitro adipocyte models are essential tools for studying the regulation of adipogenesis and adipokine secretion, which fundamentally influence insulin sensitivity and the body's metabolic processes. Although a variety of stromal stem cells derived from rodent and human adipose tissue are available, their limited proliferative capacity restricts their use. Moreover, many existing models are either transformed or originate from tumorigenic sources, which can affect the reliability of experimental outcomes. One of the most widely used human cell models is the SGBS cell line, derived from a rare X-linked overgrowth syndrome. SGBS cells exhibit enhanced proliferative and differentiation capacity, maintaining the ability to differentiate into adipocytes for at least 50 generations. During this time, they continue to express adipocyte-specific genes stably, and their metabolic functions — such as glucose transport, lipogenesis, and lipolysis — closely resemble those of primary human adipocytes. Their increased proliferative potential is thought to result from an imbalance between pro-growth and inhibitory mechanisms, leading to impaired growth inhibition and apoptotic regulation. This may be partly due to mutations or deletions in the *glypican-3* gene, which encodes a cell-surface proteoglycan involved in the regulation of organ growth. In SGBS cells, the expression of key adipogenic genes — such as *adiponectin*, *leptin*, *PPAR γ* , and *C/EBP* — is 10–15 times higher than in primary SuC white adipocytes, although their metabolic profile differs from each other. Overall, the SGBS cell line provides a reliable and versatile model for investigating human adipocyte function.

1.5. Vitamin-B1

Thiamine, also known as vitamin B1, was the first B vitamin to be discovered and was isolated in 1912. It is a water-soluble vitamin, meaning it is excreted in the urine and does not accumulate in the body to toxic levels. Structurally, thiamine consists of two heterocyclic rings: a pyrimidine and a thiazole, connected by a methylene bridge. Due to the positive charge on the thiazole ring, the thiamine molecule itself carries a net positive charge. In nature, it is most commonly found in free form in plants, whereas in animal tissues it predominantly exists as thiamine diphosphate (TDP). Ingested thiamine is converted into its free form by phosphatases in the intestinal tract, making it bioavailable for cellular uptake. Upon entering the cells, it is

phosphorylated by the enzyme thiamine pyrophosphokinase-1 (TPK1) to thiamine pyrophosphate (TPP), which is the most biologically active form of the vitamin. Thiamine may also occur as thiamine monophosphate (TMP), diphosphate (TDP), and triphosphate (TTP) in the body, each contributing to different physiological functions.

TPP acts as a critical cofactor for several key enzymes involved in carbohydrate metabolism. These include the pyruvate dehydrogenase (PDH) complex, which links glycolysis to the TCA cycle; the α -ketoglutarate dehydrogenase complex (α -KGDH), essential for producing NADH for mitochondrial electron transport; and transketolase, which plays a central role in the pentose phosphate pathway, crucial for the synthesis of nucleic acids and the generation of NADPH. NADPH is indispensable for fatty acid and steroid biosynthesis, and also contributes to maintaining redox balance in cells. Thiamine also facilitates the metabolism of BCAAs — leucine, isoleucine, and valine — through its role in the branched-chain α -ketoacid dehydrogenase (BCKDH) complex, which governs the rate of their catabolism.

The daily requirement of thiamine in an average adult is approximately 1.1–1.5 mg, though this can vary depending on age, sex, and physical activity levels. Good dietary sources include whole grains, legumes, nuts, brown rice, and animal products, particularly pork and beef. Thiamine is absorbed in the small intestine through active transport at low concentrations, while at higher concentrations passive diffusion also plays a role. Once absorbed, it is distributed primarily to the brain, heart, liver, and kidneys. Given its short biological half-life of about 12 hours, the body requires a continuous supply. Thiamine stores can become depleted within two to three weeks, particularly in conditions that accelerate metabolic turnover such as alcohol withdrawal, seizures, infections, diabetes mellitus (DM), or rapidly progressing malignancies, especially those affecting the gastrointestinal tract or hematopoietic system. Thiamine deficiency may arise from various factors. Inadequate intake is often associated with chronic alcoholism, malnutrition, or after bariatric surgery. Malabsorption syndromes, such as persistent vomiting, gastrointestinal cancers, or inflammatory bowel disease, also contribute to deficiency states. Increased renal or gastrointestinal losses due to diarrhea, vomiting, dialysis, use of diuretics, or systemic infections such as sepsis further exacerbate thiamine depletion. Additionally, certain physiological states like pregnancy, lactation, hyperthyroidism, or elevated enzymatic activity may increase thiamine demand. Prolonged deficiency leads to substantial neurological impairment due to the disruption of TPP-dependent enzymatic activity. Impaired PDH activity, for instance, causes excessive lactate accumulation, promoting oxidative stress and microglial damage. Disruption in the synthesis of fatty acids, nucleic acids, and steroids leads to demyelination and impaired neuronal signaling. Beyond its coenzymatic

roles, thiamine also modulates synapse formation, supports axonal growth, and contributes to myelin production. It influences the synthesis and reuptake of key neurotransmitters such as acetylcholine and serotonin, particularly within the cerebellum, hippocampus, and hypothalamus.

Clinically, the classic consequence of thiamine deficiency is beriberi, which exists in two main forms. Wet beriberi affects the cardiovascular system, leading to edema, heart failure, and potentially infarction. Dry beriberi primarily presents with neurological symptoms, such as peripheral neuropathy and muscle weakness. Another severe neurological manifestation is Wernicke-Korsakoff syndrome, which features memory deficits, ataxia, and recurrent hypothermia. This condition involves damage to the hypothalamus, the regulatory center for thermoregulation and appetite. Case reports have demonstrated that thiamine replacement can reverse hypothermia within 48 hours. Thiamine deficiency also causes significant metabolic disturbances, particularly in glucose metabolism. In DM, urinary thiamine excretion increases markedly — by up to 24-fold in type 1 DM and 16-fold in type 2. Correspondingly, lower TDP levels in erythrocytes are associated with reduced transketolase activity. Clinical trials have shown that supplementing with 150 mg of thiamine daily for one month significantly lowered fasting blood glucose levels in newly diagnosed, untreated type 2 DM patients. Improvements in lipid profiles and creatinine levels were also noted. In another study, administration of 300 mg thiamine daily for six weeks improved glucose tolerance in hyperglycemic individuals.

Once absorbed, thiamine circulates in the bloodstream and is taken up by erythrocytes, leukocytes, and platelets, which facilitate its transport to vital organs such as the liver, myocardium, and central nervous system. Within the brain, thiamine crosses the blood-brain barrier primarily through the cerebrospinal fluid. Transport is mediated by members of the SLC family of transporters, most notably the SLC19 group. This includes thiamine transporter 1 (ThT1, encoded by *SLC19A2*), thiamine transporter 2 (ThT2, encoded by *SLC19A3*), and the reduced folate carrier (RFC, encoded by *SLC19A1*), which exports TMP, TDP, and TTP from cells to maintain homeostasis of phosphorylated forms. ThT1 and ThT2 transport only free thiamine into cells and do not bind folate. Another critical transporter is the mitochondrial TPP transporter (TPC, encoded by *SLC25A19*), which supplies the PDH and α -KGDH complexes with their required cofactor. The *SLC19A2* gene encodes a protein with 12 transmembrane domains which is ubiquitously expressed in human tissues. Although its protein product possesses potential N-glycosylation sites, these are not essential for its transport function. The transport mechanism is a thiamine/H⁺ antiport with high specificity, excluding other organic cations. Knockout studies in mice have shown that loss of *SLC19A2* expression results in DM,

hearing loss, and megaloblastic anemia on a thiamine-deficient diet. In humans, mutations in this gene cause thiamine-responsive megaloblastic anemia (Rogers syndrome), which is treatable with high-dose thiamine supplementation. *SLC19A3* encodes a structurally and functionally similar transporter. Thiamine antagonists such as amprolium, a structural analog, are useful in experimental models to study thiamine transport and function. *In vitro* studies commonly utilize the Caco-2 intestinal epithelial cell line to evaluate ThT inhibitors. Fedratinib, for example, inhibits ThT2 with markedly higher potency ($IC_{50} = 1.36 \mu M$) than ThT1 ($IC_{50} = 7.10 \mu M$), opening potential pharmacological avenues for further research.

2. AIMS OF THE STUDY

Investigation of thermogenic activity in human neck-derived adipocytes following long-term thiamine treatment

The aim of this study was to investigate the effect of long-term, gradually increasing concentrations of thiamine on the thermogenic activity of human neck-derived adipocytes. We assessed the thermogenic capacity of these adipocytes by measuring oxygen consumption and evaluating the expression of the UCP1. Our research focused on clarifying the impact of thiamine on thermogenic mechanisms, particularly from the perspective of mitochondrial function.

- During the study, we also monitored the expression of mitochondrial complex subunits to understand how varying concentrations of thiamine affect mitochondrial functions therefore, the thermogenic capacity.
- Additionally, we examined the expression of ThTs, thermogenic markers, and browning markers to understand how thiamine influences thermogenesis and adipocyte browning.

Short-term thiamine treatment and inhibition of thiamine transporters on the thermogenesis of SGBS adipocytes

The aim of this part of the study was to explore the impact of short-term thiamine treatment and ThT inhibition on the thermogenic processes in the SGBS adipocyte cell line.

- We investigated the effect of increasing thiamine concentrations and ThT inhibition on oxygen consumption and thermogenesis in SGBS adipocytes, in combination with adrenergic stimulation induced by dibutyl (db)-cAMP, focusing particularly on UCP1 activity.
- One goal of the experiments was to assess the expression of mitochondrial complex subunits to determine how thiamine treatment and transporter inhibition affect mitochondrial processes.
- We also examined how the inhibition of ThT function, along with thiamine treatment, influences the expression of genes involved in thermogenesis and browning processes.

3. MATERIALS AND METHODS

3.1. Ethics statement

The acquisition of human adipose tissue biopsies and the study protocol were accepted by the Medical Research Council of Hungary (20571-2/2017/EKU) followed by the EU Member States' Directive 2004/23/EC on presumed consent practice for tissue collection. All experiments were performed in accordance with the accepted ethical guidelines of the Helsinki Declaration. Written informed consent from all volunteers was obtained before the surgical procedure.

3.2. Aquired samples and isolation of subcutaneous (SuC) and deep neck (DeN) derived human adipose-derived stromal cells (hASCs)

In order to rule out inter-individual variations, a pair of SuC and DeN adipose tissue biopsy was obtained from patients who underwent thyroid surgery. Biopsies were originated from patients aged 20-65, with a Body Mass Index (BMI) <29.9. The selection of the samples was made based on the BMI values but not on the gender. Patients with abnormal thyroid hormone levels, known DM, or malignant tumor at the time point of the surgery were excluded. The adipose tissue samples were immediately transported to the laboratory and processed after being removed. During the processing of the tissues, the adipose tissue specimens were carefully cut and separated from connective tissues and blood vessels and transferred into 15 mL centrifuge tubes. The tissue pieces were digested in 120 U/mL Collagenase from *Clostridium histolyticum*-containing 1x sterile Phosphate-Buffered Saline (PBS) for 1 hour in 37°C water bath. Every 15 minutes the tubes were gently shaken. In order to remove any remaining tissue debris, the tissues were filtered by a filter with a pore size of 140 µm. After that, the cell suspension was centrifuged at 200 g for 10 minutes and the pellet of stromal vascular fraction was re-suspended in DMEM-F12-HAM medium containing 10% FBS, 100 U/mL penicillin-streptomycin, 33 µM biotin, and 17 µM pantothenic acid. hASCs were seeded onto Ibidi eight-well µ-slides or 6-well plates at a density of 15000 cells/cm² and cultured in serum-containing medium at 37°C in 5% CO₂ for 24 hours to attach. After 24 hours, floating cells were removed with PBS and the attached hASCs were cultured until they reached proper confluency. The absence of mycoplasma was checked by polymerase chain reaction (PCR) analysis.

3.3. Cell culture and differentiation of hASCs and SGBS preadipocytes into mature white (ADCY) and „beige” (B-ADCY) adipocytes

For the experiments, hASCs and SGBS preadipocytes have been used as adipocyte progenitors. SGBS human cell line was kindly provided by our collaborating partners, Pamela Fischer-Posovszky and Martin Wabitsch (University Medical Center Ulm, Ulm, Germany) who previously established it. hASCs and SGBS preadipocytes were differentiated into ADCY under regular adipogenic medium and B-ADCY under long-term rosiglitazone treatment. ADCY differentiation was induced for three days using DMEM-F12-HAM medium supplemented with 33 μ M biotin, 17 μ M pantothenic acid, 10 μ g/mL human apo-transferrin, 20 nM human insulin, 100 nM hydrocortisone, 0.2 nM T3, 2 μ M rosiglitazone, 25 nM dexamethasone, and 0.5 mM IBMX. B-ADCY differentiation was induced for three days using DMEM-F12-HAM medium supplemented with 33 μ M biotin, 17 μ M pantothenic acid, 10 μ g/mL apo-transferrin, 850 nM human insulin, 0.2 nM T3, 1 μ M dexamethasone, and 0.5 mM IBMX. After three days, dexamethasone and IBMX were omitted from both differentiation media. From day 4-14, hydrocortisone was constantly present in the ADCY differentiation cocktail. Rosiglitazone and higher concentration of insulin were present in the B-ADCY differentiation cocktail since day 4-14 of differentiation.

3.4. Treatment of hASCs and SGBS adipocytes during and after adipocyte differentiation

The differentiation of hASCs was executed in the presence of abundant or gradually increasing concentrations of thiamine from the third day of the differentiation until the fourteenth day. In the experiments with abundant amounts of thiamine, cells were differentiated using DMEM-F12-HAM medium with or without the addition of excess (25 000 nM or 50 000 nM) thiamine hydrochloride throughout the last 11 days of adipocyte differentiation. In these experiments, the control (Ctrl) cells were differentiated only in the presence of thiamine found in the DMEM-F12-HAM, which was 6400 nM, throughout the entire differentiation process. In case of the thiamine concentration-dependence experiments, we used a specific custom-made thiamine-free culture fluid for differentiating the adipocytes supplemented with gradually increasing concentrations (40 nM, 200 nM, 1 nM, 5000 nM, or 25 000 nM) of thiamine hydrochloride throughout the last 11 days of differentiation. In these experiments, the Ctrl cells were differentiated in the absence of thiamine throughout the entire differentiation process. At the end of the differentiation, cells were lysed in TRIzol or Sodium dodecyl sulfate (SDS) for further experiments. In case of differentiated SGBS adipocytes in DMEM-F12-HAM, ADCYs and B-ADCYs were treated with a single bolus of 0.5 mM db-cAMP for 10 h to mimic *in vivo*

cold-induced thermogenesis. Fedratinib at 1 μ M or Amprolium at 300 μ M was administered also for 10 h to inhibit ThT activity. Db-cAMP was also administered with the combination with Fedratinib or Amprolium for 10 h. In the experiments with excess thiamine, differentiated SGBS ADCYs and B-ADCYs were treated using DMEM-F12-HAM medium with or without the addition of excess (25 000 nM or 50 000 nM) thiamine hydrochloride for 10 h. In these experiments, the control cells were differentiated only in the presence of thiamine found in the DMEM-F12-HAM. After the 10 h treatment, cells were lysed in TRIzol or SDS for further experiments. Both hASCs and SGBS adipocytes were incubated at 5% CO₂ and 37°C and medium were changed every 3 days.

3.5. Analysis of publicly available single nuclei RNA-sequencing (snRNA-seq) data

Available snRNA-seq dataset was explored by using a public webtool (<https://batnetwork.org/>) as described by Sun *et al.*, 2020.

3.6. Total RNA isolation and TaqMan[™] reverse transcription-coupled quantitative PCR (RT-qPCR)

After collecting cells in TRIzol, total RNA was isolated by chloroform-isopropanol extraction. The concentration and purity of the isolated RNA was checked by using Nanodrop 2000 Spectrophotometer after DNase treatment. RNA was diluted to 100 ng/ μ L for all samples and was reverse transcribed to cDNA by using reverse transcription kit following the manufacturer's instructions. Validated TaqMan assays used in qPCR were designed and supplied by Thermo Fisher Scientific. qPCR was performed in Light Cycler® 480 II. The following conditions were set to perform the reactions: initial denaturation step at 95 °C for 60 sec followed by 50 cycles of 95 °C for 12 sec and 60 °C for 30 sec. Gene expression values were calculated by the comparative threshold cycle (Ct) method. Δ Ct represents the Ct of the target minus the geometric mean of endogenous controls, which were *ACTB* and *GAPDH*. Normalized gene expression levels equal $2^{-\Delta Ct}$.

3.7. Immunoblotting and densitometry

In case of both differentiated hASCs and SGBS adipocytes, ADCYs and B-ADCYs were washed with cold 1x sterile PBS and collected in SDS buffer. The samples were boiled at 100 °C for 10 minutes and loaded onto SDS polyacrylamide gel. Proteins were transferred onto PVDF Immobilon-P Transfer Membrane followed by blocking in Tris-buffered saline (TBS) containing 0.05% Tween-20 TBS (TTBS) and skimmed milk for 1 hour. Membranes were

probed by primary antibodies in specific working dilutions overnight at 4 °C. Next, membranes were washed with TTBS 3x15 minutes and followed by incubation with horseradish-peroxidase-conjugated species-corresponding secondary antibodies for 1 h at room temperature. Immunoblots were developed with Immobilon western chemiluminescent substrate. Densitometry analysis of immunoblots was performed by using FIJI Image J software, where the density value of the target protein of interest was divided by the density value of the tubulin or actin household protein, thus obtaining the normalized optical density.

3.8. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurement by Seahorse

OCR and ECAR of hASC-derived and SGBS adipocytes, which were differentiated as described in 3.3, were measured using an XF96 oximeter according to previously optimized protocols. When the hASCs were differentiated in the presence of excess thiamine, cells were pre-incubated for 1h and treated in Agilent Seahorse XF Base Medium, which is optimized for extracellular flux assays and contains thiamine at 6400 nM concentration. When the adipocytes were differentiated at thiamine concentrations close to the physiological blood plasma levels, the measurement was performed in thiamine-free medium to carry out the assay with the previously obtained thiamine pool of the cells which was gained during differentiation. Since thiamine-free medium was used instead of the buffer-free Agilent Seahorse XF Base Medium during the measurement, it was not possible to determine ECAR in this setting. In both types of experimental settings, after recording the baseline OCR, 0.5 mM db-cAMP was injected to mimic an adrenergic cue; then stimulated OCR was recorded every 20 min.

In case of differentiated SGBS adipocytes, after recording the baseline OCR, 0.5 mM db-cAMP, 1 μ M Fedratinib, 300 μ M Amprolium, or combination of db-cAMP and SLC inhibitor were injected to the cells. Then, stimulated OCR was recorded every 30 minutes. For thiamine excess experiment, SGBS preadipocytes were differentiated into ADCY and B-ADCY at regular culture conditions, then OCRs were detected for 10 h following the injection of 0.5 mM db-cAMP in the presence or absence of excess thiamine (25 000 nM or 50 000 nM). Stimulated OCR was recorded every 30 minutes.

In all settings, proton-leak respiration was monitored after injecting Oligomycin at 2 μ M concentration. Finally, adipocytes received a single bolus of Antimycin A at 10 μ M concentration for baseline correction (measuring non-mitochondrial respiration). The OCR was normalized to protein content.

3.9. Quantification of adipogenic differentiation ratio using laser-scanning cytometry

SuC and DeN derived hASCs were plated and differentiated in 8-well Ibidi μ -chambers (Ibidi GmbH, Gräfelfing, Germany). Cells were washed with PBS prior to fixation by 4% paraformaldehyde. Cells were permeabilized with 0.1% saponin, followed by blocking with 5% skimmed milk as per previously described protocols. The cells were incubated overnight with the anti-UCP1 (1:200, U6382) primary antibody at room temperature. Incubation with the Alexa 488 goat anti-rabbit IgG (1:1000, A11034) secondary antibodies was kept for 3 h at room temperature. Nuclei were labeled with Propidium Iodide (1.5 μ g/mL, 1 h). Scanning was done by an iCys Research Imaging Cytometer. The images were processed and analyzed (n=4, 2000 cells per sample) by our high-throughput automatic cell-recognition protocol using the iCys companion software (iNovator Application Development Toolkit version 7.0), Cell Profiler, and Cell Profiler Analyst. Cells were identified according to their nuclear staining. Then, the fluorescence signal intensity of the UCP1 immunostaining and the texture sum variance of the light scatter signal of lipid droplets were quantified in each cell within the 30-pixel immediate outward vicinity of the nucleus contour by the Cell Profiler software. Afterward, based on these fluorescence and light scatter signal of single cells, a semi-automated classification and enumeration of the differentiated adipocytes and undifferentiated preadipocytes were carried out applying the trained classification “Fast Gentle Boosting” of the Cell Profiler Analyst software. The image analysis was performed by Dr. Zsolt Bacsó (University of Debrecen, Faculty of Medicine, Department of Biophysics and Cell Biology).

3.10. Statistical analysis

The results are expressed as mean $\pm$ SD. The normality of the distribution of the data was tested by Shapiro–Wilk test. For multiple comparisons of each group, one-way or two-way ANOVA and Tukey’s *post hoc* test were used. The data were visualized and analyzed by using GraphPad Prism 8.

4. RESULTS

4.1. Extracellular thiamine concentration modulates the thermogenic capacity of human adipocytes derived from the cervical region

4.1.1. Excess thiamine levels during adipogenesis enhance thiamine transporter expression

Our previous findings revealed that the upregulation of ThTs and the resulting increase in thiamine uptake support efficient thermogenic activation in adipocytes derived from the cervical region. Here, we primarily investigated the effects of excess thiamine concentrations (25 000 nM and 50 000 nM) on transporter expression in adipocytes of SuC and DeN origin, differentiated into white (ADCY) or beige (B-ADCY) adipocytes using standard adipogenic medium or long-term rosiglitazone treatment, respectively. Differentiation was performed under standard conditions (6400 nM thiamine), with or without the addition of extra thiamine-hydrochloride. Thiamine supplementation did not affect the differentiation efficiency of ADCYs. ThT2 expression — unlike that of ThT1 — was significantly higher at both mRNA and protein levels in DeN ADCYs compared to their SuC counterparts. In B-ADCYs, 25 000 nM thiamine significantly increased ThT1 expression in SuC cells, whereas both 25 000 nM and 50 000 nM thiamine enhanced ThT1 expression similarly in DeN cells. Furthermore, 50 000 nM thiamine significantly increased ThT2 mRNA levels in DeN B-ADCYs. At protein level, ThT2 expression was markedly elevated in SuC B-ADCYs exposed to 25 000 nM thiamine, while ThT1 protein levels remained largely unaffected by thiamine supplementation.

4.1.2. Thiamine supplementation during adipogenesis promotes thiamine transporter expression

To further explore the effects of thiamine availability, we analyzed the impact of increasing thiamine concentrations (40 nM, 200 nM, 1000 nM, and 5000 nM) on transporter expression during the differentiation of human adipocytes from SuC and DeN fat depots. Cells were differentiated in thiamine-free medium (Th 0) or supplemented with the aforementioned concentrations. The presence of thiamine had no effect on the differentiation efficiency of ADCYs. However, at 1000 nM and 5000 nM, thiamine induced significant changes compared to Th 0 and 40 nM conditions. Both ThT1 and ThT2 mRNA levels increased in a concentration-dependent manner during differentiation in the presence of thiamine.

4.1.3. Thiamine availability regulates thermogenic capacity in a concentration-dependent manner during adipogenesis

We assessed how varying thiamine concentrations (40 nM – 25 000 nM) during differentiation affect the thermogenic capacity of ADCYs. db-cAMP, a membrane-permeable cAMP analog, significantly enhanced mitochondrial respiration. Increasing thiamine concentrations led to a dose-dependent rise in basal respiration, maximal cAMP-stimulated respiration, and proton leak respiration, particularly in DeN ADCYs. SuC ADCYs showed significant differences even at 200 nM thiamine. Thiamine supplementation did not affect oligomycin-inhibited, ATP-coupled respiration in either adipocyte type.

4.1.4. Thiamine availability during differentiation influences mitochondrial biogenesis in cervical adipocytes

SuC ADCYs differentiated in increasing thiamine concentrations showed elevated protein levels of mitochondrial complex I, II, and III subunits. Thiamine concentrations of 1000 nM and 5000 nM led to statistically significant increases compared to 40 nM or thiamine-free conditions. However, complex IV and V subunit expressions remained unchanged. In DeN adipocytes, which inherently exhibit high mitochondrial protein levels, only complex II expression was affected by thiamine supplementation.

4.1.5. Thiamine supplementation during differentiation enhances thermogenic gene expression in a dose-dependent manner

As mitochondrial respiration, particularly proton leak respiration, increased in response to elevated thiamine concentrations, we next examined thermogenic gene expression. mRNA levels of *UCP1* and *PGC1A* were elevated in a concentration-dependent manner in both SuC and DeN ADCYs. A similar trend was observed at the protein level for UCP1, whereas PGC1A protein levels remained unaffected. In contrast to the expression of the white adipocyte marker *LEP*, several thermogenic markers (*DIO2*, *TBX1*, *CKMT2*, *CIDEA*) increased with elevated thiamine concentration. Notably, *CITED1* expression decreased under both thiamine-deficient and high-thiamine conditions, peaking at 40 nM.

4.1.6. High thiamine levels promote both coupled and uncoupled respiration during adipocyte differentiation

We then evaluated thermogenesis in ADCYs from SuC and DeN depots differentiated in the presence of additional thiamine (beyond the standard 6400 nM in the differentiation medium).

Basal respiration was significantly elevated in SuC adipocytes exposed to 25 000 nM thiamine compared to standard conditions. A similar trend was observed in DeN adipocytes, though the differences were not statistically significant. Maximal cAMP-stimulated respiration increased in both adipocyte types in the presence of excess thiamine. Both proton leak and ATP-coupled respiration were higher in SuC and DeN adipocytes differentiated with extra thiamine. cAMP-induced increases in ATP-coupled and proton leak respiration were particularly pronounced in DeN adipocytes. While proton leak respiration was significantly elevated in SuC cells under high thiamine conditions, ATP-coupled respiration was enhanced in DeN adipocytes. cAMP-stimulated extracellular acidification increased in both cell types, but high thiamine levels during differentiation did not significantly affect this parameter. In SuC ADCYs, 25 000 nM or 50 000 nM thiamine significantly increased protein expression of mitochondrial complex I, II, and IV subunits, while in DeN adipocytes, 50 000 nM thiamine selectively increased complex I expression.

4.1.7. Elevated thiamine levels during differentiation promote thermogenic gene expression

We hypothesized that elevated thiamine levels during differentiation would further enhance the expression of genes related to thermogenesis and mitochondrial biogenesis. Supporting this, elevated thiamine increased mRNA expression of both *UCP1* and *PGC1A* compared to control ADCYs and B-ADCYs. However, in DeN adipocytes, UCP1 upregulation was not statistically significant. In both ADCYs and B-ADCYs, 50 000 nM thiamine resulted in higher gene expression irrespective of anatomical origin. UCP1 protein levels were also elevated in B-ADCYs differentiated with extra thiamine. In SuC B-ADCYs, PGC1A protein levels were increased, and further upregulated by 50 000 nM thiamine.

4.1.8. Excess thiamine enhances brown/beige marker and thermogenic gene expression in cervical adipocytes

We next assessed the expression of *CKMT2*, *CIDEA*, *DIO2*, *TBX1*, *CITED1*, and *LEP* in response to extra thiamine supplementation during differentiation. Except for *LEP*, the expression of all analyzed genes was upregulated in SuC and DeN B-ADCYs. In SuC ADCYs, *CKMT2* and *TBX1* were significantly upregulated by high thiamine, whereas in DeN ADCYs, *DIO2*, *TBX1*, and *CITED1* expression increased.

4.2. The functional role of thiamine in the thermogenic activity of human adipocytes

4.2.1. Human white adipocytes exhibit the highest expression of thiamine transporters

Using publicly available snRNA-seq datasets, we analyzed the expression profiles of ThT1 and ThT2 in human WAT. ThT1 was primarily expressed in preadipocytes and mature adipocytes, whereas ThT2 was exclusively found in mature adipocytes. Other cell types — such as fibroblasts, endothelial cells, and CD4⁺ T lymphocytes — did not show detectable expression. These data suggest a critical role for thiamine in WAT function, particularly in adipocyte browning and thermogenesis. The anatomical clustering of ThT-expressing adipocytes from the cervical region further underscores the physiological relevance of vitamin B1 in thermogenically active fat depots.

4.2.2. Thiamine transporter expression is induced by adrenergic stimulation and excess thiamine in differentiated SGBS adipocytes

We previously observed that high thiamine levels during the adipogenesis of cervical-derived stromal cells promoted ThT expression. Accordingly, we examined how abundant thiamine concentrations (25 000 nM and 50 000 nM) affect basal and db-cAMP-induced transporter expression in differentiated SGBS adipocytes. Cells were treated with 0.5 mM db-cAMP with or without extra thiamine. db-cAMP stimulation significantly upregulated *SLC19A2* mRNA expression in both ADCYs and B-ADCYs. Protein levels of ThT1 were also significantly increased following db-cAMP treatment. In B-ADCYs, 25 000 nM thiamine further enhanced transporter expression under db-cAMP stimulation. Treatment with 50 000 nM thiamine significantly increased both basal and stimulated ThT1 protein levels in differentiated adipocytes. In contrast, no significant changes were observed in ThT1 expression in B-ADCYs. *SLC19A3* mRNA and ThT2 protein levels were unaffected by either db-cAMP or thiamine supplementation.

4.2.3. Activation of thiamine transporters by db-cAMP is attenuated in the presence of transporter inhibitors

Next, we examined whether administration of the ThT inhibitors, fedratinib or amprolium, after differentiation, affects the basal or db-cAMP-stimulated expression of ThTs in ADCYs and B-ADCYs. Differentiated adipocytes were treated with 0.5 mM db-cAMP in the presence or absence of transporter inhibitors. Our observations revealed that db-cAMP stimulation significantly increased both ThT mRNA and protein levels in ADCYs, whereas in B-ADCYs, this effect was confined to mRNA levels. Fedratinib significantly reduced db-cAMP-induced

SLC19A2 and *SLC19A3* mRNA expression in both ADCYs and B-ADCYs. At the protein level, this inhibitory effect was only detectable in db-cAMP-treated B-ADCYs for both transporters, whereas in ADCYs, only ThT2 expression was reduced. Treatment with amprolium significantly decreased mRNA expression of both transporters in db-cAMP-stimulated B-ADCYs; however, at the protein level, this effect was exclusively observed for ThT2.

4.2.4. Inhibition of thiamine transporters reduces both coupled and uncoupled respiration in differentiated SGBS adipocytes

We further explored the potential role of ThTs in the thermogenic activation of differentiated SGBS adipocytes. ADCYs and B-ADCYs were treated with db-cAMP in the presence of transporter inhibitors. Basal oxygen consumption was initially recorded, followed by db-cAMP injection to assess maximal stimulated respiration. Subsequently, oligomycin was injected to determine proton leak respiration, which correlates with UCP1-mediated thermogenesis. Oxygen consumption significantly increased upon db-cAMP treatment in both cell types. We observed that the db-cAMP-induced elevation in oxygen consumption and proton leak respiration was significantly attenuated by both fedratinib and amprolium in ADCYs and B-ADCYs. In contrast, extracellular acidification rates induced by db-cAMP remained unaffected by transporter inhibition in either cell type.

4.2.5. Inhibition of thiamine transporters after adipocyte differentiation reduces mitochondrial biogenesis in SGBS ADCYs and B-ADCYs

We also investigated the effect of transporter inhibitors on the expression of mitochondrial complex subunits. Our results demonstrated that db-cAMP stimulation markedly enhanced the expression of subunits of complexes I, II, III, and IV, while expression of complex V remained unchanged in ADCYs. Both fedratinib and amprolium significantly inhibited the db-cAMP-induced expression of complex I subunits. Moreover, amprolium reduced basal expression of complexes III and IV, whereas fedratinib selectively decreased basal expression of complex IV. Additionally, both inhibitors reduced expression of complex II subunits, although these reductions were not statistically significant.

4.2.6. Inhibition of thiamine transporters reduces thermogenic marker expression in differentiated SGBS adipocytes

After observing reduced thermogenic activity in SGBS ADCYs in the presence of transporter inhibitors, we further assessed how inhibition of thiamine transport — and thereby thiamine

availability — affects expression of thermogenic markers. Our data showed that db-cAMP elevated UCP1 mRNA and protein levels in both ADCYs and B-ADCYs. However, fedratinib suppressed this increase, except in B-ADCYs where *UCP1* mRNA levels remained unchanged. Fedratinib also reduced UCP1 expression at both mRNA and protein levels in B-ADCYs. Similarly, amprolium inhibited db-cAMP-induced UCP1 protein expression in both cell types. Both inhibitors reduced *UCP1* mRNA levels in B-ADCYs and protein levels in ADCYs. Furthermore, fedratinib suppressed db-cAMP-stimulated *PGC1A* expression in both adipocyte types, while amprolium had a similar effect in B-ADCYs. Protein levels of PGC1A were also reduced by both inhibitors under db-cAMP stimulation in ADCYs and B-ADCYs.

4.2.7. Inhibiting thiamine transporters suppresses expression of thermogenic genes in differentiated SGBS adipocytes

We also assessed how ThT inhibition affects the mRNA expression of various thermogenesis-related genes in SGBS adipocytes. Our findings revealed that db-cAMP significantly increased expression of *CITED1*, *DIO2*, and *TBX1* in both cell types, while *CIDEA* and *CKMT2* were upregulated only in B-ADCYs. Fedratinib significantly reduced db-cAMP-stimulated expression of all these markers in both ADCYs and B-ADCYs.

4.2.8. Excess thiamine enhances coupled and uncoupled respiration after adipogenesis

We previously observed that excess thiamine during adipocyte differentiation enhances the thermogenic potential of human adipocytes. Based on this, we aimed to evaluate the short-term effects of elevated thiamine levels on thermogenic capacity in differentiated SGBS ADCYs and B-ADCYs, both in unstimulated and db-cAMP-stimulated conditions. In both cell types, treatment with 50 000 nM thiamine significantly increased oxygen consumption and extracellular acidification under basal conditions. Although additional thiamine did not further augment the db-cAMP-induced response, db-cAMP alone significantly increased maximal stimulated oxygen consumption, proton leak respiration, and extracellular acidification in both cell types compared to untreated controls, consistent with prior observations.

4.2.9. High thiamine levels during differentiation enhance mitochondrial biogenesis in SGBS adipocytes

Next, we assessed how treatment with elevated thiamine after adipocyte differentiation influences the expression of mitochondrial complex subunits in ADCYs. We found that db-cAMP significantly increased protein expression of complexes I, II, III, and IV, while having

no effect on complex V, in line with earlier findings. Treatment with 25 000 nM thiamine significantly elevated expression of complexes I and IV, while no changes were observed in complexes II, III, or V. Treatment with 50 000 nM thiamine further enhanced protein expression of complexes I–IV. Notably, complex II protein levels were strongly increased following db-cAMP stimulation. Neither thiamine nor db-cAMP affected the expression of complex V.

4.2.10. Elevated thiamine levels after differentiation enhance the expression of thermogenic markers in white adipocytes

Given the observed enhancement in cellular respiration under high-thiamine conditions, we hypothesized that excess thiamine might further promote the expression of genes related to mitochondrial biogenesis and thermogenesis in differentiated SGBS adipocytes. Our data showed that db-cAMP-stimulated *PGC1A* mRNA expression in ADCYs and *UCP1* expression in B-ADCYs were further elevated with thiamine supplementation. Treatment with 25 000 nM thiamine increased PGC1A protein levels in untreated ADCYs, while 50 000 nM thiamine enhanced *PGC1A* mRNA in B-ADCYs and PGC1A protein levels in ADCYs. Furthermore, 50 000 nM thiamine increased UCP1 protein expression in ADCYs following db-cAMP treatment.

4.2.11. Elevated thiamine increases expression of beige/brown markers and thermogenic genes in differentiated SGBS adipocytes

Finally, we investigated how increased thiamine availability affects the expression of additional thermogenic markers. Our results showed that db-cAMP stimulation combined with excess thiamine further enhanced *TBX1*, *DIO2*, and *CIDEA* mRNA expression in ADCYs. In B-ADCYs, increased thiamine levels elevated *CKMT2*, *CITED1*, *DIO2*, and *TBX1* expression. Notably, even in the absence of db-cAMP, expression of *CITED1* and *DIO2* was elevated in ADCYs, while *CKMT2* and *CITED1* expression increased in B-ADCYs.

5. DISCUSSION

A previous study investigated differentiated adipocyte populations derived from DeN and SuC adipose tissues of nine independent human donors. Gene expression profiles of these adipocytes were analyzed via bulk RNA-seq to elucidate molecular pathways regulating thermogenesis. During adipocyte differentiation, using either a standard or a browning-inducing differentiation medium was applied. Regardless of the differentiation protocol, cells originating from DeN fat exhibited a markedly higher proportion of brown adipocyte-like characteristics and an enhanced tendency for browning compared to those derived from SuC tissue. These observations were confirmed by results from both the BATLAS and ProFAT tools. The study identified several genes encoding SLC transporter membrane proteins crucial for nutrient uptake and cellular energy regulation. Given the high energy demands of brown and beige adipocytes, upregulation of these nutrient transporters likely plays an essential role in sustaining their metabolic activity. Notably, the thiamine transporter ThT2, encoded by the *SLC19A3* gene, was found to be highly expressed in DeN-derived adipocytes, particularly during the differentiation process. Previous findings suggested that elevated vitamin B1 levels are necessary for these cells to elicit an effective thermogenic response to adrenergic stimulation. Other studies have also highlighted the importance of micronutrients — such as thiamine, pantothenic acid, and riboflavin — in promoting the browning of human dermal fibroblasts. Our study aimed to investigate how thiamine availability influences the differentiation and thermogenic capacity of human neck-derived adipocytes. ThT2 has previously been implicated in intestinal thiamine absorption and systemic energy homeostasis. Reduced ThT2 expression has been observed in SuC WAT from obese individuals compared to lean controls — with gene expression levels positively correlating with weight loss — suggesting a potential role in metabolic regulation. Animal studies further indicated that ThT2 can partially compensate for ThT1 deficiency, as ThT2 knockout mice exhibit impaired thiamine absorption, whereas ThT1 deficiency alone does not alter plasma thiamine levels.

In our experiments, increasing thiamine concentrations during differentiation upregulated both SLC19A2 (ThT1) and SLC19A3 (ThT2) gene and protein expression in adipocytes from both anatomical depots, regardless of the medium used. Although dietary sources of thiamine include whole grains, legumes, and meats, daily intake may not always meet physiological requirements. Recommended daily thiamine intake is 1.2 mg for men and 1.1 mg for women, with increased needs during pregnancy. Thiamine deficiency primarily affects the nervous, cardiovascular, and digestive systems, often arising from inadequate intake or increased

excretion — commonly due to alcoholism or malnutrition — leading to conditions such as beriberi and Wernicke–Korsakoff syndrome, which are frequently associated with memory impairment, coordination deficits, and hypothermia. Some studies suggest that hypothermia may arise from hypothalamic dysfunction, given its role in thermoregulation. Clinically, thiamine supplementation can reverse low body temperature within just two days in cases of deficiency.

We observed that human DeN-derived adipocytes differentiated under thiamine-deficient conditions exhibited significantly impaired thermogenic function. This manifested as reduced basal respiration, lower levels of proton leak respiration — an indicator of UCP1-mediated thermogenesis — and decreased expression of thermogenic genes. In contrast, continuous thiamine supplementation during differentiation improved these parameters in a dose-dependent manner. Higher thiamine levels led to more effective browning and increased expression of thermogenic markers. Interestingly, while differentiation media commonly contain 10 000 nM thiamine, circulating plasma levels are typically in the nanomolar range. Supplementation with 25 000 nM thiamine markedly enhanced basal and maximal respiration, as well as proton leak respiration, suggesting an elevated thermogenic potential. This was accompanied by increased expression of specific subunits of the mitochondrial electron transport chain. Furthermore, DeN adipocytes differentiated in the presence of high thiamine levels displayed greater thermogenic activity under *ex vivo* conditions.

Given their high glucose and fatty acid uptake capacity, brown and beige adipocytes are considered promising targets in the prevention and treatment of obesity and type 2 DM. Transplantation of *in vitro* differentiated human brown adipocytes into mice has been shown to improve glucose clearance and energy expenditure. However, optimizing browning *in vivo* remains a challenge. Our findings suggest that thiamine availability during differentiation alone may be sufficient to enhance thermogenic capacity.

The SGBS cell line provides a robust *in vitro* model that resembles human SuC white adipocytes. These cells display key characteristics of primary human adipocytes, including browning potential and UCP1 expression, and are widely used to study adipose tissue metabolism and metabolic disease pathophysiology under controlled conditions. Due to inter-donor variability, cell line-based systems are essential to validate findings obtained from primary human cells. While hASCs are commonly used, their properties are significantly influenced by individual factors such as lifestyle, metabolic status, and genetic background. Analysis of publicly available snRNA-seq data from SuC WAT confirmed that ThT expression is largely restricted to adipocytes, underscoring their essential role in adipose cell function.

The SGBS cell line thus offers a practical alternative, minimizing variability and access limitations associated with primary samples. Previous studies have shown that inhibition of ThTs impairs thermogenic capacity in human DeN adipocytes. Consistent with these findings, we observed that thiamine uptake inhibitors, such as fedratinib and amprolium, suppressed the expression of thermogenic genes and reduced respiration in SGBS adipocytes. Fedratinib primarily targets ThT2, while being less effective on ThT1, and amprolium — a structural analog of thiamine — competitively blocks cellular thiamine uptake, inducing functional thiamine deficiency. Although fedratinib also inhibits JAK2, which may affect thermogenesis in immune cell-rich environments, this is unlikely in our adipocyte *in vitro* system. This is further supported by the similar inhibitory effects observed with amprolium.

TPP, the active form of thiamine, is a critical cofactor for mitochondrial enzymes such as PDH and α -KGDH. Inhibition of thiamine uptake reduces PDH activity and consequently NADH production, impairing cellular energy metabolism. Additionally, the uptake and catabolism of BCAAs by brown adipocyte mitochondria, mediated by the TPP-dependent BCKDH complex, may also be influenced by thiamine availability.

WAT constitutes a significant portion of adult body mass and is strongly associated with obesity. ThT2 expression is lower in the SuC fat of obese individuals. Transcriptomic data suggest that thermogenic adipocytes upregulate *SLC19A2* and *SLC19A3* in response to adrenergic stimulation. Similarly, db-cAMP-induced adrenergic activation increased ThT1 expression in SGBS adipocytes, suggesting a role for ThTs in mounting an adequate thermogenic response. Our data also showed that thiamine supplementation during db-cAMP stimulation enhanced the expression of *UCP1* and other thermogenic genes in SGBS cells.

The mechanisms by which thiamine promotes thermogenic gene activation remain incompletely understood. It is hypothesized that thiamine may influence the transcription of *UCP1* through cAMP-responsive transcription factors and the PGC-1 α coactivator, which act on regulatory elements in the gene's non-coding regions. Phosphorylation of transcription factors such as CREB and ATF2 — activated by cAMP signaling — may further regulate *UCP1* expression.

Although thiamine deficiency is relatively rare in industrialized countries, it remains prevalent among individuals with high alcohol consumption. It can also arise following bariatric surgery — especially gastric bypass — and is common among patients with DM. Thiamine deficiency has been linked to oxidative stress, endothelial dysfunction, and hyperglycemia-induced cellular damage. Several studies have reported reduced transketolase activity in erythrocytes or

widespread thiamine deficiency in type 2 DM patients, particularly in those with microalbuminuria. Low thiamine levels have also been observed in individuals with type 1 DM. In developed countries, including the United States, thiamine deficiency has been documented even among children, often associated with obesity, DM, or excessive intake of sugary beverages. Thiamine supplementation has been shown to improve endothelial function, potentially reducing vascular complications in DM. These findings suggest that thiamine deficiency contributes to metabolic disturbances associated with obesity and DM, and that thiamine supplementation may be a valuable strategy for prevention and treatment. A six-week regimen of thiamine administered three times daily significantly improved glucose tolerance in hyperglycemic individuals, while 150 mg daily thiamine reduced fasting blood glucose in treatment-naïve type 2 diabetics. Supplementation also had beneficial effects on serum lipid and creatinine levels.

Additional risk factors for thiamine deficiency include immune dysfunction, hyperthyroidism, heart failure, and dialysis-dependent renal failure. Once converted to its active form, TPP, thiamine is essential for mitochondrial enzyme activity. While animal models have established its significance, data on its role in human adipocyte differentiation remain limited. However, studies in other cell types, such as T lymphocytes, have shown that thiamine supports differentiation and TGF- β production, suggesting a broader role in immune development. In a clinical study involving overweight or obese individuals, a high-protein diet combined with 2.8 mg/day thiamine over 16 weeks resulted in significant weight loss, despite unchanged erythrocyte thiamine levels. Based on the results, it can be concluded that optimizing extracellular thiamine availability may promote the differentiation of thermogenic adipocytes, which could be particularly important for future transplantation-based therapies. Regular thiamine supplementation could provide a safe and cost-effective approach for the prevention and treatment of obesity and associated metabolic diseases. Overall, these findings reinforce the potential of thiamine as a dietary supplement for enhancing thermogenesis and combating obesity.

6. SUMMARY

BAT plays an important role in non-shivering thermogenesis, primarily through the activity of UCP1. Located in the inner mitochondrial membrane, UCP1 enables the release of energy as heat by uncoupling oxidative phosphorylation from ATP synthesis. In humans, especially in the cervical region, BAT contains both classical brown and inducible beige adipocytes, which are metabolically active and represent potential targets for the treatment of obesity and related metabolic disorders. As a result, strategies aimed at stimulating the activation and thermogenic function of these adipocytes have attracted increasing research interest.

In this study, we investigated how extracellular thiamine influences the differentiation and thermogenic function of human adipocytes. Primary adipogenic progenitor cells were isolated *ex vivo* from SuC and DeN fat depots and differentiated for 14 days in the presence of increasing concentrations of thiamine to examine dose-dependent effects. We found that higher thiamine levels during differentiation significantly increased the expression of SLC transporters ThT1 and ThT2 at both mRNA and protein levels, particularly in adipocytes derived from DeN tissue. Functional assays showed that thiamine enhanced mitochondrial respiration, including basal oxygen consumption, db-cAMP-stimulated respiration, and proton leak, all of which suggest improved thermogenic capacity. Thiamine also supported mitochondrial biogenesis and dose-dependently increased the expression of key thermogenic genes such as *UCP1*, *PGC1 α* , and *CKMT2* in both SuC and DeN adipocytes.

We also used the human SGBS cell line, a widely accepted model for studying adipocyte browning and mitochondrial function. In this system, we examined how thiamine availability — either by supplementation or transporter inhibition — affects thermogenic activation during adrenergic stimulation. Blocking ThT1 and ThT2 reduced the db-cAMP-induced expression of UCP1 and PGC1 α , as well as proton leak respiration. In contrast, thiamine supplementation enhanced thermogenic gene expression and increased transporter expression in response to db-cAMP, indicating a potential positive feedback mechanism. Interestingly, thiamine also boosted mitochondrial respiration even without adrenergic stimulation, suggesting a role in maintaining basal mitochondrial function. Moreover, it increased the expression of respiratory chain complexes, indicating enhanced mitochondrial biogenesis and efficiency.

Overall, our findings suggest that thiamine contributes not only as a coenzyme in cellular metabolism, but also actively regulates thermogenic programming in human adipocytes. Thiamine availability shapes both transcriptional and functional responses to adrenergic signals, opening new possibilities for dietary or pharmacological approaches to enhance

thermogenesis and energy expenditure. Given that thiamine deficiency is common among individuals with obesity and type 2 DM, our results support the idea that long-term thiamine supplementation could serve as a simple and accessible strategy to promote adipose tissue browning and improve metabolic health.

7. NEW RESULTS

- Extracellular thiamine concentration modulates the thermogenic capacity of human adipocytes derived from the cervical region.
- Thiamine supplementation during adipogenesis promotes thiamine transporter expression.
- Thiamine availability regulates thermogenic capacity in a concentration-dependent manner during adipogenesis.
- Thiamine availability during differentiation influences mitochondrial biogenesis in cervical-derived adipocytes.
- Thiamine supplementation during differentiation enhances thermogenic gene expression in a dose-dependent manner.
- High thiamine levels promote both coupled and uncoupled respiration during adipocyte differentiation.
- Elevated thiamine levels during differentiation promote thermogenic gene expression.
- Excess thiamine enhances brown/beige marker and thermogenic gene expression in cervical-derived adipocytes.
- Thiamine transporter expression is induced by adrenergic stimulation and excess thiamine in differentiated SGBS adipocytes.
- Activation of thiamine transporters by dibutyryl-cAMP is attenuated in the presence of transporter inhibitors.
- Inhibition of thiamine transporters reduces both coupled and uncoupled respiration in differentiated SGBS adipocytes.
- Inhibition of thiamine transporters after adipocyte differentiation reduces mitochondrial biogenesis in SGBS adipocytes differentiated either regularly or towards a thermogenic phenotype.
- Inhibition of thiamine transporters reduces thermogenic marker expression in differentiated SGBS adipocytes.
- Inhibiting thiamine transporters suppresses expression of thermogenic genes in differentiated SGBS adipocytes.
- Excess thiamine enhances coupled and uncoupled respiration after adipogenesis.
- High thiamine levels during differentiation enhance mitochondrial biogenesis in SGBS adipocytes.

- Elevated thiamine levels after differentiation enhance the expression of thermogenic markers in white-differentiated adipocytes.
- Abundant thiamine increases expression of beige/brown markers and thermogenic genes in differentiated SGBS adipocytes.



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List of publications related to the dissertation

1. **Vinnai, B. Á.**, Arianti, R., Fischer-Posovszky, P., Wabitsch, M., Fésüs, L., Kristóf, E.: The importance of thiamine availability in the thermogenic competency of human adipocytes. *Mol. Cell. Endocrinol.* 599, 1-14, 2025.
DOI: <http://dx.doi.org/10.1016/j.mce.2025.112483>
IF: 3.8 (2023)
2. **Vinnai, B. Á.**, Arianti, R., Györy, F., Bacsó, Z., Fésüs, L., Kristóf, E.: Extracellular thiamine concentration influences thermogenic competency of differentiating neck area-derived human adipocytes. *Front. Nutr.* 10, 1-15, 2023.
DOI: <http://dx.doi.org/10.3389/fnut.2023.1207394>
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List of other publications

3. Arianti, R., **Vinnai, B. Á.**, Alrifai, R., Karadsheh, G., Qais, A. K. Y., Póliska, S., Györy, F., Fésüs, L., Kristóf, E.: Upregulation of inhibitor of DNA binding 1 and 3 is important for efficient thermogenic response in human adipocytes. *Sci. Rep.* 14 (1), 1-13, 2024.
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4. Arianti, R., **Vinnai, B. Á.**, Györy, F., Guba, A., Csósz, É., Kristóf, E., Fésüs, L.: Availability of abundant thiamine determines efficiency of thermogenic activation in human neck area derived adipocytes. *J. Nutr. Biochem.* 119, 1-13, 2023.
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FEBS Lett. 595 (16), 2085-2098, 2021.
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