

DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

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

by

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1. ABBREVIATIONS

ACTB – Actin Beta

ADCY – White-like adipocyte

ATP – Adenosine triphosphate

B-ADCY – “Beige”-like adipocyte

BAT – Brown adipose tissue

BCAA – Branched-chain amino acid

BCKDH – Branched-chain α -ketoacid dehydrogenase

Ca²⁺ – Calcium

CIDEA – Cell death-inducing DFFA-like effector A

CITED1 – CBP/p300-interacting transactivator with Glu/Asp-rich tail 1

CKB – Creatine kinase B

Ct – Threshold cycle

Db-cAMP – Dibutryl-3',5'-cyclic adenosine monophosphate

DM – Diabetes mellitus

DMEM – Dulbecco's modified Eagle's medium

DeN – Deep neck

ECAR – Extracellular acidification rate

EnE – Energy expenditure

EPA – Eicosapentaenoic acid

ER – Endoplasmic reticulum

ETC – Electron transport chain

FA – Fatty acid

FADH – Flavin Adenine Dinucleotide

GAPDH – Glyceraldehyde-3-Phosphate Dehydrogenase

hASC – Human adipose-derived stromal cell

IBMX – 3-Isobutyl-1-methylxanthine

LD – Lipid droplet

NAA – N-acyl amino acid

NAD(P)H – Nicotinamide Adenine Dinucleotide (Phosphate)

NE – Norepinephrine

NST – Non-shivering thermogenesis

OCR – Oxygen consumption rate

PBS – Phosphate-Buffered Saline

PCR – Polymerase chain reaction
PDH – Pyruvate dehydrogenase
PGC1A – Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha
PM20D1 – Peptidase M20 Domain Containing 1
PPAR γ – Peroxisome Proliferator-Activated Receptor Gamma
PRDM16 – PR/SET Domain 16
PrL – Proton leak
RFC – Reduced folate carrier
RT-qPCR – Reverse transcription-coupled quantitative polymerase chain reaction
RyR – Ryanodine receptor
SuC – Subcutaneous
SDS – Sodium dodecyl sulphate
SERCA – Sarco/endoplasmic reticulum calcium ATPase
SGBS – Simpson-Golabi-Behmel Syndrome
SLC – Solute carrier
SLC19A1/2/3 – Solute Carrier Family 19 Member 1/2/3
SLC25A19/44 – Solute Carrier Family 25 Member 19/44
SR – Sarcoplasmic reticulum
 β 3-AR – β 3-adrenergic receptor
TAG – Triacylglycerol
ThT – Thiamine transporter
ThTI – Thiamine transporter inhibitor
TMP – Thiamine monophosphate
TNAP – Tissue non-specific alkaline phosphatase
TPK1 – Thiamine pyrophosphokinase-1
TPP – Thiamine pyrophosphate
TRPV1 – Transient receptor potential vanilloid type 1
TTBS – Tween-20 Tris-buffered saline
TTP – Thiamine triphosphate
UCP1 – Uncoupling protein 1
WAT – White adipose tissue
WKS – Wernicke-Korsakoff syndrome
 α -KGDH – α -ketoglutarate dehydrogenase

2. KEYWORDS

Human brown and “beige” fat cells, SGBS adipocytes, deep cervical region, subcutaneous, non-shivering thermogenesis, adrenergic activation, expression of thermogenesis-related genes, UCP1 (uncoupling protein-1), solute carrier transporter family, thiamine, thiamine transporter protein, fedratinib, amprolium.

Humán barna/bézs adipociták, SGBS adipociták, mély nyaki régió, szubkután, nem-didergéses termogenezis, adrenerg stimulus, termogenikus génexpresszió, szétkapcsoló fehérje 1, oldott anyag transzporter család, tiamin, tiamin transzporter, fedratinib, amprolium.

3. INTRODUCTION

Based on the most recent report from the World Health Organization, in 2024, approximately one in eight individuals across the globe were affected by obesity. For 35 years, the global prevalence of obesity has ascended to more than twice its former level in adults and to roughly four times its previous level in adolescents. Recently, an estimated 2.5 billion adults (older than 18 years) were classified as overweight, of whom about 890 million fulfilled the diagnostic threshold for obesity. Additionally, approximately 37 million children aged less than five years were identified as being overweight [World Health Organization, 2024]. Obesity raises the likelihood of the incidence of type 2 diabetes mellitus (DM), various cardiovascular disorders, malignancies, and metabolic syndrome. While genetic, neurological, and endocrine disorders play a role, major drivers of obesity include decreased physical activity and frequent access to high-fat, high-carbohydrate, and fast foods [Hruby *et al.*, 2016; Hurt *et al.*, 2010]. During the last ten years, research attention has increasingly focused on the mechanisms regulating energy homeostasis in mammals, especially those involving adipose tissue. Adipocytes can be grouped into three main types — white, brown, and “beige” — each serving distinct roles based on their structure and function in energy management. White adipocytes are primarily designed for energy preservation, containing an expanded unilocular lipid droplet (LD) and relatively few mitochondria (**Figure 1**) [Ziqubu *et al.*, 2023]. In contrast, brown adipocytes contain numerous mitochondria and small, multilocular LDs, which, along with high Uncoupling Protein 1 (UCP1) expression, drive thermogenesis and facilitate energy expenditure (EnE). Likewise, “beige” adipocytes, located within white adipose tissue (WAT), can develop thermogenic capabilities under certain conditions (**Figure 1**) [Ziqubu *et al.*, 2023].

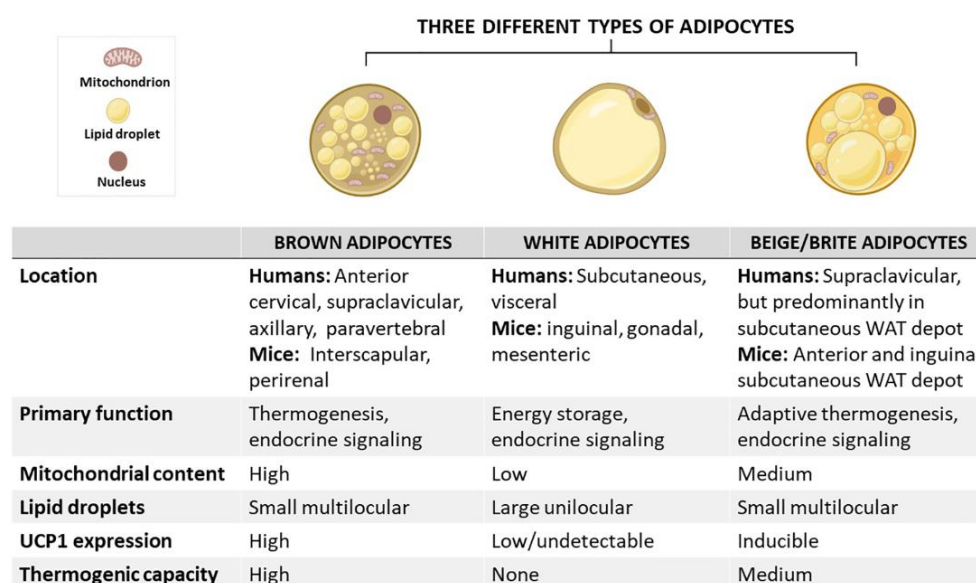


Figure 1. Characteristics and functions of adipocytes [Ziqubu *et al.*, 2023]

Thermogenic adipocytes require essential metabolites for generating heat, as they activate processes that lead to increased EnE. Among these processes, the UCP1 in brown and “beige” fat cells plays a pivotal role by dissipating the proton gradient in mitochondria, effectively converting stored energy into heat rather than adenosine triphosphate (ATP) synthesis. These adipocytes primarily utilize glucose, free fatty acids (FAs), branched-chain amino acids (BCAAs), and other molecules as substrates. This thermogenic mechanism, facilitated by UCP1, has become an appealing target in anti-obesity strategies, as enhancing the function of these adipocytes could potentially increase metabolic rate and aid in combating obesity [Xue *et al.*, 2022].

Members of the solute carrier (SLC) transporter family are essential for various biological functions, primarily by transporting vital molecules into cells. However, the role of SLCs in brown and “beige” adipocytes during thermogenic activation remains poorly understood, especially in humans. In my PhD projects, the significant role of thiamine transporters (ThTs) in driving the thermogenic response of human adipocytes following stimulation by an adrenergic cue is highlighted. Blocking these transporter proteins in the presence of dibutyryl-cAMP (db-cAMP) resulted in reduced heat generation, as indicated by decreased mitochondrial proton leak (PrL) respiration. Additionally, blocking thiamine influx by ThT inhibitors (ThTIs), namely fedratinib and amprolium, resulted in a significant loss of the db-cAMP-triggered increase in thermogenic marker expression. Moreover, administering sufficient or increased levels of thiamine — either during adipocyte differentiation through long-term supplementation or at the end of the differentiation process alongside db-cAMP treatment — was associated with elevated mRNA and protein levels of major thermogenic markers, accompanied by an elevation in PrL respiration. Our results suggest a promising approach for obesity treatment by targeting the regulatory mechanisms of thiamine utilization to boost heat production in thermogenically active adipocytes in people with obesity.

4. THEORETICAL BACKGROUND

4.1. White adipose tissue

WAT represents the most prevalent form of adipose tissue that is critically involved in storing surplus energy as triacylglycerols (TAGs), thereby contributing to the regulation of whole-body energy homeostasis [Cypess *et al.*, 2022]. The main WAT depots can be categorized according to their anatomical distribution as visceral or subcutaneous (SuC) depots. Visceral WAT of humans is localized within the peritoneal cavity, particularly in the mesenteral and omental regions [Chusyd *et al.*, 2016]. SuC WAT can be found directly under the skin, concentrated mainly within the gluteofemoral and abdominal depots. It accounts for approximately $\geq 80\%$ of an individual's overall fat mass [Karastergiou and Fried, 2017]. In rodents, WAT depots comprise visceral (mesenteric, gonadal, and perirenal) as well as SuC (axillary and inguinal) stores. An important distinction is that in rodents, venous blood drained from gonadal WAT flows into the systemic circulation, while in humans, visceral WAT is drained into the portal vein [Rytka *et al.*, 2011].

Adipocytes of WAT are spherical in shape, with their size primarily determined by one prominent LD occupying the majority of the cell volume and are characterized by relatively low mitochondrial content (**Figure 2, left panel**). The principal function of these adipocytes is to preserve and mobilize energy according to fluctuations in whole-body energy status. These functions are carried out by lipolysis (FA release) and lipogenesis (FA uptake/synthesis) [Sakers *et al.*, 2022]. In addition to maintaining energy balance, WAT is also recognized as an endocrine organ because it secretes several hormones and substances, collectively known as adipokines. These adipokines are able to regulate metabolism by affecting insulin signaling (e.g. leptin and adiponektin) and promoting glucose intolerance (e.g. resistin and retinol-binding protein 4) or inflammatory processes (e.g. IL-1 β , IL-6, IL-8, and TNF- α) [Funcke and Scherer, 2019].

4.2. From brown to “beige”: the spectrum of thermogenic adipocytes

4.2.1. Localization of brown adipose tissue (BAT) and “beige” adipocytes in humans

Brown and “beige” adipocytes, although they account for a much smaller proportion of total fat mass, exert a significant metabolic effect on the body as a whole, through their thermogenic capacity. Activated thermogenic adipocytes are able to raise total-body EnE by up to 100% in rodents and by approximately 40–80% in humans [Ouellet *et al.*, 2012; Angueira *et al.*, 2020]. In contrast to white adipocytes (**Figure 2, left panel**), brown (**Figure 2, right panel**) and “beige” (**Figure 2, middle panel**) adipocytes are polygonal cells of variable diameter which

are recognized by the presence of multiple and small LDs and a high density of mitochondria along with robust expression of UCP1, their major molecular marker protein.

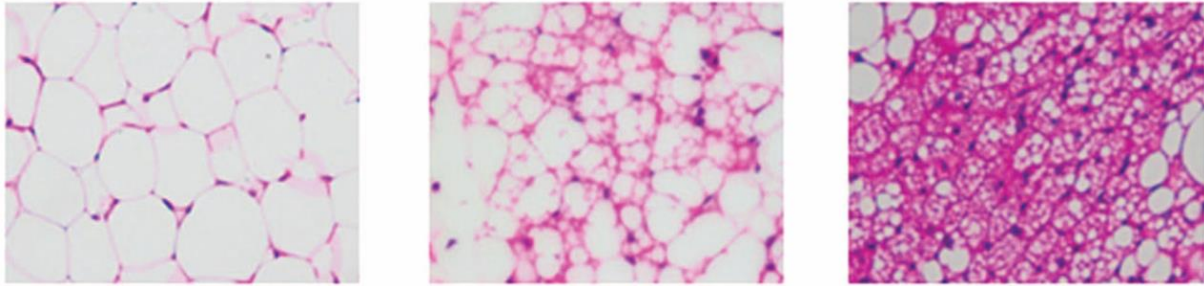


Figure 2. Morphology of white adipocytes in WAT (left panel), “beige” adipocytes scattered in WAT (middle panel), and adipocytes of BAT (right panel) [Kwok et al., 2016]

UCP1 plays a pivotal role in non-shivering thermogenesis (NST) by uncoupling nutrient catabolism from ATP synthesis. This function is achieved primarily through its ability to disrupt the proton motive force across the inner membrane of the mitochondria [Cannon and Nedergaard, 2004].

Brown adipocytes arise within dedicated BAT depots that are established prenatally. In human newborns, these depots are present in the cervical, axillary, periadrenal, and perirenal regions. Later, the decay of brown adipocytes can be observed, therefore, for a long period of time, BAT was thought to be typically absent in adults [Wu *et al.*, 2013]. In contrast, “beige” adipocytes emerge within WAT, predominantly as an adaptation to a cold stimulus [Ikeda *et al.*, 2018]. Although BAT was previously believed to be largely restricted to newborns and negligible or absent in adults, independent studies utilizing positron emission tomography (PET) demonstrated more than a decade ago that adults retain variable quantities of BAT in the cervical and supraclavicular regions, exhibiting high glucose uptake [Virtanen *et al.*, 2009; Cypess *et al.*, 2009]. Follow up biopsy analyses from this region have revealed adipocytes with numerous LDs. Advancements in PET-computed tomography have allowed for more precise localization of these thermogenic depots, helping researchers to map the distribution of BAT throughout the body and estimate its ability to produce heat. These thermogenic reservoirs are found at multiple anatomical sites, including the cervical, axillary, supraclavicular, paraspinal, mediastinal, and abdominal regions (**Figure 3**) [Leitner *et al.*, 2017].

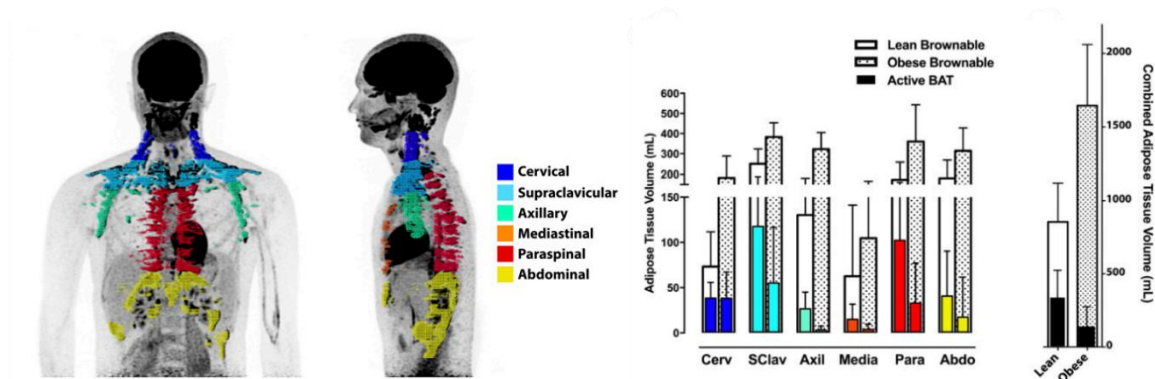


Figure 3. The distribution of BAT and “beige” adipocytes in humans (left panel) and the quantity of active and recruitable BAT in specific depots in lean subjects compared with individuals with obesity (right panel). BAT was distributed across six distinct anatomical depots, with the supraclavicular depot containing the largest proportion of total BAT volume. [Leitner et al., 2017]

4.2.2. The development of thermogenic adipocytes

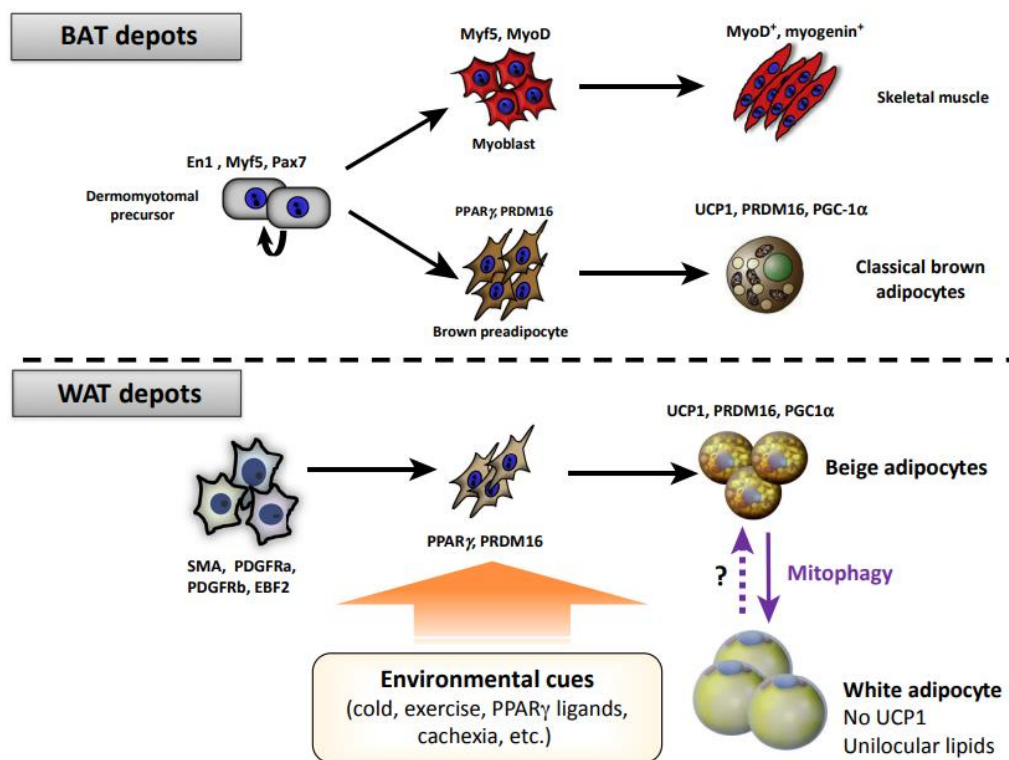


Figure 4. Developmental pathways of thermogenic adipocytes.

The figure illustrates the developmental lineage of classical brown adipocytes and the inducible nature of “beige” adipocytes in adult white adipose tissue. While brown adipocytes arise during embryogenesis from specific myogenic precursors, “beige” adipocytes are recruited in response to environmental stimuli and can be reverted to a white adipocyte phenotype upon stimulus withdrawal.

En1: Engrailed 1, Myf5: Myogenic Factor 5, Pax7: Paired Box Domain 7, MyoD: Myoblast Determination Protein 1, PPAR γ : Peroxisome Proliferator-Activated Receptor Gamma, PRDM16: PR/SET Domain 16, PGC-1 α : Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Alpha, SMA: Smooth Muscle Actin, PDGFR α /b: Platelet Derived Growth Factor Receptor Alpha/Beta, EBF2: Early B Cell Factor 2 [Ikeda et al., 2018]

During embryogenesis, the development of BAT precedes that of WAT. This developmental sequence supports the notion that BAT is primarily responsible for NST, which is crucial to maintain the constant core body temperature in neonates after birth [Sidossis and Kajimura, 2015]. Classical brown adipocytes within BAT depots originate from dermomyotomal precursors, which express key transcription factors influencing development, such as Paired Box Domain 7, Engrailed 1, and Myogenic Factor 5. According to the expression pattern of genes characteristic of particular cell lineages, these precursors can differentiate into two distinct cell lineages. One pathway leads to the formation of brown preadipocytes that express PPAR γ and PRDM16. These adipocyte precursors subsequently give rise to classical brown adipocytes, characterized by the expression of UCP1, PRDM16, and PGC1A. The alternative lineage involves the differentiation of dermomyotomal precursors into myoblasts, which subsequently develop into skeletal muscle cells (**Figure 4, top panel**).

In contrast, “beige” adipocytes, dispersed within WAT, follow a distinct developmental pathway. They arise from progenitors that express Smooth Muscle Actin, Platelet Derived Growth Factor Receptor α and β , and Early B Cell Factor 2. The development of these adipocytes is significantly influenced by various environmental cues, such as cold exposure, increased physical activity, the presence of PPAR γ ligands, and extensive tissue damage. In response to these external factors, precursor cells can differentiate into “beige” adipocytes. Upon the cessation of external stimuli, “beige” adipocytes undergo a selective autophagy process called mitophagy, transforming into inactive “beige” adipocytes with white morphology. These cells are characterized by low UCP1 expression and unilocular lipid storage (**Figure 4, bottom panel**) [Ikeda et al., 2018; Altshuler-Keylin et al., 2016].

4.3. Thermogenesis in brown and “beige” adipocytes: UCP1-dependent and UCP1-independent pathways

“Beige” adipocytes, as well as adipocytes of the BAT, critically contribute to the process of heat production, which is majorly achieved through their mitochondrial uncoupling ability. By being able to express genes contributing to thermogenesis, they contribute to the energy metabolism of the mammalian body through NST [Magro and Dias, 2024]. Heat-generating

processes can occur via mechanisms that either require UCP1-dependent or UCP1-independent ways.

In thermogenic adipocytes, both cold- and diet-induced NST are conducted primarily by UCP1. During the 1970s, researchers identified UCP1 within the mitochondria of mammalian BAT, linking the tissue's thermogenic function to the expression of this protein [Ricquier *et al.*, 1976]. UCP1, belonging to the SLC25 carrier family, is located in the inner membrane of the mitochondria and is responsible for heat production because of its ability to uncouple mitochondrial respiration [Fedorenko *et al.*, 2012]. The thermogenic activity of adipocytes is primarily governed by adrenergic signaling. Adipose tissue, particularly BAT, is characterized by dense sympathetic neural innervation [Morrison, 2016]. In response to a cold stimulus, sympathetic nerves release the catecholamine neurotransmitter norepinephrine (NE). This results in the activation of the β_3 -adrenergic receptor (AR) –cAMP–Protein Kinase A signaling cascade in adipocytes. This amplified pathway triggers lipolysis (thereby promoting UCP1-dependent NST) and drives the transcription of genes that contribute to heat-generating processes in brown and “beige” adipocytes [Bertholet and Kirichok, 2017; Villarroya *et al.*, 2017]. Upon its activation — which is allosterically induced by long-chain FAs, the primary oxidative fuel of brown adipocytes — UCP1 facilitates PrL across the inner mitochondrial membrane, thereby dissipating the proton motive force generated by electron transfer. As a result, the electrochemical energy gained by proton pumping associated with the electron transport chain (ETC) is dissipated as heat instead of ATP production [Fedorenko *et al.*, 2012].

Upon activation, UCP1 uncouples mitochondrial oxygen consumption from ATP synthesis, resulting in heat production. The associated energy demand is met by the oxidation of metabolic substrates, primarily FAs and glucose. Key fuels include glucose, FAs, and amino acids, which fuel and maintain flux of the tricarboxylic acid cycle in order to yield NADH and FADH₂ for the ETC. In response to cold, BAT in humans and rodents additionally catabolizes BCAAs [Yoneshiro *et al.*, 2019]. These are transported into mitochondria via a BCAA transporter encoded by *SLC25A44* and oxidized by the enzyme complex, branched-chain α -ketoacid dehydrogenase (BCKDH), thereby producing extra NADH. Furthermore, succinate is actively imported into BAT. Its oxidation boosts citric acid cycle activity and produces reactive oxygen species potentiating UCP1 function [Mills *et al.*, 2018; Chouchani *et al.*, 2016]. Additionally, glucose taken up by BAT during cold exposure is metabolized to pyruvate, which is then converted to acetyl-CoA via pyruvate dehydrogenase (PDH) to fuel further metabolic processes [Panic *et al.*, 2019]. These mechanisms underscore the metabolic versatility of BAT in sustaining UCP1-driven NST (**Figure 5A**). The literature also highlights the presence of

thermogenic mechanisms that function independently of UCP1. Evidence for these alternative pathways emerged from observations that mice lacking UCP1 were capable of adapting to cold conditions when the surrounding temperature gradually declined [Ukropec *et al.*, 2006]. These processes include the futile cycling of calcium (Ca^{2+}), creatine/creatine-phosphate, and TAGs (Figure 5B) [Kazak *et al.*, 2015; Ikeda *et al.*, 2017; Chouchani and Kajimura, 2019].

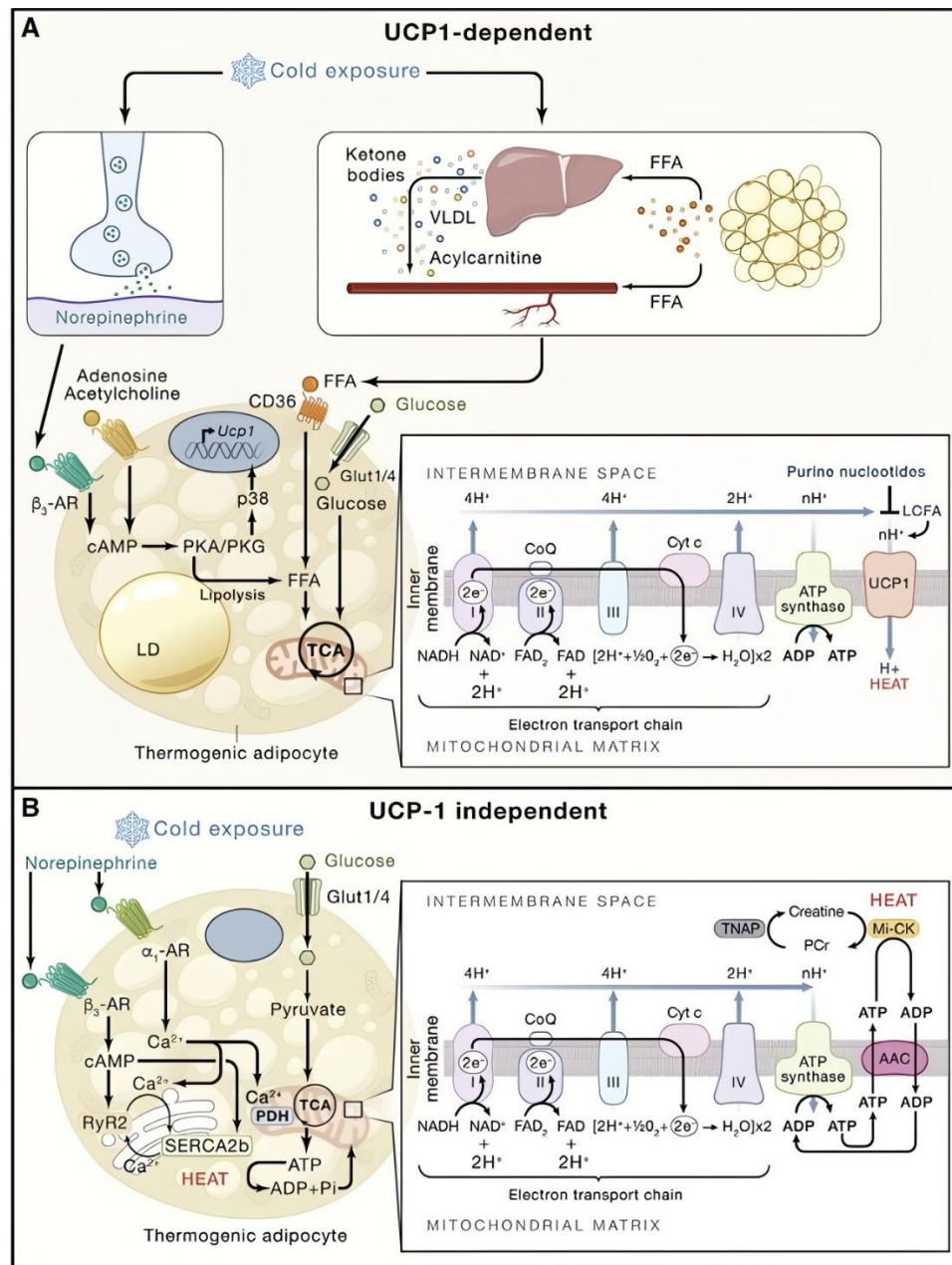


Figure 5. Metabolic flexibility in thermogenesis performed by UCP1-dependent (A) and UCP1-independent mechanisms (B).

The figure summarizes both UCP1-dependent and UCP1-independent pathways of non-shivering thermogenesis in brown/"beige" adipocytes. While UCP1-dependent pathways rely on mitochondrial uncoupling via adrenergic signaling and increased fuel oxidation, UCP1-

independent pathways utilize energy-consuming futile cycles, such as Ca^{2+} and creatine cycling, to generate heat during cold exposure.

AR: Adrenergic Receptor, AAC: ATP/ADP carrier, LD: lipid droplet, Mi-CK: Mitochondrial Creatine Kinase, PCr: Phosphocreatine, PDH: Pyruvate Dehydrogenase, RyR2: Ryanodine Receptor 2, SERCA2b: Sarco/endoplasmic Reticulum Ca^{2+} ATPase 2b, TNAP: Tissue-nonspecific Alkaline Phosphatase, UCP1: Uncoupling Protein 1 [Sakers et al., 2022]

Creatine is a key contributor to UCP1-independent NST, as evidenced by various mouse studies [Hepler *et al.*, 2022; Rahbani *et al.*, 2021; Rahbani *et al.*, 2022; Sun *et al.*, 2021]. The prevailing concept of creatine-driven NST relies on the futile creatine cycle, in which creatine is repeatedly phosphorylated and dephosphorylated in an ATP-dependent fashion [Kazak *et al.*, 2015; Rahbani *et al.*, 2021]. This mechanism requires two key mitochondrial proteins, creatine kinase (CK) B and tissue non-specific alkaline phosphatase (TNAP), which are essential for NST in adipocytes [Rahbani *et al.*, 2021; Rahbani *et al.*, 2022; Sun *et al.*, 2021]. In the absence of these proteins, mice exhibit expanded fat mass in response to high-fat diet and impaired glucose tolerance, further highlighting the importance of these proteins in thermogenic pathways. Loss of either CKB or TNAP impairs NE-driven heat generation in brown adipocytes, lending support to the futile creatine-cycle model, which connects CKB and TNAP activity to ATP production during NST (**Figure 6**) [Rahbani *et al.*, 2022]. Mechanistically, the futile creatine cycle contributes to NST by increasing mitochondrial ATP turnover, which in turn stimulates the ETC. Complexes I, III, and IV of the ETC pump protons (H^+ s) from the mitochondrial matrix into the intermembrane space, resulting in the proton motive force. The subsequent flow of protons into the matrix — either via UCP1 or via ATP-consuming processes such as the creatine cycle — dissipates this gradient. This mitigates the thermodynamic backpressure on the ETC, thereby promoting sustained substrate oxidation, mitochondrial respiration, and heat generation (**Figure 6**) [Kazak, 2023].

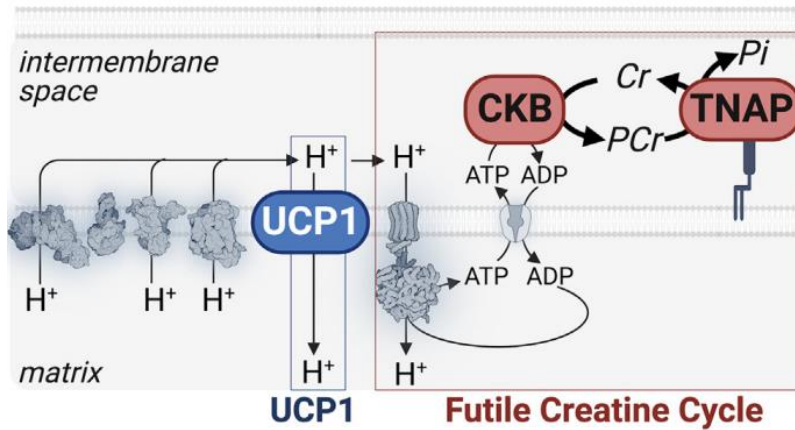


Figure 6. Heat generating mechanisms by UCP1 (left panel) and futile creatine cycle (right panel). Cr: Creatine, PCr: Phosphocreatine, CKB: Creatine Kinase B, TNAP: Tissue Non-specific Alkaline Phosphatase [Lawrence Kazak, 2023]

In striated muscle, intracellular Ca^{2+} is stored in the sarcoplasmic reticulum (SR), whereas in majority of other cells it resides in the endoplasmic reticulum (ER). Ryanodine receptors (RyRs) function as Ca^{2+} -release channels positioned in the membrane of the SR/ER. Both RyRs and the sarco/endoplasmic reticulum calcium ATPase (SERCA) are crucial for UCP1-independent NST in “beige” adipocytes. Upon a cold stimulus, NE binds to the $\alpha 1$ - and $\beta 3$ -ARs, which in turn elevate intracellular Ca^{2+} levels by stimulating SERCA2b and RyR2. SERCA2b pumps Ca^{2+} backwards to the ER and potentially into the mitochondria, where it enhances the activity of PDH phosphatase, thereby activating PDH and promoting ATP production. When UCP1 is absent, “beige” adipocytes rely on glucose as the main fuel for NST, by channeling it through glycolysis, the tricarboxylic acid cycle, and the mitochondrial ETC. This Ca^{2+} cycling process uncouples Ca^{2+} transport from ATP production, contributing to heat generation in a noncanonical manner [Denton, 2009; Ikeda *et al.*, 2017]. Tajima *et al.* observed recently that enhancing Ca^{2+} influx elevated NST in both wild-type and UCP1^{-/-} SuC adipocytes. This effect depended on the expression of *ATPase Sarcoplasmic/Endoplasmic Reticulum Ca²⁺ Transporting 2 (ATP2A2)*, the gene encoding SERCA2 [Tajima *et al.*, 2020].

Ball and Jungas also described a process in which a kind of exothermic cycle occurs in WAT by the continuous hydrolysis and re-esterification of TAGs. During this cycle, following lipolysis, TAGs are hydrolyzed to FAs and glycerol, or monoacylglycerols, or diacylglycerols, which are then re-esterified to regenerate monoacylglycerols, diacylglycerols, or TAGs, respectively. Re-esterification depends on glycerol phosphate, which can be obtained in two ways: either during glucose metabolism, with an investment of ATP, or through the activity of the enzyme glycerol kinase. In addition, this process involves the activation of FAs to FA-CoA,

which also requires ATP. Since the process is ATP-demanding, this process dissipates energy and contributes to heat production (**Figure 7, right panel**) [Ball and Jungas, 1961]. Interestingly, Cell death–inducing DFFA-like effector A (CIDEA) is a critical regulatory protein of the metabolism of adipose tissues, especially in BAT. It is primarily associated with LDs and promotes their growth, which is critical for NST by these cells. Brown adipocytes are specialized in heat production, a process that relies heavily on stored lipids. CIDEA supports the fusion of LDs, forming larger storage units that can be mobilized more efficiently for NST. Studies have shown that when CIDEA function is disrupted, LD fusion is impaired, leading to reduced heat-producing capacity of brown fat cells. This indicates that CIDEA is not only involved in lipid storage but also in regulating how energy reserves are organized and used within the cell. Interestingly, similar roles for CIDEA have been observed in other species, such as yeast, suggesting that its function is evolutionarily conserved [Barneda *et al.*, 2015].

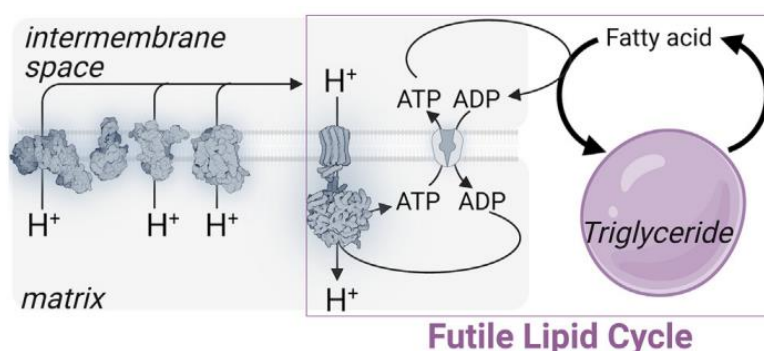


Figure 7. Heat generating mechanisms by UCP1 (left panel) and futile lipid cycle (right panel) [Lawrence Kazak, 2023]

Interestingly, Peptidase M20 Domain Containing 1 (*PM20D1*) gene encodes an enzyme released predominantly by brown and “beige” adipocytes where it makes substantial contribution to NST. The enzyme's primary function is to catalyze the conjugation of FAs, such as arachidonic acid and oleic acid, with amino acids, predominantly phenylalanine, resulting in the formation of intracellular metabolites known as N-acyl amino acids (NAAs). These metabolites directly affect mitochondrial function and NST. In mice, $UCP1^+$ adipocytes express *PM20D1* predominantly, while the expression of this enzyme is minimal in $UCP1^-$ adipocytes. Beyond adipose tissue, high *PM20D1* expression is observed in the liver and kidneys. Cold exposure upregulates *PM20D1* expression and therefore the synthesis of NAA products. Notably, NAAs bind directly to mitochondria and act as endogenous uncouplers, independently disrupting cellular respiration and mitochondrial ATP synthesis. Through this mechanism, they

promote energy dissipation as heat, essentially contributing to the sustained metabolic and thermal homeostasis. Studies in mice demonstrate that increased circulating PM20D1 levels elevate NAA production, enhancing glucose homeostasis and EnE, thereby mitigating obesity. Conversely, *PM20D1* knockout mice exhibit greater susceptibility to insulin resistance and glucose intolerance compared to wild-type counterparts (**Figure 8**) [Long *et al.*, 2016; Long *et al.*, 2018].

Another mentionable marker named CBP/p300-interacting transactivator with Glu/Asp-rich tail 1 (CITED1) is a transcriptional co-regulator showing high expression in brown and „beige” adipocytes. Studies show its expression is significantly higher in SuC fat from subjects with normal body weight compared to those with obesity, indicating a strong link with leanness. In fact, its expression negatively correlates with body mass index, indicating a role in healthy fat metabolism. *CITED1* is considered a marker of „beige” adipocytes. Functionally, CITED1 is implicated in promoting the “browning” of WAT. Its higher levels may facilitate the differentiation or stimulation of „beige” adipocytes in SuC fat. Although the mechanistic details require further investigation, CITED1 likely acts as a regulatory switch in adipocyte differentiation and metabolic activation [Al-Amrani *et al.*, 2018].

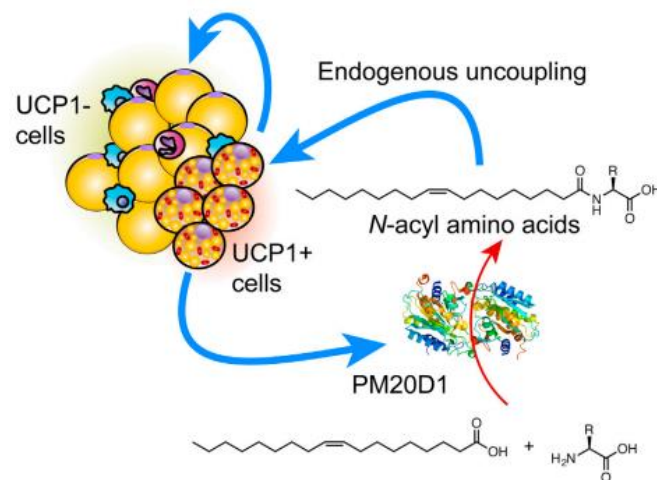


Figure 8. The PM20D1 mediated uncoupling in thermogenic adipocytes [Long *et al.*, 2016]

Mitochondrial uncoupling mechanisms are responsible for approximately 20% of total mitochondrial respiration, which supports the importance of NST in the overall EnE of the body [Rolfe and Brown, 1997].

4.4. Activators of thermogenesis

In the last decade, much research has been conducted on how to stimulate BAT activity in various ways. These include activation by sympathetic neurons (in response to cold exposure), various endocrine factors, or auto/paracrine substances produced by the body, for example as a result of physical activity or diet (**Figure 9**) [Broeders *et al.*, 2015].

Cold exposure activates BAT NST through NE binding to β -ARs, stimulating UCP1 transcription and heat production [Cannon and Nedergaard, 2004]. A sustained cold stimulus significantly enhances BAT function, increasing oxidative metabolism by 2.6-fold and promoting dietary FA uptake and redistribution [Coolbaugh *et al.*, 2019; Blondin *et al.*, 2017]. In humans, four weeks of moderate cold acclimation ($\sim 18^{\circ}\text{C}$) was observed to expand the metabolically active BAT mass by 45% and to boost oxidative metabolism by 182% [Blondin *et al.*, 2017]. Even brief cold exposure (e.g. 16°C for 2 hours daily) can raise glucose uptake in BAT by 12-fold, exceeding insulin's effect and improving EnE through increased perfusion [van Marken Lichtenbelt *et al.*, 2009].

The impact of habitual exercise on human BAT is still unclear, because endurance-trained athletes do not exhibit recruitment of brown or „beige” adipocytes, in contrast to what is observed in animal studies [Vosselman *et al.*, 2015; De Matteis *et al.*, 2013]. However, other studies indicate that exercise induces an oxidative phenotype in BAT, increasing its mass, reducing weight gain, and altering metabolic markers, without promoting WAT browning or significantly improving metabolic health [Aldiss *et al.*, 2020; Otero-Díaz *et al.*, 2018]. Physical exercise triggers UCP1 expression in WAT, driven by muscle-derived signals like IL-6 and myokines, such as meteorin-like, which enhance mitochondrial biogenesis in BAT [Knudsen *et al.*, 2014; Amano *et al.*, 2020]. Irisin is also a well-characterized myokine, which is released during skeletal muscle contraction and stimulates mitochondrial biogenesis and the expression of UCP1 in WAT, enhancing heat production [Kozak and Young, 2012]. Exercise also fosters heart-BAT endocrine communication, with BAT-derived vesicles protecting against myocardial ischemia/reperfusion injury, underscoring its potential cardioprotective role [Zhao *et al.*, 2022].

Capsinoids, omega-3 FAs, and caffeine also play significant roles in enhancing NST and promoting metabolic health. Capsinoids, which are analogues of capsaicin, activate BAT by stimulating transient receptor potential vanilloid 1 (TRPV1) receptor in the gastrointestinal tract, thereby increasing daily EnE by roughly 120 kcal in healthy individuals [Ono *et al.*, 2011]. Long-term capsinoid consumption has been demonstrated to improve BAT vascular density and resting EnE, even in individuals with low or negligible BAT amounts. While capsinoid intake

results in smaller increases in EnE compared to cold exposure, it still enhances FA oxidation, alleviates insulin resistance, and improves lipid profiles [Fuse *et al.*, 2020; Sun *et al.*, 2018]. Capsaicin, another TRPV1 agonist, can induce body weight reductions by up to 8% through the activation of BAT and WAT NST, alongside the upregulation of *Sirtuin 1* expression, helping to combat diet-induced obesity [Joo *et al.*, 2010; Baskaran *et al.*, 2017]. Omega-3 FAs, such as docosahexaenoic acid and eicosapentaenoic acid (EPA), also facilitate NST by activating TRPV1 and promoting UCP1-dependent mechanisms, which contribute to reduced fat accumulation, enhanced mitochondrial biogenesis, and increased BAT activity [Kim *et al.*, 2015]. EPA, in particular, has been found to be more effective than combined EPA and docosahexaenoic acid supplementation in activating BAT and improving lipid metabolism [Bargut *et al.*, 2019]. Furthermore, omega-3 FAs reduce inflammation, enhance FA oxidation, and lower leptin levels in overweight individuals [LeMieux *et al.*, 2015; Soni *et al.*, 2019; Huerta *et al.*, 2015]. Similarly, caffeine was found to acutely enhance BAT NST, with effects lasting up to 60 minutes post-consumption. By activating UCP1 and critical regulators of BAT and WAT browning, such as PRDM16 and PGC1A, caffeine promotes NST and fat metabolism, even with low doses [Pérez *et al.*, 2021; Velickovic *et al.*, 2019].

β 3-AR agonists, such as mirabegron, have emerged as potential agents for enhancing NST and promoting weight loss. Mirabegron, commonly prescribed for hyperactive bladder, has been demonstrated to enhance metabolic activity in BAT, raising resting EnE by approximately 203 kcal per day and facilitating glucose uptake and lipolysis captured from WAT [Cypess *et al.*, 2015]. Chronic use of mirabegron may lead to significant weight loss, with estimates suggesting a reduction of around 5 kg within the first year. Further research has demonstrated that β 3-AR agonists increase BAT NST, improve overall EnE, and beneficially affect the metabolism of lipids and carbohydrates in both animal models and human studies [Ghorbani *et al.*, 1997; O'Mara *et al.*, 2020; Nahon *et al.*, 2020]. While cold stimulus can similarly activate BAT and influence lipid levels, mirabegron does not affect serum lipids, making it a unique alternative for stimulating NST [Nahon *et al.*, 2020]. Additionally, glucagon-like peptide-1 receptor agonists, such as exenatide and liraglutide, have been demonstrated to activate BAT NST, promote weight loss, and increase EnE through central nervous system signaling and UCP1 expression in both types of adipose tissue [Beiroa *et al.*, 2014; Janssen *et al.*, 2020]. Mineralocorticoid receptor antagonists, including spironolactone, also promote BAT activation and WAT browning, with studies indicating increased EnE and potential therapeutic benefits against excess adiposity [Armani *et al.*, 2021; Marzolla *et al.*, 2021; Thuzar *et al.*, 2019].

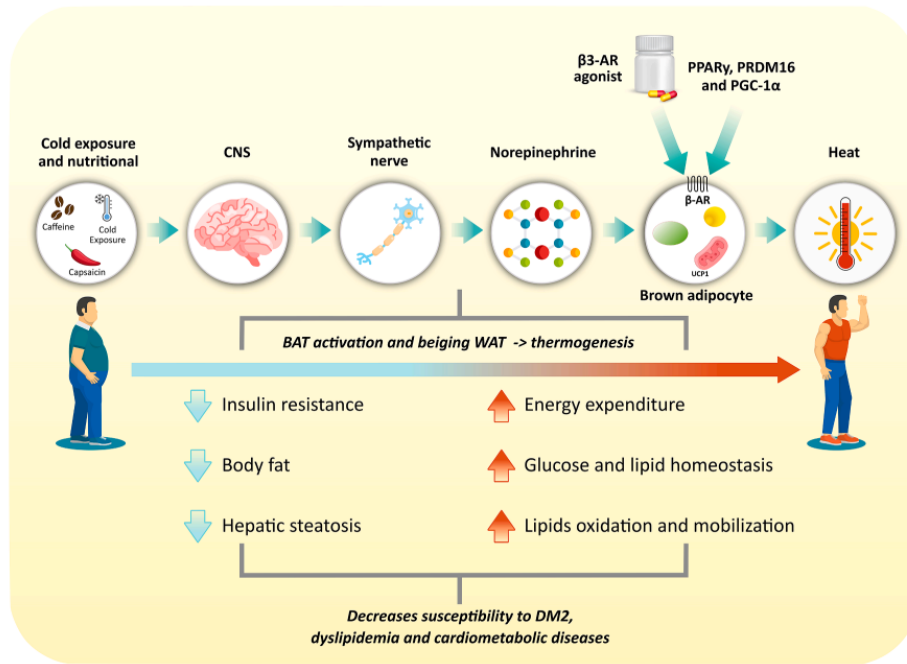


Figure 9. Different stimulators of BAT thermogenesis. CNS: Central nervous system, DM2: Type 2 diabetes mellitus [Magro and Dias, 2024]

4.5. Simpson-Golabi-Behmel Syndrome (SGBS) cells - a widely applicable experimental model of human adipocytes

In vitro adipocyte models are widely used to investigate how adipogenesis and adipokine secretion are regulated, which overall affect the physiological insulin response and the proper metabolic processes of the body. To date, a variety of rodent and human adipose tissue-derived stromal cells (hASCs) are available, providing the opportunity to study both adipogenesis and the metabolic and endocrine functions of adipocytes. A limitation of these models is that the cells undergo a cellular aging process which provides a limited source of material. There are many available adipocyte models, but they are either transformed or derived from tumorigenic individuals, which is another factor that can affect the results of the experiments. One of the most commonly applied human cell models today is the so-called SGBS cells, which have an increased proliferation and differentiation capacity [Tews *et al.*, 2022].

SGBS cells were originally described in 2001, when Wabitsch and co-workers studied a human preadipocyte line derived from SuC WAT of an infant with SGBS, a rare X-linked condition marked by excessive growth before and after birth. Phenotypic features of the syndrome include fetal overgrowth, widespread hemangiomatosis after birth, a prominent forehead, a short, broad upturned nose, six lumbar vertebrae, postaxial hexadactyly of both hands and the right foot, as well as hydramnios [Wabitsch *et al.*, 2001; Poetke *et al.*, 2002]. They demonstrated that SGBS cells are capable of adipocyte differentiation for at least 50

generations. In addition to their enhanced proliferation and differentiation capacity, they are able to stably express adipocyte-specific genes, and their glucose transport, lipogenesis, and lipolysis processes do not differ from human primary adipocytes.

The augmented proliferation may presumably result from an imbalance between factors that promote and restrain cell growth, leading to dysregulated cell cycle and apoptosis [Wabitsch *et al.*, 2001; Li *et al.*, 1998; Gonzalez *et al.*, 1998]. This may be due to point mutations or deletions in the *glypican-3* gene at Xq26, which encodes a proteoglycan located on the cell surface and involved in the regulation of organ growth [Lindsay *et al.*, 1997; Weksberg and Templeton, 1996; Song *et al.*, 1997; Tenorio *et al.*, 2014]. Interestingly, it was observed that SGBS cells show a more pronounced upregulation of adipogenic genes during adipocyte differentiation; nonetheless, their metabolic profile differs from that of white adipocytes of SuC origin. Expression levels of genes implicated in adipocyte differentiation, such as adiponektin, leptin, PPAR γ , and CCAAT/enhancer-binding proteins, were 10–15-fold more pronounced in SGBS cells than in primary human SuC white adipocytes [Yeo *et al.*, 2017].

4.6. Thiamine metabolism

Vitamin B1, also referred to as thiamine, was the first B-vitamin to be isolated and characterized, in 1912. It is known as a water-soluble vitamin, which get expelled through the urinary system therefore it has no toxic effect since it does not accumulate in the body [Ali *et al.*, 2022; Wuest, 1962]. In terms of its structure, thiamine consists of two heterocyclic rings—a thiazole and a pyrimidine — connected by a short methylene bridge. Because the thiazole ring carries an uneven positive charge, the whole molecule bears a net positive charge (**Figure 10**) [Mrowicka *et al.*, 2023].

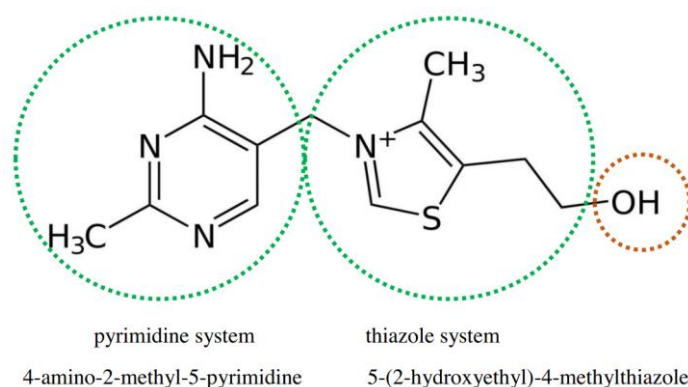


Figure 10. The structure of the thiamine molecule [Mrowicka *et al.*, 2023]

Efficient absorption requires dietary thiamine to be dephosphorylated to free thiamine by phosphatases. Thiamine that has crossed the cell membrane is phosphorylated to thiamine pyrophosphate (TPP) by thiamine pyrophosphokinase-1, whereas the subsequent

dephosphorylation of TPP to thiamine monophosphate (TMP) and free thiamine is catalyzed by prostatic acid phosphatase (**Figure 11**) [Gangolf *et al.*, 2010]. In humans, vitamin B1 is additionally present in the triphosphate (TTP) form [Mrowicka *et al.*, 2023]. **Table 1** summarizes the distribution of vitamin B1 derivatives in human whole blood and plasma.

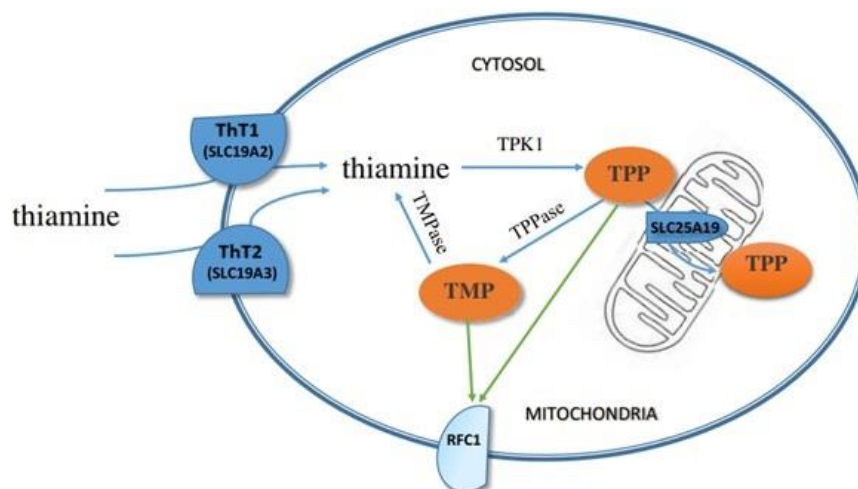


Figure 11. Transport of thiamine through thiamine membrane transporters.

Thiamine uptake into cells is primarily mediated by **ThT1 (encoded by SLC19A2)** and **ThT2 (encoded by SLC19A3)** transporters. Once internalized, thiamine is converted to its active coenzyme form, **TPP**, by **TPK1**. TPP can be dephosphorylated to **TMP** by **TPPase**, and further to free thiamine by **TMPase**, enabling intracellular recycling. **RFC1** also contributes to homeostasis by exporting phosphorylated thiamine derivatives from the cytosol.

TPK1: Thiamine Pyrophosphokinase 1, **TMP**: Thiamine Monophosphate, **TPP**: Thiamine Pyrophosphate, **RFC1**: Reduced Folate Carrier 1/SLC19A1, **TMPase**: Thiamine Monophosphatase, **TPPase**: Thiamine Pyrophosphatase, **ThT1 (SLC19A2)**: Thiamine transporter 1, **ThT2 (SLC19A3)**: Thiamine transporter 2 [Mrowicka *et al.*, 2023]

Specimen	Thiamine (nM)	TMP (nM)	TPP (nM)	TTP (nM)
Whole blood	4 ± 3 (2.4%)	10 ± 4 (6.1%)	138 ± 33 (83.6%)	13 ± 4 (7.9%)
Plasma	11 ± 3 (68.7%)	5 ± 2 (31.3%)	n.d	n.d

Table 1. Vitamin B1 derivatives in human whole blood and plasma, including their percentage contribution to total thiamine derivatives in each sample type, n.d: not detectable [Withfield *et al.*, 2018]

The most widely recognized thiamine derivative is TPP, which acts as an essential cofactor in numerous enzyme-catalyzed reactions. Such enzymes include PDH (which catalyzes the reaction that connects glycolysis to the Krebs cycle), the α -ketoglutarate dehydrogenase (α -KGDH) complex (a multicomponent enzyme complex that specifically interacts with complex I of the mitochondrial ETC), the transketolase enzyme (which contributes to pentose metabolism by providing ribose 5-phosphates for nucleotide synthesis and salvage, and NADPH for the production of FAs and mevalonate-derivates), and the BCKDH complex (which is the rate-limiting enzyme in BCAA catabolism) (**Figure 12**) [Tylicki *et al.*, 2011].

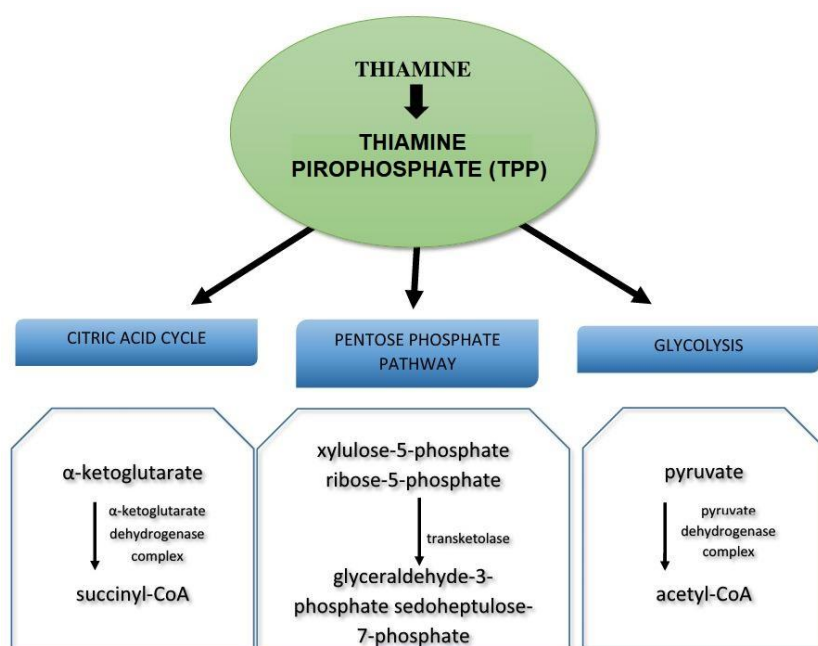


Figure 12. Biochemical pathways involving thiamine

TPP is the active cofactor form of vitamin B1. TPP plays a critical role as a coenzyme in several essential metabolic pathways, including the citric acid cycle (specifically in the α -ketoglutarate dehydrogenase complex), the pentose phosphate pathway (in transketolase), and glycolysis (in the pyruvate dehydrogenase complex) [Mrowicka *et al.*, 2023]

Dietary thiamine requirements are influenced by individual factors such as gender, age, and physical condition. In adults, the recommended daily intake is approximately 1.1–1.5 mg. Thiamine occurs in plant-derived foods predominantly as TMP (e.g. bread, nuts, whole grains, legumes, and brown rice), whereas in animal products it is present mainly as TPP, as in beef and pork [Jarosz *et al.*, 2020].

Thiamine from various sources is absorbed in smaller amounts by active transport, and in larger doses by passive diffusion. The converted, phosphorylated thiamine is stored primarily

in the central nervous system, liver, cardiac muscle, and kidneys. Because vitamin B1 has a relatively limited half-life of about 12 hours, it must be regularly replenished. If intake is insufficient, thiamine reserves of the body may become exhausted within 14-21 days. Increased thiamine turnover occurs in hypermetabolic conditions, including alcohol withdrawal, seizures, infections, DM, and rapidly progressive malignancies, particularly those affecting the gastrointestinal tract or hematopoietic system.

Thiamine deficiency may arise from a variety of causes. Insufficient intake is often the result of chronic ethanol abuse, insufficient diet, or gastric bypass surgery. Absorption disorders may also play a significant role, such as malnutrition, vomiting, neoplastic hyperplasia, or malabsorption syndrome. Thiamine loss can be further accelerated by vomiting or diarrhoea, haemodialysis, use of diuretics, and by systemic illnesses, infections or sepsis. In addition, reduced utilization of the vitamin may contribute to deficiency, such as in pregnancy, lactation, reduced enzyme activity, or hyperthyroidism [Isenberg-Grzeda *et al.*, 2012]. Prolonged omission of thiamine from the diet can lead to severe neurological complications. Thiamine is one of the most important vitamins that supports the physiological functioning of the nervous system through both enzyme-dependent and non-enzymatic mechanisms. When thiamine level is deficient, the activity of enzymes that require TPP as a cofactor becomes compromised. The resulting enzymatic dysfunction manifests first in the nervous system. For instance, thiamine depletion diminishes the activity of the PDH complex, leading to elevated lactate production and enhanced oxidative stress because of the generation of free radicals, which in turn may induce microglial alterations [Frank *et al.*, 2008; Wang and Hazell, 2010]. Moreover, thiamine deficiency has been shown to disrupt the synthesis of FAs, nucleic acids, and steroids, potentially causing demyelination of nerve fibers.

Beyond its enzymatic roles, thiamine is involved in cell differentiation, synapse formation, axonal outgrowth, and the promotion of myelin formation (myelinogenesis). It also contributes to the biosynthesis of neurotransmitters, *e.g.* acetylcholine or serotonin; in the case of serotonin, thiamine not only facilitates its production but also enhances its uptake. In addition, thiamine influences the function of the cerebellum, hippocampus, and hypothalamus [Mrowicka *et al.*, 2023; Butterworth, 1982; Van Woert *et al.*, 1979].

One of the consequences of thiamine deficiency can be Beriberi, which can cause muscle wasting, reduced cardiac contractility, hypotension, and paralysis of the nervous system. Clinically, Beriberi is classified into two forms: dry and wet. Dry Beriberi is associated with nervous system dysfunctions, such as polyneuropathy, Wet Beriberi, on the other hand, is characterized by marked fluid accumulation (edema), which can lead to circulatory failure and

myocardial infarction due to involvement of the cardiovascular system. [Hassan *et al.*, 2018; Mikkelsen and Apostolopoulos, 2019]. Another severe consequence of thiamine deficiency is Wernicke-Korsakoff Syndrome (WKS). The clinical picture of WKS is characterized by ataxia, memory impairment, and hypothermia; the latter frequently occurring alongside other symptoms. Experimental and clinical studies indicate that thiamine deficiency can injure the hypothalamus, a key region of the central nervous system that controls body temperature and appetite. Case studies demonstrated that thiamine supplementation reverses hypothermia in as little as two days [Mateos-Díaz *et al.*, 2022; Tanev *et al.*, 2008; Hansen *et al.*, 1984].

Vitamin B1 is crucial for carbohydrate metabolism as a cofactor for enzymes, and its deficiency can lead to severe metabolic disorders, including DM, due to impaired glucose metabolism. The excretion of thiamine was markedly elevated in diabetic patients compared to healthy subjects, with increases of 24-fold in type 1 DM and 16-fold in type 2 DM, respectively [Thornalley *et al.*, 2007]. It was observed in 17-79% of patients with DM that low TPP levels due to vitamin deficiency reduced erythrocyte transketolase activity [Jermendy, 2006; Page *et al.*, 2011]. Interestingly, results of a clinical trial showed that administering 150 mg of vitamin B1 per day for 30 days led to a robust fall in fasting plasma glucose levels in untreated patients with type 2 DM. It was also observed that vitamin B1 administration may have a positive effect on the patients' condition by contributing to improvements in lipid profiles and creatinine levels [González-Ortiz *et al.*, 2011; Al-Attas *et al.*, 2014]. Supplementation of 100 mg of thiamine three times per day (around 100 times more than its recommended daily intake) for 42 days improved glucose tolerance in patients with hyperglycemia [Alaei Shahmiri *et al.*, 2013].

Thiamine uptake occurs predominantly across the intestinal mucosa, after which it enters the circulation and is consumed by erythrocytes, leukocytes, and thrombocytes [Tallaksen *et al.*, 1997; Casirola *et al.*, 1990]. It is subsequently transported into cells of multiple tissues, including the liver, myocardium and nervous tissue. In neurons, entry takes place from the bloodstream via the cerebrospinal fluid and involves passage across the blood–brain barrier. Several transporters participate in the transport of thiamine, of which members of the SLC transporter family are the best characterized [Rindi and Laforenza, 2000].

4.7. Thiamine transporters

The SLC19 family of transporters is a subgroup of the SLC superfamily involved in the import of thiamine and folate (**Figure 13**). This subgroup comprises the transmembrane ThT1 (SLC19A2), ThT2 (SLC19A3), and the RFC/SLC19A1. RFC contributes to the transport of TMP, TPP, and TTP out of cells, thereby helping to maintain the balance of phosphorylated

vitamin B1 derivatives [Zhao *et al.*, 2002; Liu *et al.*, 2017]. SLC19A2 and SLC19A3, in turn, import thiamine across the plasma membrane, however, they do not transport folate [Gangolf *et al.*, 2010; Zhao and Goldman, 2013]. Another important transporter is the TPP carrier transporter (SLC25A19) which provides cofactors for PDH and α -KGDH across the inner mitochondrial membrane (**Figure 13**) [Ruprecht and Kunji, 2020].

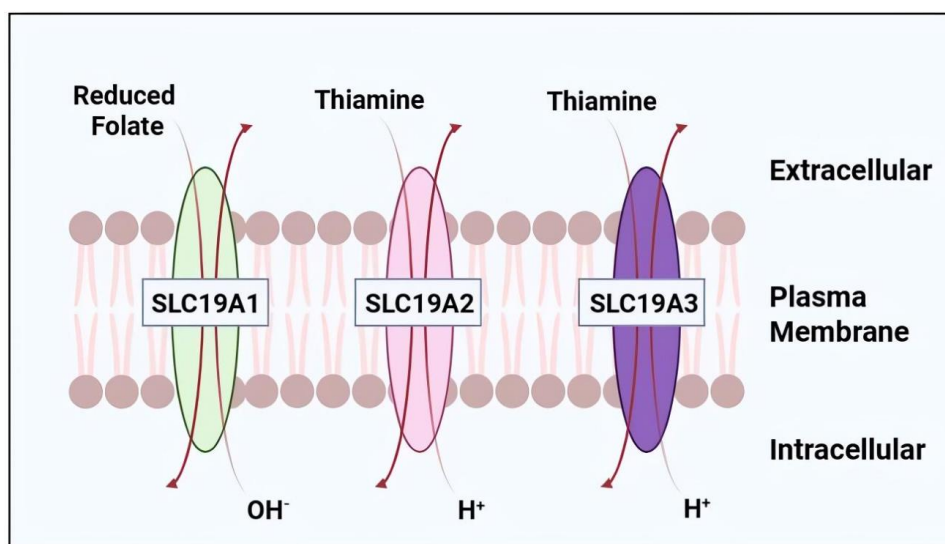


Figure 13. Proposed transport mechanisms of SLC19 family members (created with BioRender)

SLC19A2 encodes a protein with 12 transmembrane segments and two N-glycosylation sites, and it is expressed ubiquitously in human tissues [Dutta *et al.*, 1999; Diaz *et al.*, 1999; Labay *et al.*, 1999; Fleming *et al.*, 1999]. These N-glycosylation motifs are not essential for its transporter activity. [Balamurugan *et al.*, 2002]. Thiamine influx proceeds through a thiamine/H⁺ antiport mechanism, and the carrier shows high specificity for thiamine, failing to recognize other organic cations as substrates [Dutta *et al.*, 1999]. The gene is made up of six exons. In mouse models, targeted knock out of this gene has been demonstrated to cause DM, sensorineural deafness, and megaloblastosis when the animals were kept on a thiamine-deficient diet [Oishi *et al.*, 2002]. In humans, mutations in *SLC19A2* give rise to thiamine-responsive megaloblastic anemia (Rogers syndrome), a condition that can be effectively managed with vitamin B1 supplementation [Neufeld *et al.*, 2001; Raz *et al.*, 2000]. The *SLC19A3* gene is similar to *SLC19A2* in both structure and function [Eudy *et al.*, 2000; Rajgopal *et al.*, 2001]. Thiamine is a widely used pharmacological agent and nutritional supplement, and its antagonists are employed to clarify the role of the vitamin in metabolism and to explore the consequences of its deficiency [Withfield *et al.*, 2018]. Various ThTIs have been investigated, primarily using the CACO2 cell line, an *in vitro* model of intestinal epithelial

cells [Giacomini *et al.*, 2017; Zhang *et al.*, 2014]. Fedratinib shows a stronger inhibitory effect on SLC19A3 ($IC_{50} = 1.36 \mu M$) than on SLC19A2 ($IC_{50} = 7.10 \mu M$) [Giacomini *et al.*, 2017; Zhang *et al.*, 2014]. Amprolium is a structural analogue of thiamine which competitively inhibits thiamine transport. Therefore, this compound can block vitamin B1 uptake and reduce thiamine levels intracellularly [Park *et al.*, 2000; Bettendorff *et al.*, 1995].

5. AIM OF THE STUDY

To clarify the role of ThTs in regulating NST in **human neck-derived** and **SGBS adipocytes**

To examine how prolonged exposure to high and gradually increasing thiamine concentrations affects the thermogenic capacity of human neck-derived adipocytes

- Investigating how high and progressively increasing thiamine concentrations influence thermogenic activity in human neck-derived adipocytes by measuring oxygen consumption and UCP1-mediated uncoupling.
- Determining the impact of prolonged thiamine excess and rising thiamine levels on the expression of mitochondrial respiratory-chain complex subunits.
- Assessing how sustained and gradually increased thiamine availability modulates the expression of ThTs, as well as thermogenic and browning marker genes.

To investigate the impact of short-term thiamine excess and ThT inhibition, along with db-cAMP-induced thermogenic activation, in the SGBS adipocyte model

- Determining how ThT inhibition combined with brief high-dose thiamine exposure alters thermogenic activity in SGBS adipocytes, using measurements of oxygen consumption and UCP1-dependent NST.
- Evaluating the effects of short-term thiamine treatment and ThT blockade on the expression of mitochondrial respiratory-chain complex subunits.
- Assessing how short-term thiamine exposure and ThT inhibition influence the expression of ThTs and of thermogenic and browning marker genes in SGBS adipocytes under db-cAMP-stimulated and unstimulated conditions.

6. MATERIALS AND METHODS

6.1. Ethics statement

hASCs were isolated from paired SuC and deep neck (DeN) adipose tissue biopsies collected during elective thyroid surgeries. Donors had a body mass index below 29.9 and were aged between 20 and 65 years. Individuals with DM, malignancies, or pathological thyroid hormone levels were excluded from the study. The study protocol was approved by the Medical Research Council of Hungary (20571-2/2017/EKU), in compliance with the EU Member States' Directive 2004/23/EC on presumed consent practice for tissue collection. All procedures were conducted in accordance with the ethical principles set forth in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the surgical intervention.

6.2. Materials

The compounds used in cell culture and differentiation, inhibition of ThTs, and Seahorse measurements, gene primers and probes, as well as antibodies used in immunoblotting are listed in the Appendix, Section 11. All reagents were purchased from Sigma-Aldrich (Munich, Germany) unless stated otherwise.

6.3. Aquired samples and isolation of SuC and DeN-derived hASCs

To minimize inter-individual variability, paired SuC and DeN adipose tissue biopsies were collected from the same patients undergoing thyroid surgery. Sample inclusion was based on body mass index rather than on patient gender. The biopsies were promptly brought to the cell laboratory and processed after being surgically 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. Adipose tissue pieces were digested in 120 U/mL collagenase from *Clostridium histolyticum* dissolved in 1× sterile Phosphate-Buffered Saline (PBS) in a 37°C water bath for 60 minutes, with gentle mixing every 15 minutes. The digested material was then passed through a 140 µm filter to eliminate residual tissue fragments. The resulting cell suspension was centrifuged at 200 g for 10 minutes, and the stromal vascular fraction (SVF) pellet was resuspended in DMEM/F-12 HAM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin–streptomycin, 17 µM pantothenic acid, and 33 µM biotin. hASCs were plated onto Ibidi eight-well µ-slides (Ibidi GmbH, Gräfelfing, Germany), Seahorse XF96 cell culture plates (Agilent Technologies, Santa Clara, CA, USA), or 6-well plates at a density of 15000 cells/cm² and maintained in serum-containing medium at 37°C in 5% CO₂ for 24 h to allow cell attachment. After 24 h,

non-adherent cells were eliminated by washing with PBS, and adherent hASCs were cultured further until reaching the desired confluency. The cultures were verified to be absent of Mycoplasma contamination by polymerase chain reaction (PCR) analysis.

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

hASCs and SGBS preadipocytes were used as adipocyte progenitor cells. The human SGBS cell line, originally established at University Medical Center Ulm, Ulm, Germany by Martin Wabitsch and Pamela Fischer-Posovszky, was kindly provided by our collaborators.

Both hASCs and SGBS preadipocytes were differentiated into ADCY using a standard adipogenic medium, or into B-ADCY using a protocol involving prolonged rosiglitazone exposure.

For ADCY induction, cells were treated for 3 days with DMEM/F-12 HAM medium containing 17 μ M pantothenic acid, 33 μ M biotin, 10 μ g/mL human apo-transferrin, 20 nM human insulin, 0.1 μ M hydrocortisone, 0.2 nM T3, 2 μ M rosiglitazone, 25 nM dexamethasone, and 0.5 mM IBMX [Tóth *et al.*, 2020]. B-ADCY differentiation was initiated for 3 days in DMEM/F-12 HAM medium supplemented with 17 μ M pantothenic acid, 33 μ M biotin, 10 μ g/mL apo-transferrin, 850 nM human insulin, 0.2 nM T3, 1 μ M dexamethasone, and 0.5 mM IBMX.

After the initial 3-day induction period, IBMX and dexamethasone were removed from both media. From days 4–14, hydrocortisone was continuously included in the ADCY differentiation regimen [Kristof *et al.*, 2015]. In the B-ADCY protocol, rosiglitazone together with a higher insulin concentration was maintained from days 4–14 of differentiation [Elabd *et al.*, 2009; Arianti *et al.*, 2023].

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

The differentiation of hASCs was carried out in the presence of either high or progressively increasing thiamine concentrations from days 3 to 14. For the “abundant thiamine” condition, cells were cultured in DMEM/F-12 HAM with or without additional thiamine hydrochloride (25000 nM or 50000 nM) during the final 11 days of differentiation. Control (Ctrl) cells received only the thiamine naturally present in DMEM/F-12 HAM (6400 nM) throughout the entire protocol.

For concentration-dependence experiments, adipocytes were differentiated in a custom thiamine-free DMEM/F-12 HAM supplemented with increasing amounts of thiamine hydrochloride (40, 200, 1000, 5000, or 25000 nM) during the last 11 days. In this series, Ctrl

cells were differentiated in completely thiamine-free medium. At the end of differentiation, cells were harvested in TRIzol or Sodium dodecyl sulfate (SDS) lysis buffer for downstream analyses.

For SGBS adipocytes differentiated in DMEM/F-12 HAM, both ADCYs and B-ADCYs were treated with a single dose of 0.5 mM db-cAMP for 10 hours to simulate cold-induced NST *in vivo*. To block ThT activity, cells were exposed to 1 μ M Fedratinib or 300 μ M Amprolium for 10 hours [Arianti *et al.*, 2023], either alone or in combination with db-cAMP.

In thiamine-excess experiments, differentiated SGBS ADCYs and B-ADCYs were incubated for 10 hours in DMEM/F-12 HAM with or without additional thiamine hydrochloride (25000 or 50000 nM). Ctrl cells were maintained only in medium containing the basal thiamine level of DMEM/F-12 HAM. After treatment, cells were harvested in TRIzol or SDS lysis buffer.

All hASC and SGBS-derived adipocytes were cultured at 37 °C in 5% CO₂, with medium changes every 3rd day.

6.6. Analysis of publicly available single nuclei (sn) RNA-sequencing (RNA-seq) data

The available snRNA-seq dataset was interrogated using the public web tool at <https://batnetwork.org/> following the approach published by Sun *et al.* [Sun *et al.*, 2020].

6.7. Total RNA isolation and TaqManTM reverse transcription-coupled quantitative PCR (RT-qPCR)

After cell collection in TRIzol, total RNA was extracted by chloroform–isopropanol phase separation. Following DNase treatment, RNA concentration and purity were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All samples were adjusted to 100 ng/ μ L and reverse-transcribed into cDNA using a commercial reverse-transcription kit according to the manufacturer’s protocol. Validated TaqMan assays for qPCR (Thermo Fisher Scientific) are listed in **Table 4, Appendix**. Reactions were run on a LightCycler® 480 II instrument (Roche Diagnostics) with the following cycling program: 95 °C for 60 sec, then 50 cycles of 95 °C for 12 sec and 60 °C for 30 sec. Gene expression was quantified by the comparative Ct method. Δ Ct was defined as the Ct of the gene of interest minus the geometric mean of the Ct values of the reference genes, Actin Beta (*ACTB*) and Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*). Normalized expression levels were calculated as $2^{-\Delta Ct}$.

6.8. Western blotting and densitometry analysis

For both differentiated hASCs and SGBS adipocytes, ADCYs and B-ADCYs were rinsed with ice-cold 1× sterile PBS and harvested in SDS lysis buffer. Lysates were boiled at 100 °C for 10 minutes and subjected to SDS–polyacrylamide gel electrophoresis. Proteins were then transferred onto PVDF Immobilon-P membranes and blocked for 60 min in Tris-buffered saline containing 0.05% Tween-20 (TTBS) and skimmed milk.

Membranes were incubated at 4 °C overnight with primary antibodies at the working dilutions listed in **Table 5, Appendix**. After three 15-minute washes in TTBS, membranes were incubated at room temperature for 60 min with Horseradish-peroxidase-conjugated secondary antibodies corresponding to the primary antibody species. Signals were visualized using Immobilon Western chemiluminescent substrate.

Band intensities were quantified with FIJI/ImageJ software (National Institutes of Health, Bethesda, MD, USA); the optical density of each protein of interest was normalized to that of the normalizing protein (tubulin or actin).

6.9. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) quantification by Seahorse

OCR and ECAR of hASC-derived and SGBS adipocytes differentiated as described in section 6.4 were analyzed with an XF96 oximeter (Agilent Technologies) using previously optimised protocols. When hASCs were differentiated in the presence of excess vitamin B1, cells were pre-incubated for 60 min and assayed in Agilent Seahorse XF Base Medium (Agilent Technologies), which is formulated for extracellular flux measurements and contains 6400 nM vitamin B1.

When adipocytes were differentiated at vitamin B1 concentrations approximating physiological plasma levels, measurements were carried out in thiamine-free medium so that the assay reflected the intracellular thiamine pool acquired during differentiation. Because this medium was used instead of the bicarbonate-buffered Seahorse XF Base Medium, ECAR could not be determined in this setup. In both experimental designs, after baseline OCR had been recorded, 0.5 mM db-cAMP was injected to provide an adrenergic stimulus, and stimulated OCR was then monitored every 20 minutes.

For differentiated SGBS adipocytes, baseline OCR was first recorded, followed by injection of 0.5 mM db-cAMP, 1 µM fedratinib, 300 µM amprolium, or db-cAMP in combination with one of the ThTIs. OCR was then measured every 30 minutes.

In the excess thiamine experiment, SGBS preadipocytes were differentiated into ADCY or B-ADCY under standard conditions, and OCR was monitored for 10 h after addition of

0.5 mM db-cAMP in the presence or absence of extra thiamine (25000 or 50000 nM), with readings taken every 30 minutes.

In all experiments, PrL respiration was assessed after addition of Oligomycin (2 μ M). Finally, adipocytes were subjected to single dose of Antimycin A (10 μ M) to determine non-mitochondrial respiration for baseline correction. OCR values of each well were normalized to total protein content.

6.10. Quantification of adipocyte differentiation ratio by laser-scanning cytometry

SuC and DeN-derived hASCs were seeded and differentiated in 8-well Ibidi μ -chambers. After differentiation, cells were washed with PBS and fixed with 4% paraformaldehyde, permeabilised with 0.1% saponin and blocked in 5% skimmed milk as described previously [Szatmári *et al.*, 2020]. Cells were incubated overnight at room temperature with anti-UCP1 primary antibody (1:200, U6382), followed by a 3 h incubation with Alexa 488 goat anti-rabbit IgG secondary antibody (1:1000, Thermo Fisher Scientific, A11034). Nuclei were stained with 1.5 μ g/mL propidium iodide for 60 minutes. Scanning was performed by an iCys Research Imaging Cytometer (iCys, Thorlabs Imaging Systems, Sterling, VA, USA). Samples were analysed using an iCys Research Imaging Cytometer (iCys, Thorlabs Imaging Systems, Sterling, VA, USA). Images (n = 4, 2000 cells per sample) were processed with our high-throughput automated cell-recognition pipeline employing the iCys companion software (iNovator Application Development Toolkit v7.0, CompuCyte), CellProfiler (Broad Institute, MIT, MA, USA), and CellProfiler Analyst. Cells were segmented based on nuclear staining, and for each cell, UCP1 fluorescence intensity and the texture sum variance of the light-scatter signal from LDs were quantified within a 30-pixel zone surrounding the nuclear contour using CellProfiler. Subsequently, semi-automated classification and counting of differentiated adipocytes versus undifferentiated preadipocytes were performed with the “Fast Gentle Boosting” classifier implemented in CellProfiler Analyst [Kristóf *et al.*, 2015; Doan-Xuan *et al.*, 2013]. *Image analysis was carried out by Dr. Zsolt Bacsó (Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen).*

6.11. Statistical analysis

Data are presented as mean $\pm$ SD. Normality was assessed using the Shapiro–Wilk test. For comparisons among multiple groups, one-way or two-way ANOVA followed by Tukey’s *post hoc* test was applied. GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) was used for data analysis and visualization.

7. RESULTS

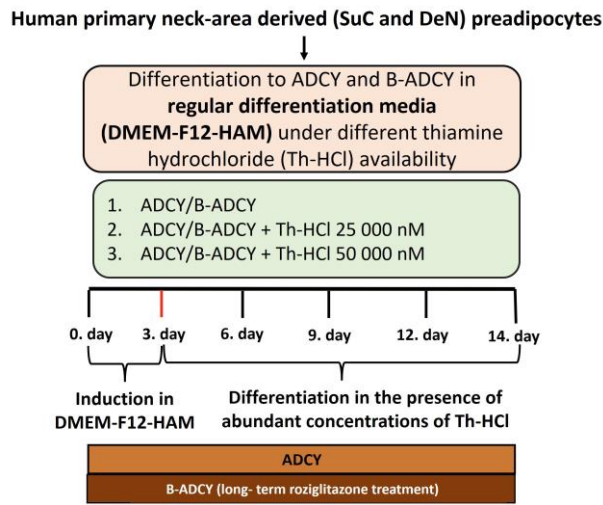
7.1. The concentration of extracellular thiamine determines the thermogenic capacity of human adipocytes originated from the neck area

7.1.1. Impact of high thiamin levels on thiamine transporter expression during adipogenesis

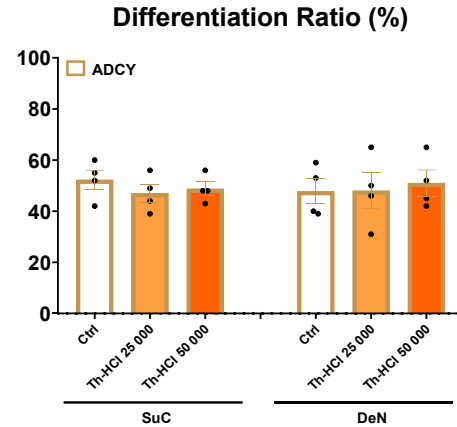
In our previous work, we showed that higher ThT expression and increased thiamine uptake support robust thermogenic activation in adipocytes cultivated from the cervical depot [Arianti *et al.*, 2023]. Here, we first analyzed how supraphysiological thiamine concentrations (25000 nM or 50000 nM) affect ThT expression in human SuC and DeN-derived adipocytes differentiated either with a standard adipogenic protocol (ADCY) or with prolonged rosiglitazone treatment to induce a thermogenic, “beige” -like phenotype (B-ADCY) [Arianti *et al.*, 2023]. In this experimental setup, adipocytes were differentiated into ADCYs or B-ADCYs under routine culture conditions with 6400 nM thiamine in DMEM/F-12, or in the same medium supplemented with additional thiamine hydrochloride (**Figure 14A**). The differentiation ratio of ADCYs derived from both SuC and DeN fat depots was unaffected by extracellular thiamine concentrations of 25000 nM or 50000 nM compared with control. Laser-scanning cytometry analysis showed no significant impact of thiamine level on differentiation efficiency in adipocytes from both anatomical sites (**Figure 14B**). In our recent work, we also demonstrated that SLC19A3, but not SLC19A2, is expressed at higher mRNA and protein levels in DeN-derived ADCYs than in those derived from SuC fat [Arianti *et al.*, 2023].

When different thiamine concentrations were tested, 25000 nM markedly increased *SLC19A2* expression in SuC-derived B-ADCYs, whereas in DeN-derived B-ADCYs a comparable upregulation was observed with both 25000 nM and 50000 nM vitamin B1 (**Figure 14C**). Moreover, 50000 nM thiamine significantly enhanced *SLC19A3* mRNA expression in DeN-derived B-ADCYs (**Figure 14D**).

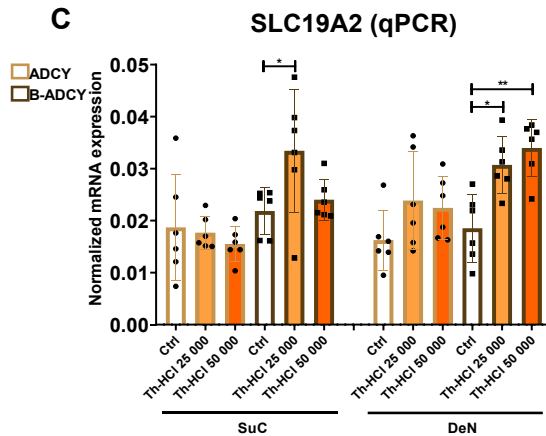
A



B



C



D

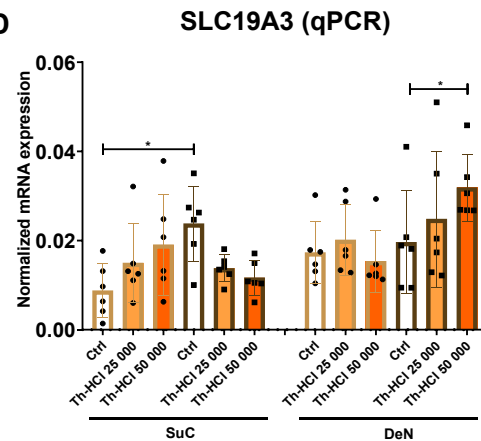


Figure 14. Effect of high thiamine hydrochloride (Th-HCl) on the expression of Th transporters (ThTs) in human subcutaneous (SuC) and deep neck (DeN)-derived white-like (ADCYs, light-brown outline) or “beige”-like adipocytes (B-ADCYs, dark-brown outline). Cells were differentiated under standard conditions (white bars) or in the continuous presence of 25000 nM (Th-HCl 25000, light-orange bars) or 50000 nM (Th-HCl 50000, dark-orange bars) Th-HCl. (A) Schematic overview of the ex vivo experimental setup. (B) Differentiation ratios quantified by laser-scanning cytometry, $n = 4$. (C, D) SLC19A2 and SLC19A3 mRNA levels determined by RT-qPCR, $n = 6$. Statistical evaluation: two-way ANOVA, $*p < 0.05$, $**p < 0.01$.

SLC19A3 protein expression was markedly increased when SuC-derived B-ADCYs were differentiated in the presence of 25000 nM vitamin B1. In contrast, SLC19A2 protein levels were not increased in response to further elevation of extracellular thiamine concentrations in the adipogenic medium (**Figure 15**).

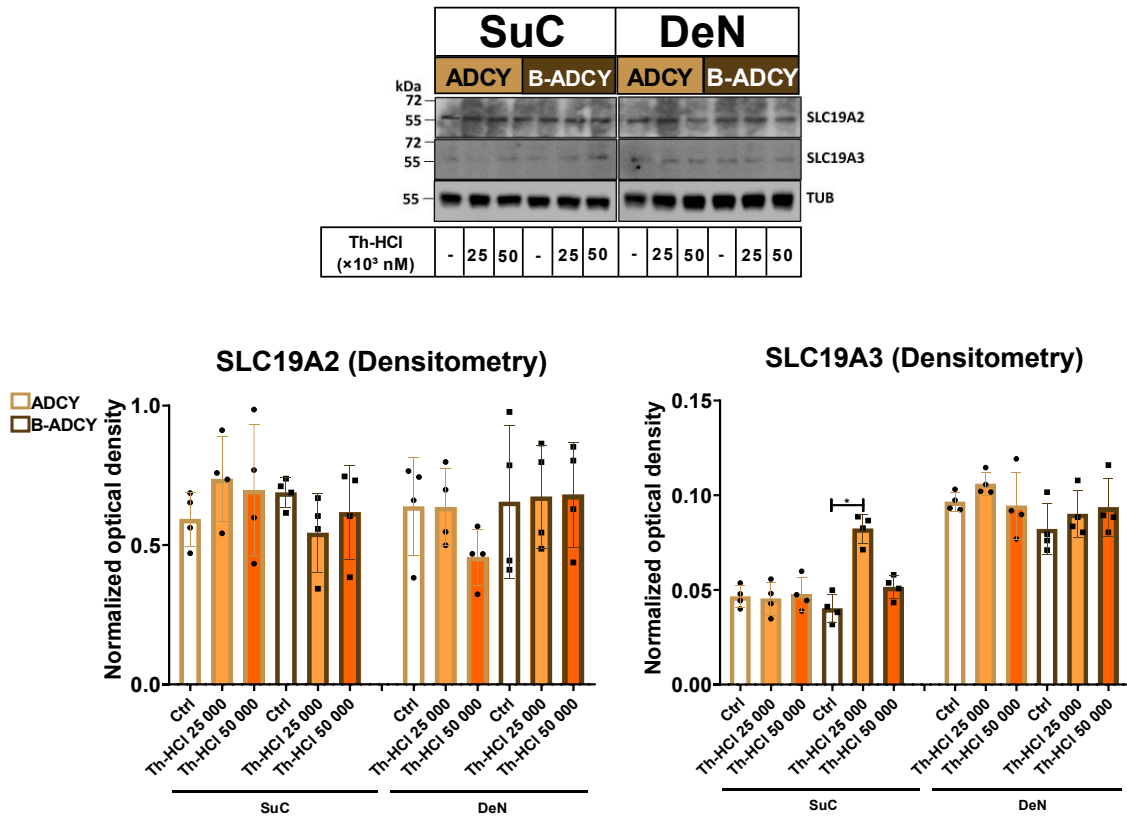


Figure 15. Effect of elevated thiamine hydrochloride (Th-HCl) on the protein levels of Th transporters (ThTs) in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs, light-brown border) and "beige"-like adipocytes (B-ADCYs, dark-brown border). Cells were differentiated as in Figure 14. SLC19A2 and SLC19A3 protein expression were assessed by western blotting, $n = 4$. Statistical evaluation: two-way ANOVA, $*p < 0.05$.

7.1.2. Impact of progressively elevated thiamine concentrations on transporter expression during adipocyte differentiation

We next examined how increases in thiamine levels (40 nM, 200 nM, 1000 nM, or 5000 nM) affect ThT expression during differentiation of human SuC and DeN-derived adipocytes. In this setup, SuC or DeN-derived hASCs were differentiated into ADCYs or B-ADCYs either in thiamine-free medium (Th 0) or in medium in which thiamine hydrochloride was reintroduced at gradually increasing concentrations. This setup allowed us to reveal the importance of thiamine availability within a range approximating physiological levels during adipocyte differentiation (**Figure 16A**). The availability of thiamine during differentiation, even when supplied in gradually increasing concentrations, did not alter the proportion of ADCYs derived from either SuC or DeN depots. Laser-scanning cytometry revealed that differentiation ratios were similar in control cells and in those subjected to 40 nM,

200 nM, 1000 nM, or 5000 nM thiamine at both anatomical sites (**Figure 16B**). By contrast, exposure to 1000 nM or 5000 nM vitamin B1 significantly affected ThT expression compared with the Th 0 condition and with 40 nM, which approximates physiological plasma levels. The mRNA levels of both ThTs increased progressively with rising thiamine concentrations (**Figures 16C, D**).

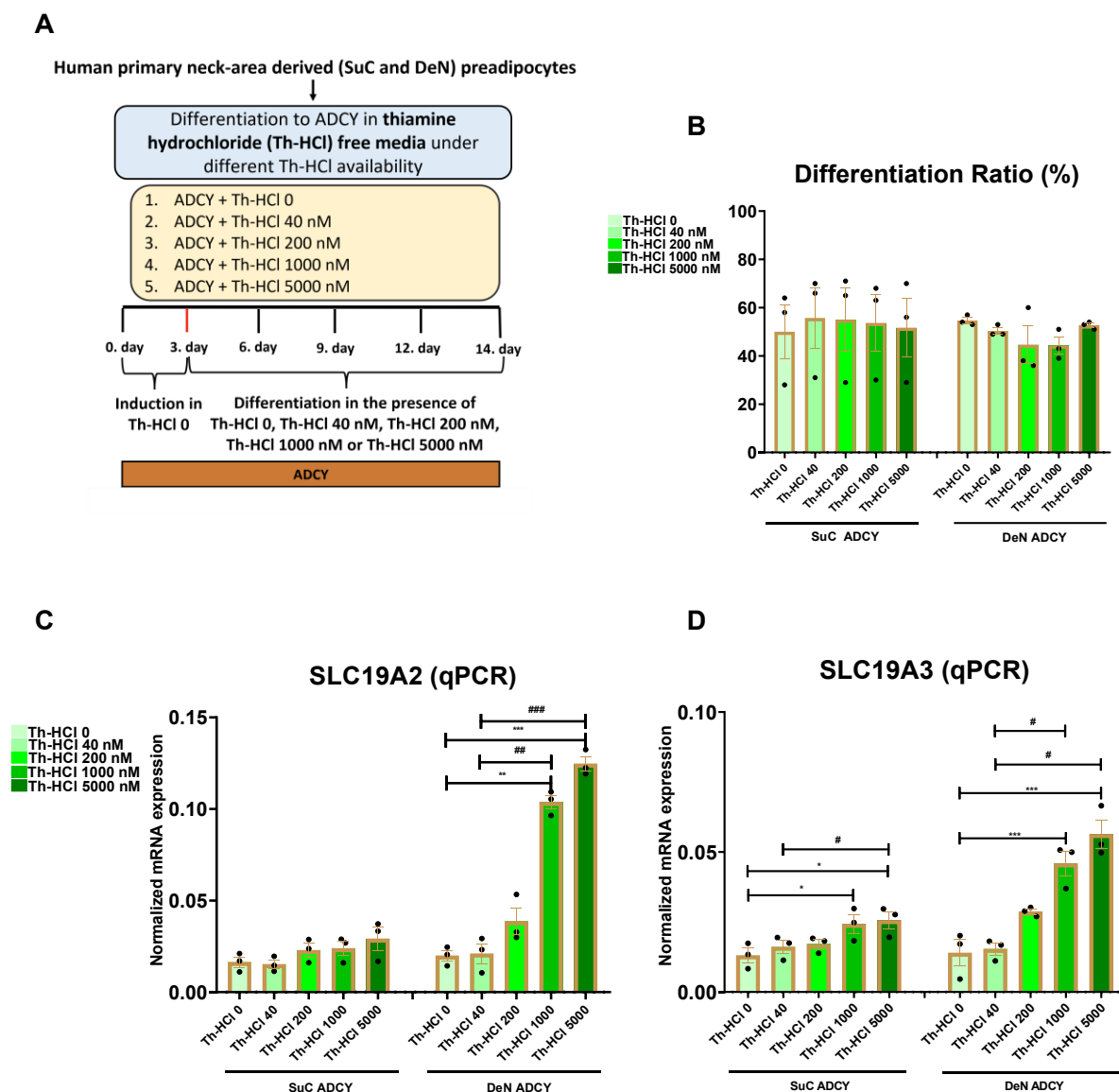


Figure 16. Effect of increases in thiamine hydrochloride (Th-HCl; 40 nM, 200 nM, 1000 nM, and 5000 nM) on thiamine transporter (ThT) gene expression in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). SuC or DeN-derived preadipocytes were differentiated into ADCYs either in Th-HCl-free medium (Th-HCl 0) or in medium supplemented with progressively higher Th-HCl concentrations. (A) Schematic overview of the ex vivo experimental setup. (B) Differentiation ratios quantified by laser-scanning cytometry, $n = 3$. (C, D) SLC19A2 and SLC19A3 mRNA levels determined by RT-qPCR, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

0.001 versus Th-free condition; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ for the indicated group comparisons.

When ThT protein expression was analyzed, a similar pattern was seen (**Figure 17**).

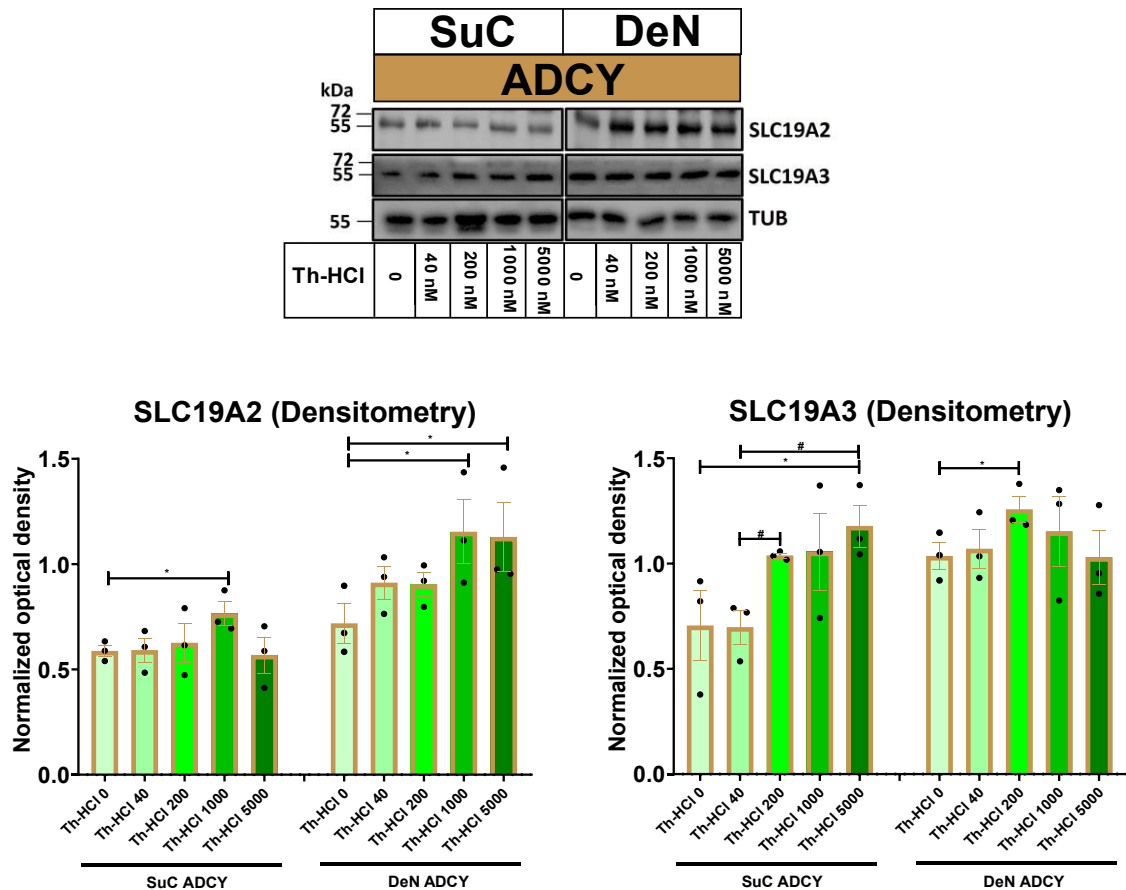


Figure 17. Effect of increases in thiamine hydrochloride (Th-HCl; 40 nM, 200 nM, 1000 nM, and 5000 nM) on the protein levels of Th transporters (ThTs) in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). SuC or DeN-derived preadipocytes were differentiated as in Figure 16. SLC19A2 and SLC19A3 protein expressions were assessed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$ versus Th-free condition and # $p < 0.05$ for the indicated group comparisons.

7.1.3. Concentration-dependent effects of thiamine availability on thermogenesis in differentiating adipocytes

We next assessed how thiamine availability influences NST in ADCYs differentiated either in Th 0 medium or in medium supplemented with higher thiamine concentrations progressively (40 nM, 200 nM, 1000 nM, 5000 nM, or 25000 nM), which were then washed out and replaced by Th-free assay medium. As described above, after recording basal OCR, cells were stimulated with db-cAMP [Arianti *et al.* 2018]. Oligomycin, an ATP-synthase

inhibitor, was subsequently added to quantify PrL respiration as a functional indicator of UCP1-dependent NST.

When thiamine levels during differentiation were raised from 40 nM to 25000 nM in both SuC and DeN-derived ADCYs, basal OCR, maximal respiration after db-cAMP administration, and db-cAMP-stimulated PrL OCR all tended to increase relative to the Th 0 condition. However, in DeN-derived ADCYs, statistically significant changes were only observed between 25000 nM and 40 nM thiamine or compared with Th 0 controls (**Figure 18B**) whereas in SuC-derived ADCYs, significant changes were already detectable when thiamine availability reached 200 nM (**Figure 18A**). The presence of vitamin B1 in the adipogenic differentiation medium of either SuC or DeN-derived ADCYs did not alter db-cAMP-stimulated, oligomycin-sensitive ATP-linked OCR (**Figures 18A, B**). It should be noted that ECAR could not be evaluated in this series of experiments, because the extracellular flux measurements were performed in Th-free assay medium, relying on the intracellular thiamine pool accumulated during differentiation.

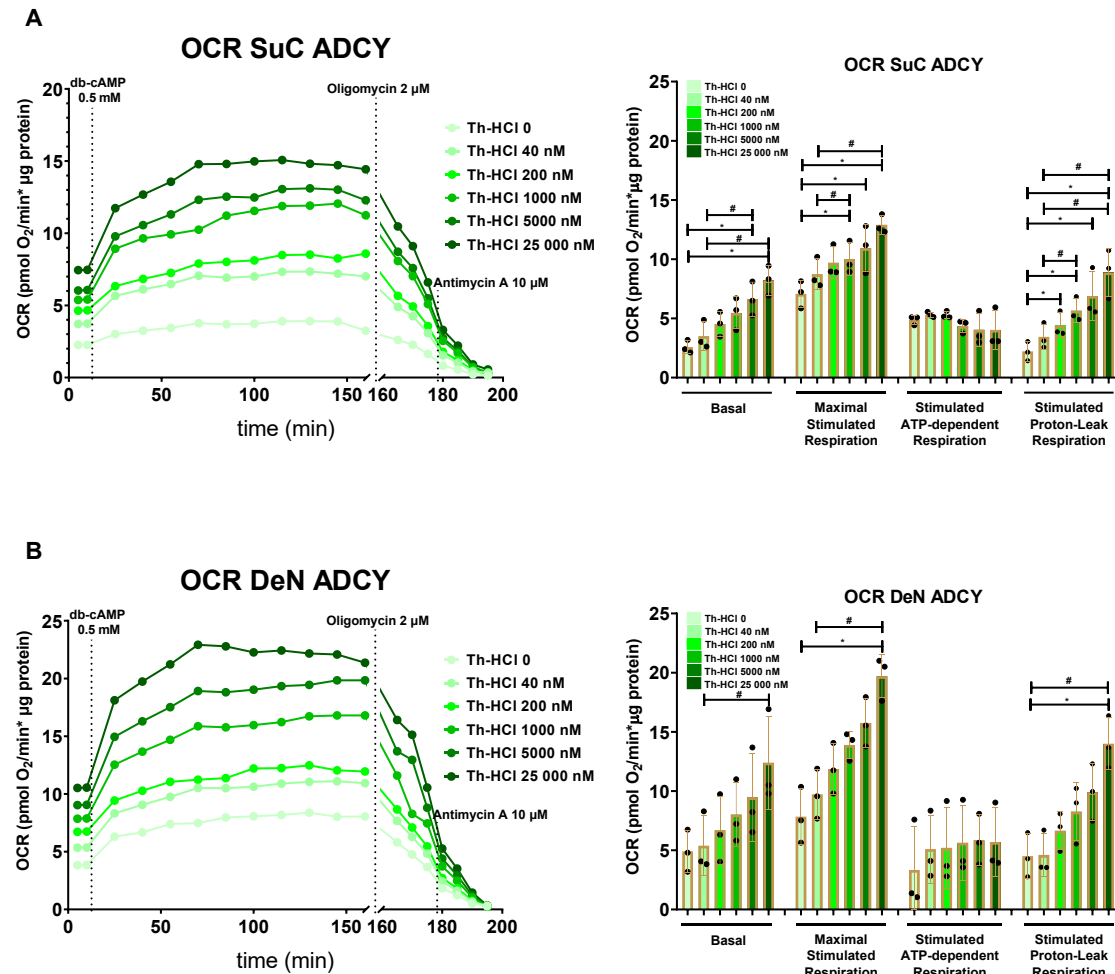


Figure 18. Effect of increases in thiamine hydrochloride (Th-HCl; 40 nM, 200 nM, 1000 nM, 5000 nM, 25000 nM) availability during differentiation on the oxygen consumption rate (OCR) of human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). (A) SuC or (B) DeN-derived preadipocytes were differentiated into ADCYs either in Th-HCl-free medium (Th-HCl 0) or in medium containing progressively higher Th-HCl concentrations. OCR was recorded under basal conditions, after stimulation with 0.5 mM dibutyryl-cAMP for 180 min and following addition of 2 µM oligomycin (to determine stimulated oligomycin-sensitive ATP-linked respiration and oligomycin-resistant proton-leak respiration). Left panels show representative traces from three independent experiments with distinct donor samples. OCR values at baseline, at maximal stimulation after cAMP injection, and after oligomycin treatment (right panels) were quantified in ADCYs derived from three independent donors. Statistical evaluation: one-way ANOVA; * $p < 0.05$ versus Th-free condition (Th 0) and # $p < 0.05$ for the indicated group comparisons.

7.1.4. Mitochondrial biogenesis of neck-derived ADCYs is significantly impacted by thiamine availability during adipocyte development

In SuC-derived ADCYs, differentiation in the presence of progressively higher thiamine concentrations led to an overall elevation in the protein levels of the subunits of mitochondrial complexes I–III. Statistically significant increases were observed for complex subunits I–III

when 1000 nM and 5000 nM vitamin B1 were applied, compared with 40 nM or Th 0 conditions. Up to 5000 nM, thiamine had no detectable impact on the subunits of complexes IV and V in SuC-derived ADCYs.

In DeN-derived ADCYs, where these respiratory-chain proteins were already highly expressed, changes in extracellular thiamine availability influenced only the abundance of complex II subunit (**Figure 19**).

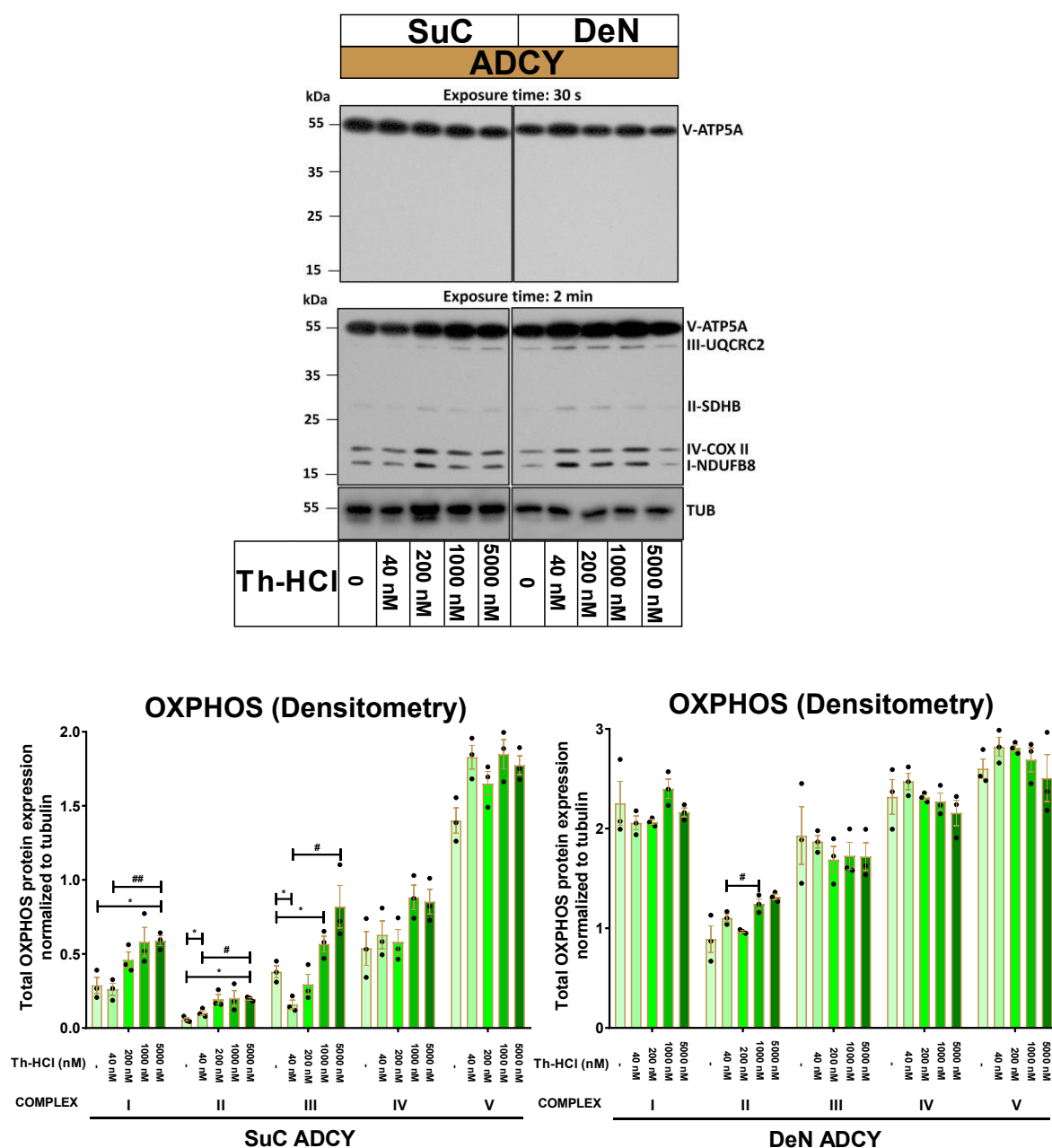


Figure 19. Effect of increases in thiamine hydrochloride (Th-HCl; 40 nM, 200 nM, 1000 nM, and 5000 nM) availability during differentiation on the expression of OXPHOS complex subunits in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). Total levels of OXPHOS protein subunits were assessed by western blotting, $n = 3$.

Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$ versus Th-free condition (Th 0), and # $p < 0.05$ for the indicated group comparisons.

7.1.5. Effect of rising thiamine concentrations on thermogenic gene expression in adipocytes

Because higher thiamine concentrations in the adipogenic differentiation medium were positively associated with enhanced mitochondrial OCR of neck-derived ADCYs, especially with db-cAMP-stimulated PrL OCR, we next examined how thiamine availability affects the expression of NST-related genes. In both SuC and DeN-derived ADCYs, *UCP1* and *PGC1A* mRNA levels increased progressively in parallel with rising thiamine concentrations (**Figures 20A, B**); significant changes were detected at 40 nM and 1000 nM vitamin B1, respectively, compared with the Th 0 condition.

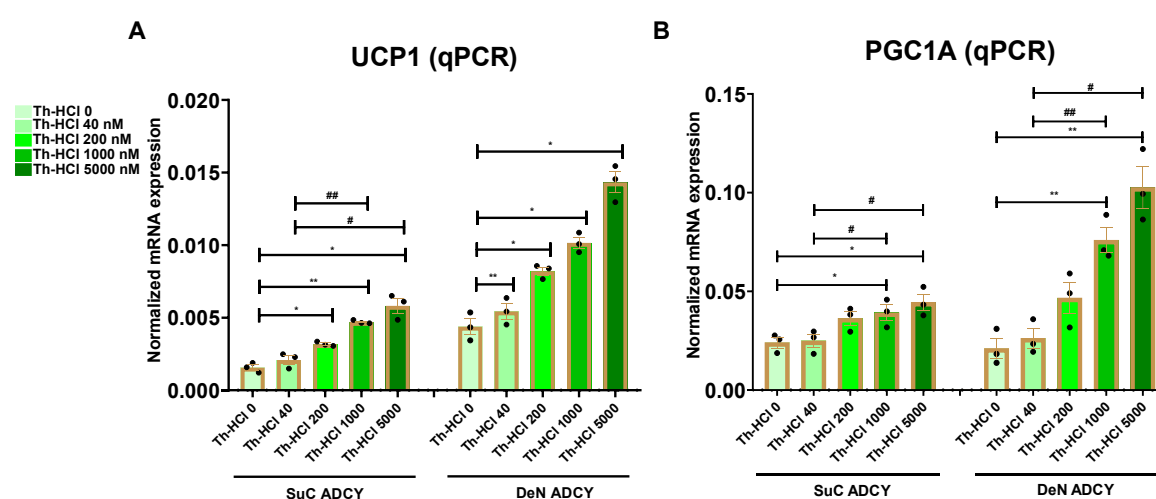


Figure 20. Effect of stepwise increases in thiamine hydrochloride (Th-HCl; 40 nM, 200 nM, 1000 nM, and 5000 nM) availability on thermogenic gene expression in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). ADCYs were differentiated as described in Figure 16. (A, B) *UCP1* and *PGC1A* mRNA expression determined by RT-qPCR, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$ versus Th-free condition (Th 0), and # $p < 0.05$ for the indicated group comparisons.

Thiamine availability had a substantial and concentration-dependent effect on UCP1 but not PGC1A protein expression in SuC and DeN-derived ADCYs (**Figure 21**).

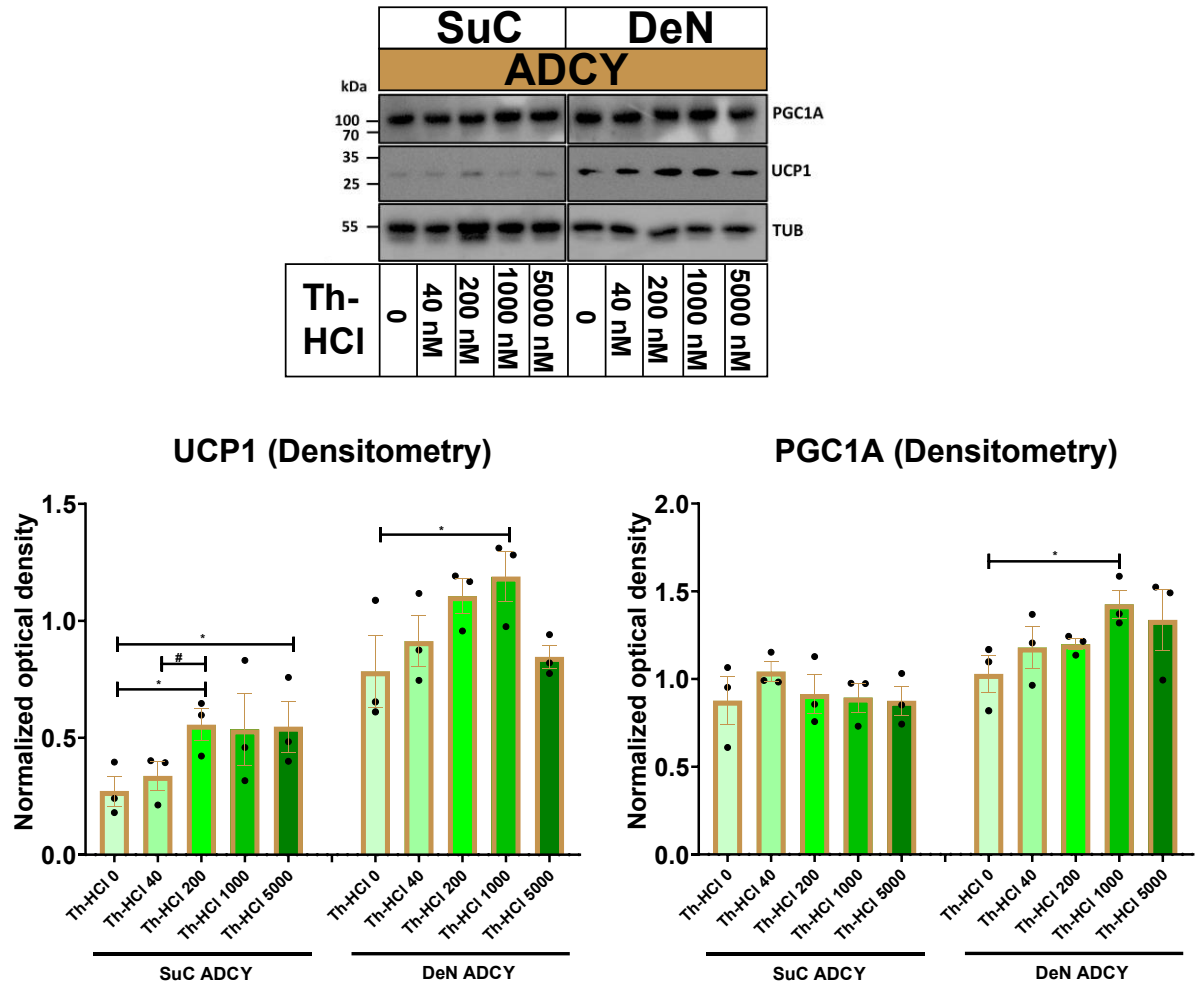


Figure 21. Effect of increases in thiamine hydrochloride (Th-HCl; 40 nM, 200 nM, 1000 nM, and 5000 nM) on thermogenic protein expression in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). ADCYs were differentiated as described in Figure 16. UCP1 and PGC1A protein levels were assessed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA; $*p < 0.05$ versus Th-free condition (Th 0), and $\#p < 0.05$ for the indicated group comparisons.

We also examined how thiamine availability influences the white adipocyte marker *LEP* and the expression of several NST-associated genes in SuC and DeN-derived ADCYs. In both depots, increasing thiamine concentrations enhanced *DIO2*, *TBX1*, *CKMT2*, and *CIDEA* mRNA levels in a dose-dependent manner. Contrarily, *CITED1* expression peaked when ADCYs of both SuC and DeN origin were differentiated with 40 nM thiamine, while both the absence of thiamine (Th 0) and higher vitamin B1 concentrations tended to lower *CITED1* mRNA in ADCYs from both anatomical sites (Figure 22).

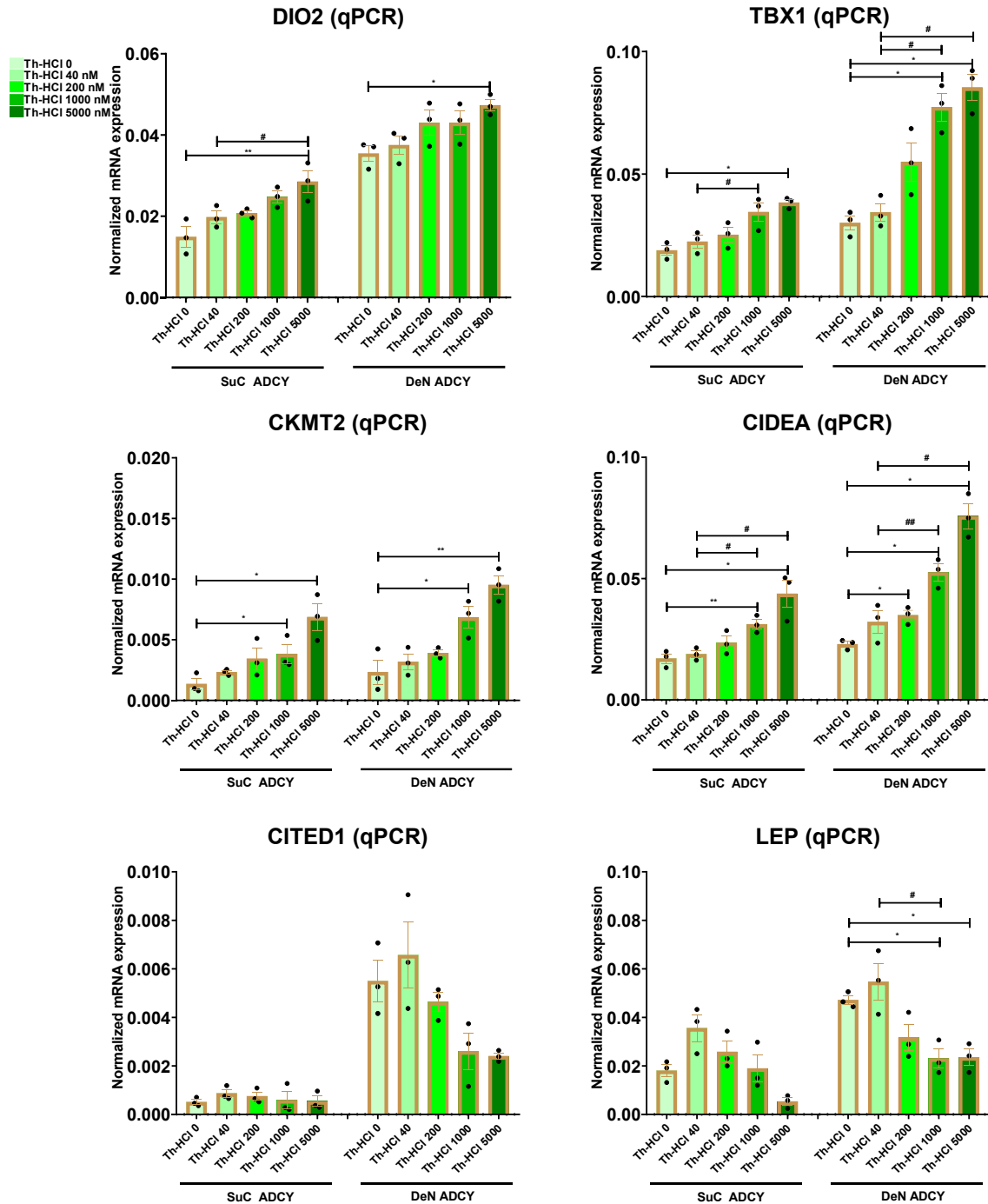


Figure 22. Effect of increases in thiamine hydrochloride (Th-HCl) availability on the induction of thermogenic genes in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). ADCYs were differentiated as described in Figure 16. mRNA levels were measured by RT-qPCR, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$ versus Th-free condition (Th 0), and # $p < 0.05$ for the indicated group comparisons.

7.1.6. Excessive thiamine treatment enhances both coupled and uncoupled respiration during adipocyte differentiation

We next analyzed NST in SuC and DeN-derived ADCYs differentiated in standard medium (6400 nM thiamine) or in the same medium supplemented with even higher vitamin B1 concentrations. In ADCYs of SuC origin, basal OCR was markedly enhanced when differentiation was carried out with an additional 25000 nM thiamine compared with the standard medium (**Figure 23A, left and middle panels**). DeN-derived ADCYs showed a similar tendency, although the differences did not reach statistical significance (**Figure 23B, left and middle panels**). In both depots, maximal cAMP-stimulated respiration was higher in ADCYs matured in the presence of extra thiamine. When hASCs were differentiated with thiamine surplus, both PrL and ATP-linked OCR were elevated in unstimulated SuC and DeN-derived ADCYs. As expected, db-cAMP stimulation further increased ATP-dependent respiration and PrL, with a more pronounced effect in DeN-derived cells. A robust enhancement of PrL OCR in response to increased thiamine during differentiation was detected in activated SuC, but not in DeN-derived ADCYs (**Figures 23A, B, left and middle panels**). Moreover, stimulated ATP-linked respiration was higher in ADCYs of DeN origin differentiated in the presence of additional vitamin B1 than in those kept under standard conditions (**Figures 23A, B, left and middle panels**). In both SuC and DeN-derived ADCYs, ECAR rose upon db-cAMP stimulation; however, high thiamine concentrations during adipogenesis had only minimal impact on ECAR values (**Figures 23A, B, right panels**).

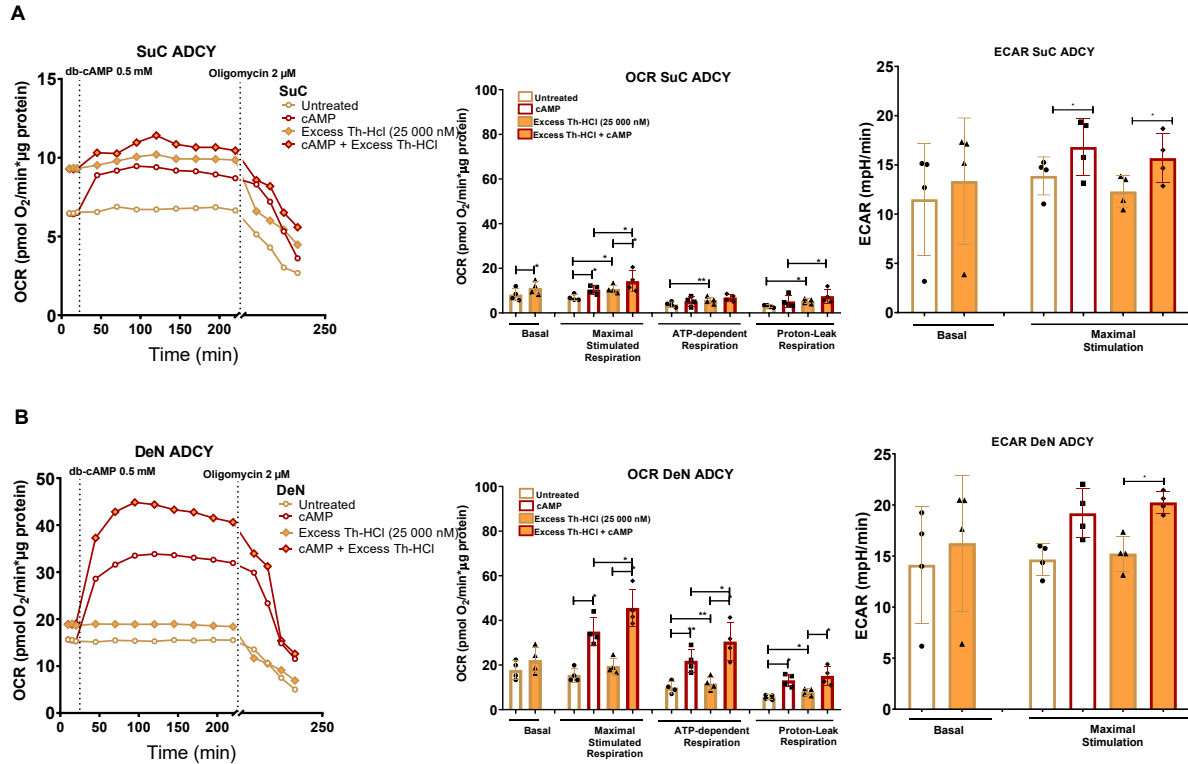


Figure 23. Surplus thiamine hydrochloride (Th-HCl) during adipogenic differentiation enhances the oxygen consumption rate (OCR) of human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). (A) SuC or (B) DeN-derived preadipocytes were differentiated into ADCYs under standard culture conditions (white bars) or in the presence of additional Th-HCl (25000 nM, orange bars). OCR was measured at baseline, after stimulation with 0.5 mM dibutyl-cAMP for 240 min and following the addition of 2 μ M oligomycin (to determine oligomycin-sensitive ATP-linked respiration and oligomycin-resistant proton-leak respiration). Left panels show representative OCR traces from four independent experiments with distinct donor samples. Basal OCR, maximal OCR after cAMP injection, OCR following oligomycin treatment (middle panels), and extracellular acidification rate (ECAR; right panels) were quantified in ADCYs from four independent donors. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$.

In SuC-derived ADCYs, differentiation in the presence of 25000 nM or 50000 nM surplus thiamine markedly increased the protein abundance of the subunits of mitochondrial complexes I, II, and IV, whereas in DeN-derived ADCYs, 50000 nM thiamine selectively enhanced complex I expression (**Figure 24**).

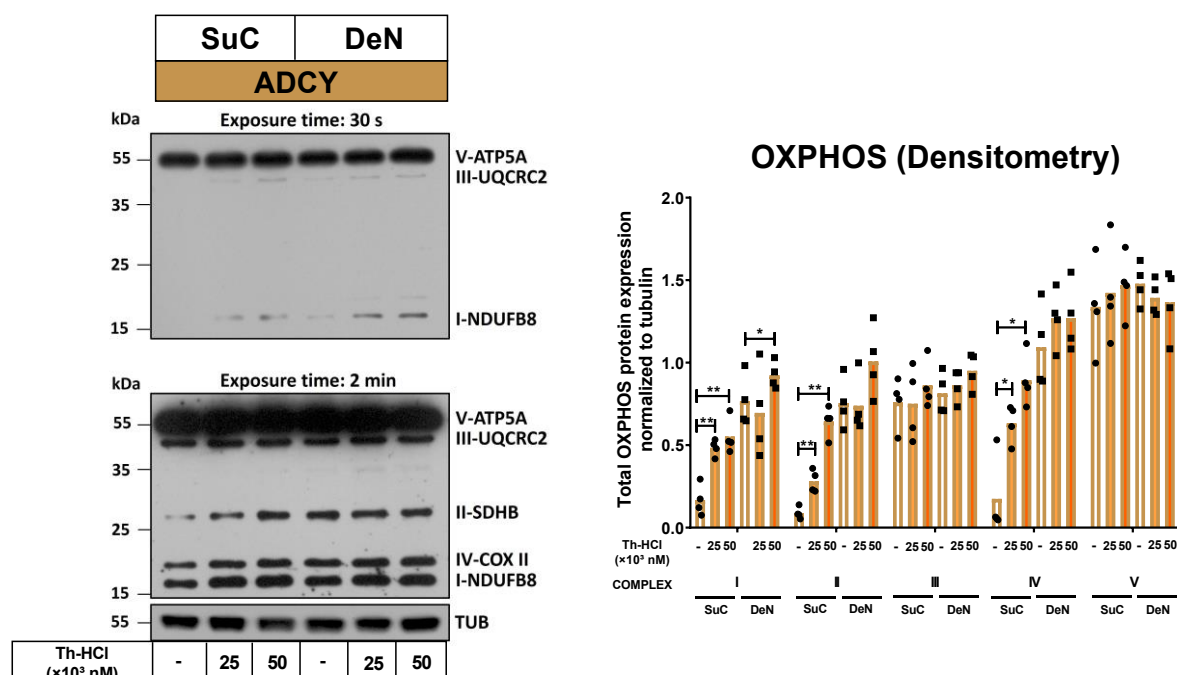


*Figure 24. Excess thiamine hydrochloride (Th-HCl) availability during adipogenic differentiation increases the expression of OXPHOS complex subunits in human subcutaneous (SuC) and deep neck (DeN)-derived white-like adipocytes (ADCYs). ADCYs were generated under standard conditions (white bars) or in medium supplemented with 25000 nM (Th-HCl 25000, light-orange bars) or 50000 nM (Th-HCl 50000, dark-orange bars) Th-HCl. Total OXPHOS subunit levels were determined by western blotting, $n = 4$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$.*

7.1.7. Effect of high thiamine levels on thermogenic gene expression during adipocyte differentiation

We hypothesized that providing excess vitamin B1 during adipogenesis would further boost the expression of genes linked to NST and mitochondrial biogenesis, in association with the observed increase in PrL respiration under high-thiamine conditions. Indeed, supplementation with additional thiamine elevated *UCPI* (**Figure 25A**) and *PGC1A* (**Figure 25B**) mRNA levels compared with control ADCYs and B-ADCYs. In ADCYs and B-ADCYs of DeN origin, however, the increase in *UCPI* transcript levels was not statistically significant. In both depots and in both phenotypes (ADCYs and B-ADCYs, respectively), 50000 nM

vitamin B1 administration resulted in the most pronounced upregulation of these markers (Figures 25A, B).

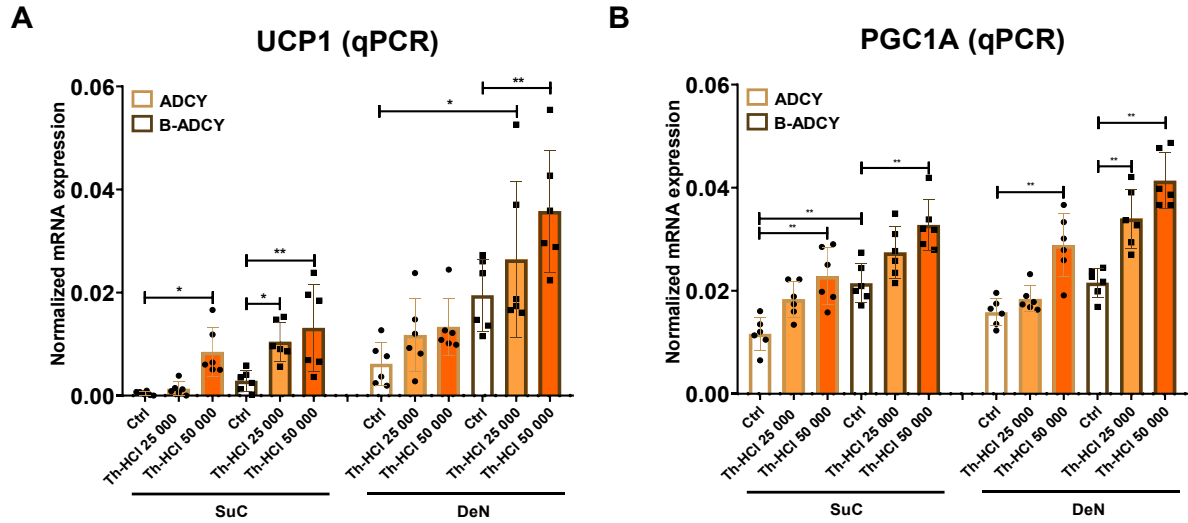


Figure 25. Excess thiamine hydrochloride (Th-HCl) availability during adipogenic differentiation enhances the expression of thermogenic markers in human SuC and DeN-derived white-like (ADCYs) and “beige”-like adipocytes (B-ADCYs). ADCYs and B-ADCYs were differentiated as described in Figure 14. (A, B) UCP1 and PGC1A mRNA expression was measured by RT-qPCR, $n = 6$. Statistical evaluation: two-way ANOVA; * $p < 0.05$, ** $p < 0.01$.

At protein level, we found that the expression of UCP1 was elevated in response to excess thiamine (25000 and 50000 nM) relative to control conditions, consistently with RT-qPCR results; this effect was statistically significant only in B-ADCYs. For PGC1A, PPAR γ -driven B-ADCY differentiation markedly enhanced its expression in SuC-derived cells, and supplementation with 50000 nM vitamin B1 further augmented the protein level of this key regulator of mitochondrial biogenesis in B-ADCYs originated from SuC area (Figure 26).

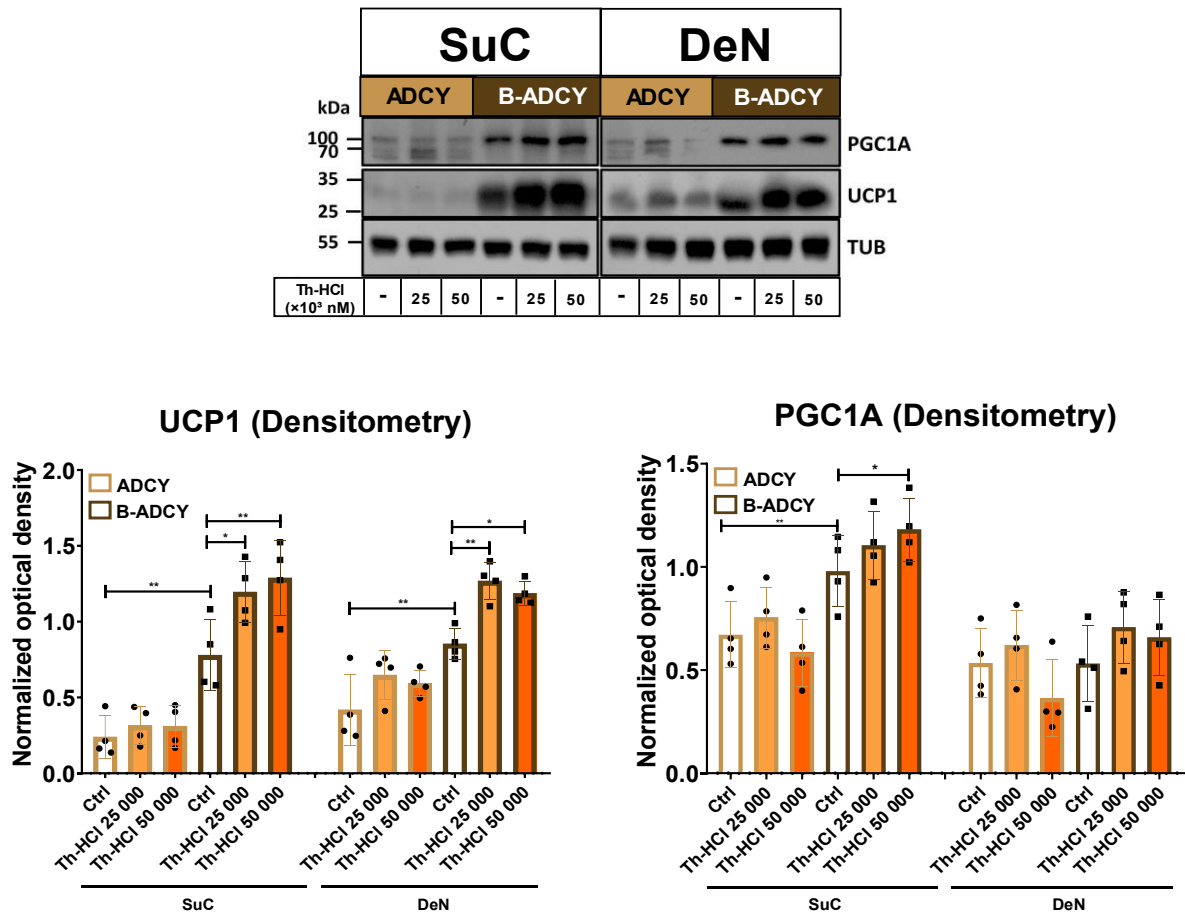


Figure 26. Excess thiamine hydrochloride (Th-HCl) availability during adipogenic differentiation enhances the expression of thermogenic markers in human subcutaneous (SuC) and deep neck (DeN)-derived white-like (ADCYs) and “beige”-like adipocytes (B-ADCYs). ADCYs and B-ADCYs were differentiated and treated as described in Figure 14. UCP1 and PGC1A protein levels were assessed by western blotting, $n = 4$. Statistical evaluation: two-way ANOVA; $*p < 0.05$, $**p < 0.01$.

7.1.8. Expression of brown/ “beige” marker and thermogenesis-related genes is enhanced by extra thiamine supplied during development of adipocytes originated from the neck region

We further analyzed the effect of surplus vitamin B1 in the differentiation medium on the mRNA expression of *CKMT2*, *CIDEA*, *DIO2*, *TBX1*, *CITED1*, and *LEP*. Except for the white adipocyte marker *LEP*, elevated thiamine increased the expression of these genes in B-ADCYs originated from both cervical depots. In SuC-derived ADCYs, *CKMT2* and *TBX1* expression rose significantly when differentiation occurred in the presence of excess vitamin B1, whereas in ADCYs originated from DeN specimens, high thiamine levels upregulated *DIO2*, *TBX1*, and *CITED1* (Figure 27).

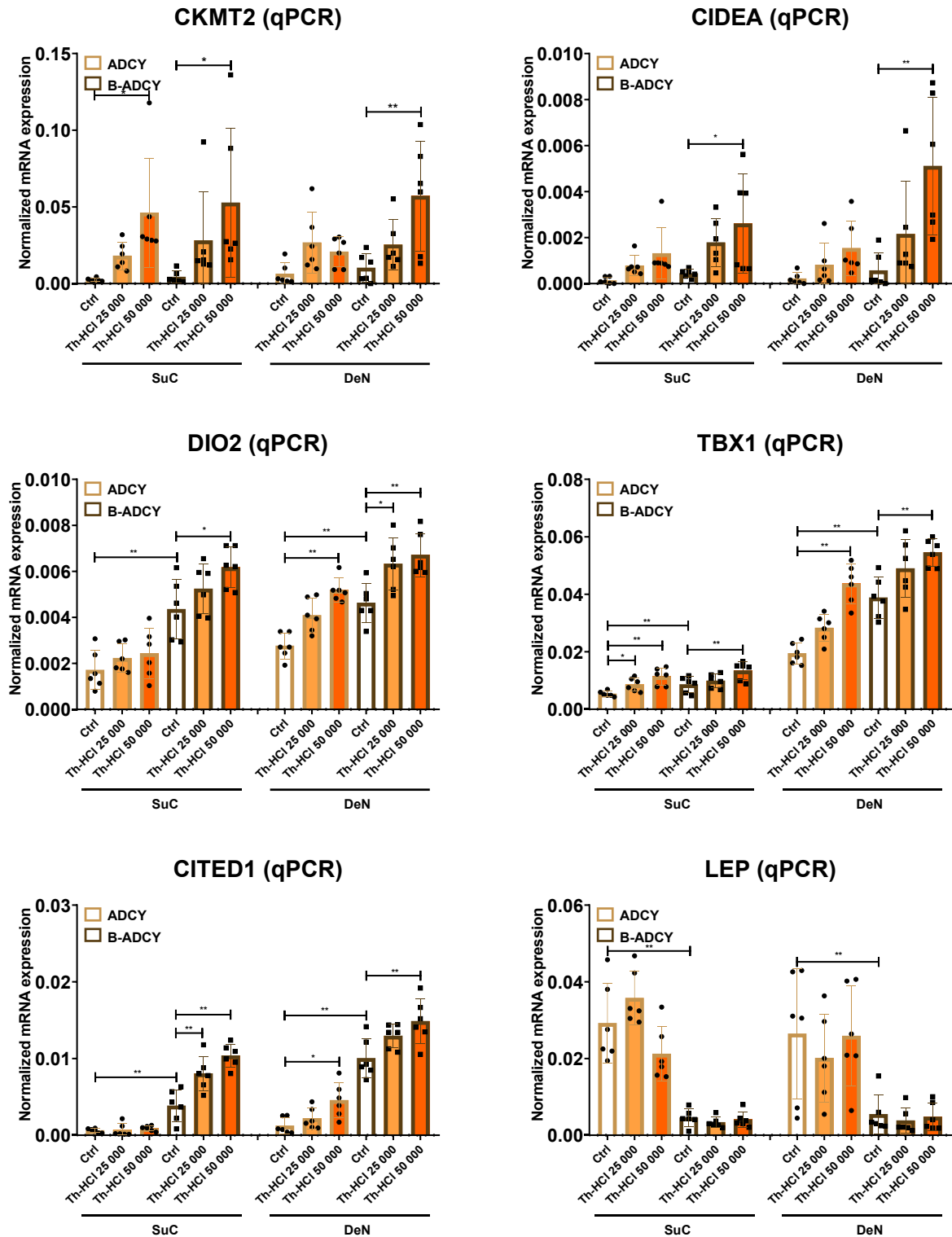


Figure 27. Excess thiamine hydrochloride (Th-HCl) availability during adipogenic differentiation increases the expression of thermogenesis-related genes in human subcutaneous (SuC) and deep neck (DeN)-derived white-like (ADCYs) and “beige”-like adipocytes (B-ADCYs). ADCYs and B-ADCYs were differentiated and treated as described in Figure 14. mRNA levels were measured by RT-qPCR, $n = 6$. Statistical evaluation: two-way ANOVA; * $p < 0.05$, ** $p < 0.01$.

7.2. The significance of vitamin B1 availability in the thermogenic activity of adipocytes in humans

7.2.1. Thiamine transporter expression is the highest in adipocytes within human WAT

By retrieving the open access snRNA-seq data, we first analyzed the expression patterns of the two ThTs in human WAT [Sun *et al.*, 2020]. *SLC19A2* expression was detected both in preadipocytes and adipocytes (**Figure 28A**), however, *SLC19A3* mRNA was only abundant in mature adipocytes (**Figure 28B**). Other cell subpopulations, such as fibroblasts, endothelium, and CD4⁺ T cells showed no detectable expression of the ThT encoding genes (**Figure 28**). This adipocyte-specific clustering of ThT expression underscores the importance of vitamin B1 for WAT function, particularly for NST and browning, in which processes thiamin accessibility was found to be pivotal for the thermogenically competent human neck-derived adipocytes.

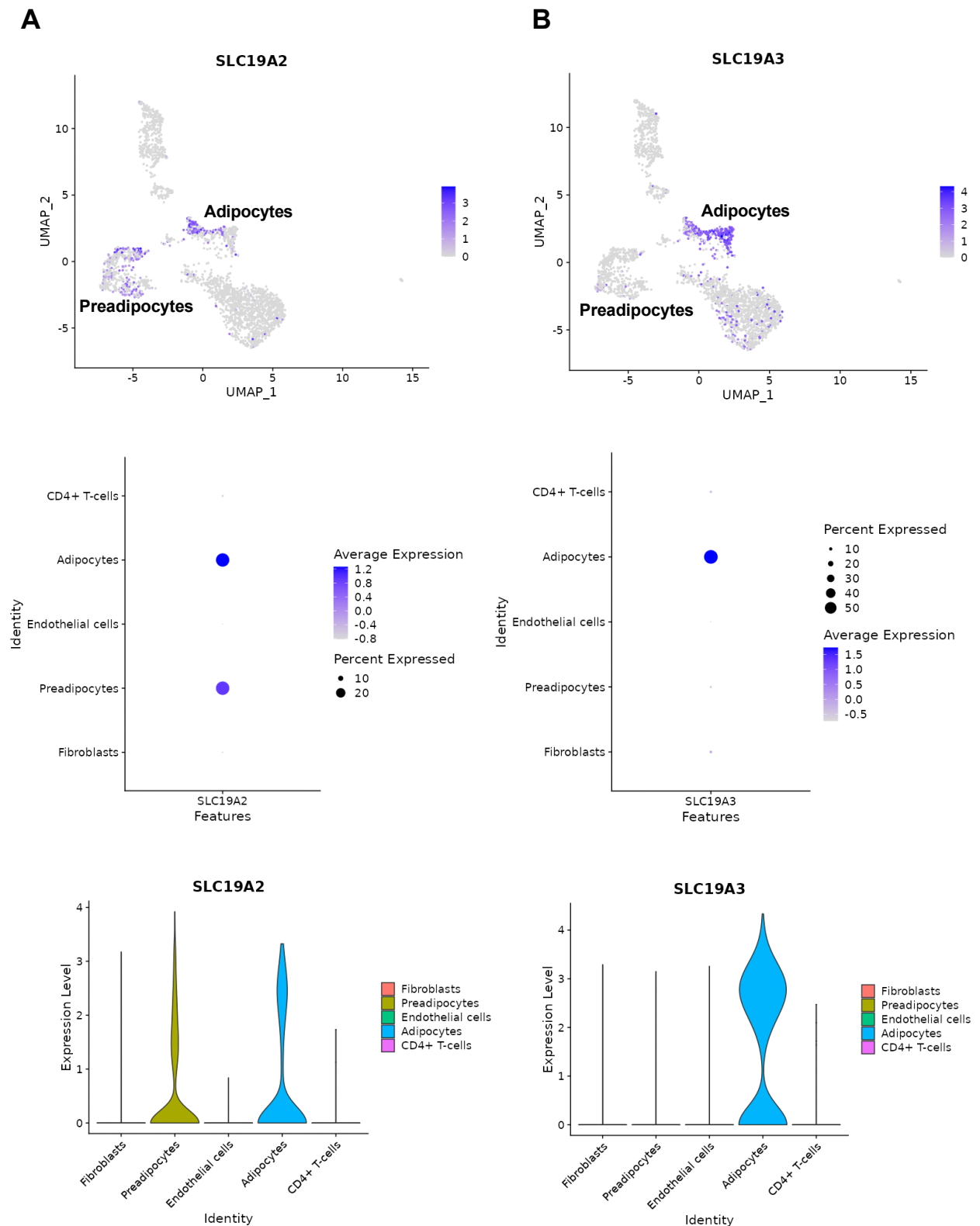


Figure 28. Expression of *SLC19A2* and *SLC19A3* in 2438 nuclei isolated from human subcutaneous white adipose tissue (SuC WAT) [Sun et al., 2020, Nature]. Panels show embedding, dot, and violin plots of single-nuclei RNA-seq data illustrating *SLC19A2* (A) and *SLC19A3* (B) expression patterns in human SuC WAT.

7.2.2. Effect of adrenergic stimulation and excess thiamine on thiamine transporter expression after SGBS adipocyte differentiation

Previously, we showed that exposing cervical-derived hASCs to higher thiamine concentrations during adipogenesis increases ThT expression (**Figures 16-17**). Accordingly, we next assessed how supplying excess thiamine (25000 or 50000 nM) after differentiation influenced basal and db-cAMP-stimulated ThT expression in SGBS adipocytes. The adipogenic differentiation was carried out under standard conditions, then cells were exposed to 0.5 mM db-cAMP with or without additional thiamine. Db-cAMP stimulation alone significantly increased *SLC19A2* mRNA levels in both ADCYs and B-ADCYs (**Figure 29A**). At protein level, db-cAMP administration resulted in a significant elevation of SLC19A2 expression in ADCYs (**Figure 29C**). When surplus vitamin B1 was provided, its inclusion at 25000 nM concentration further potentiated the adrenergic-driven upregulation of the transporter gene expression in B-ADCYs (**Figure 29A**). Treatment with 50000 nM vitamin B1 significantly enhanced both basal and db-cAMP-stimulated SLC19A2 protein expression in ADCYs, whereas in B-ADCYs, only the basal protein levels were significantly increased (**Figure 29C**). Db-cAMP-driven thermogenic activation also robustly upregulated *SLC19A3* mRNA expression in both ADCYocyte types. In ADCYs, basal *SLC19A3* transcripts were significantly higher when cells were exposed to 50000 nM vitamin B1. In B-ADCYs, db-cAMP-induced *SLC19A3* expression was further upregulated by 25000 nM or 50000 nM thiamine (**Figure 29B**). At protein level, the cell permeable cAMP analogue significantly increased SLC19A3 expression in ADCYs but not in B-ADCYs. Addition of 50000 nM vitamin B1 enhanced basal SLC19A3 protein expression in ADCYs and augmented the db-cAMP-induced level of this transporter in B-ADCYs (**Figure 29C**).

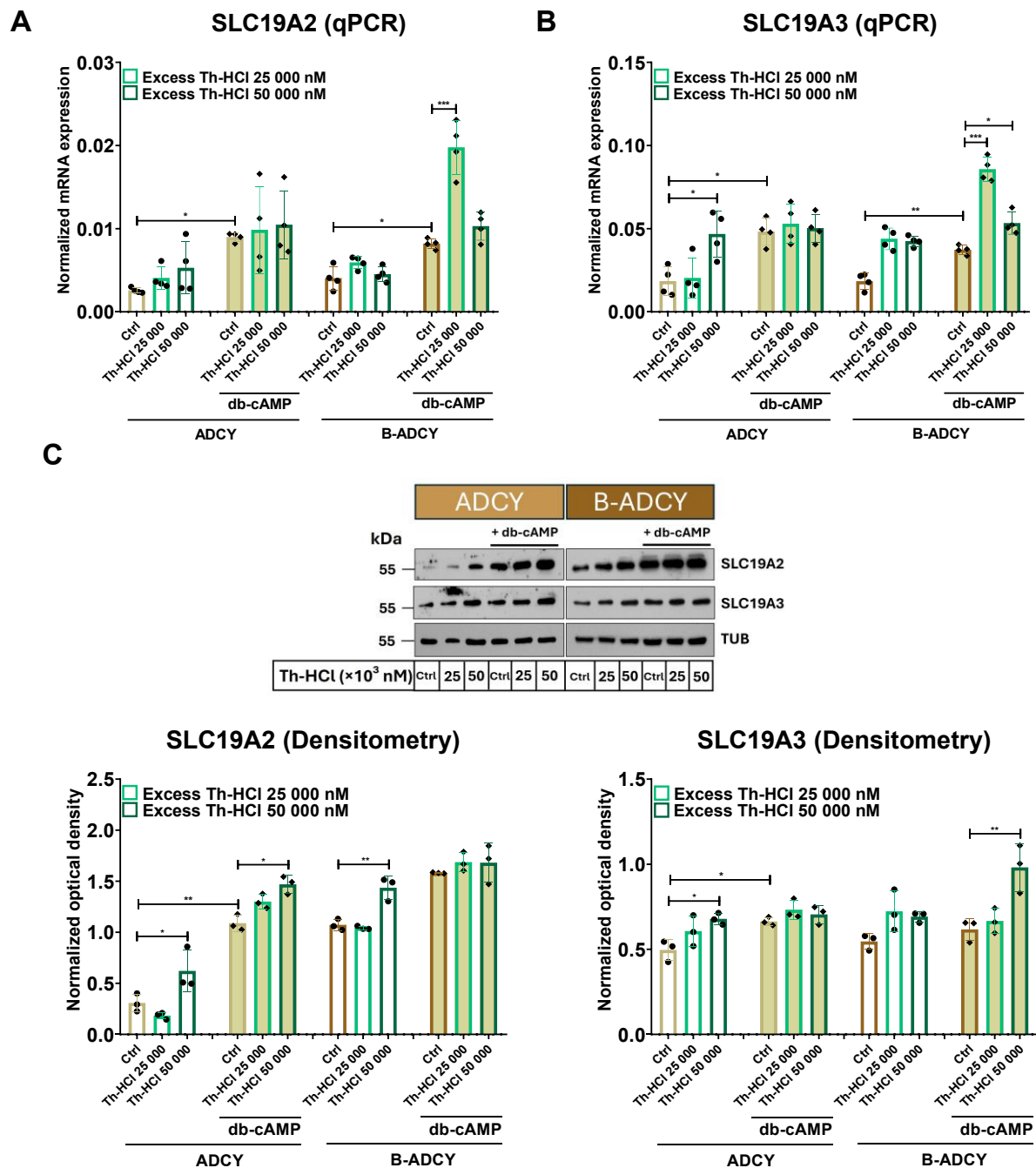


Figure 29. Effect of excess thiamine hydrochloride (Th-HCl; 25000 nM and 50000 nM) on basal and dibutyl-cAMP (db-cAMP)–stimulated ThT expression in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). Following differentiation under standard conditions, ADCYs and B-ADCYs were exposed to 0.5 mM db-cAMP (brown bars) for 10 h in the presence or absence of additional Th-HCl. (A, B) SLC19A2 and SLC19A3 mRNA levels were quantified by RT-qPCR, $n = 4$. (C) ThT protein levels were analyzed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.2.3. Reduction of db-cAMP-induced thiamine transporter activation by transporter inhibitors

We next asked whether inhibiting ThTs with fedratinib or amprolium post-adipogenesis alters basal or db-cAMP-driven ThT expression in SGBS adipocytes. ADCYs and B-ADCYs were subjected to 0.5 mM db-cAMP in the presence or absence of the ThTIs, fedratinib or amprolium After differentiation under standard condition. Db-cAMP stimulation significantly upregulated both mRNA and protein levels of the two ThTs in ADCYs, whereas in B-ADCYs the effect was confined to mRNA expression (**Figure 30A–C**). Fedratinib strongly blunted the db-cAMP-induced rise in *SLC19A2* and *SLC19A3* transcripts in both adipocyte types (**Figure 30A, B**). At protein level, this suppressive effect was evident in db-cAMP-treated B-ADCYs for both ThTs, however, in ADCYs only SLC19A3 expression was reduced (**Figure 30C**). Amprolium administration likewise markedly lowered *SLC19A2* and *SLC19A3* mRNA expression in db-cAMP-stimulated B-ADCYs (**Figure 30A, B**), while at protein level a significant decrease was detected only for SLC19A3 (**Figure 30C**).

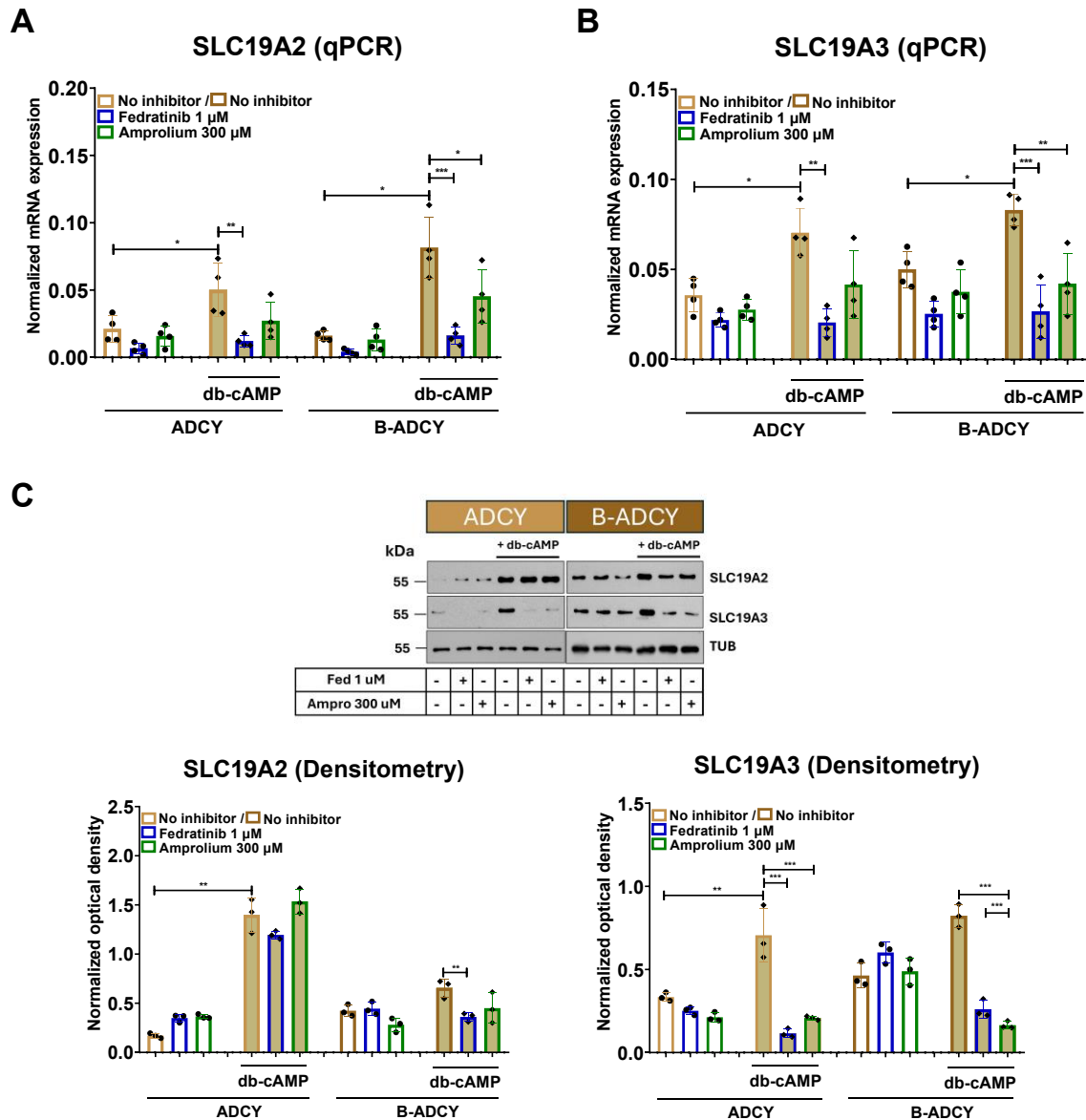


*Figure 30. Effect of thiamine transporter (ThT) inhibitors (ThTIs) on basal and dibutyryl-cAMP (db-cAMP)–stimulated ThT expression in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). Following differentiation under standard conditions, ADCYs and B-ADCYs were exposed to 0.5 mM db-cAMP (brown bars) for 10 h in the presence or absence of the ThTIs, fedratinib (1 μ M, blue outline) or amprolium (300 μ M, green outline). (A, B) SLC19A2 and SLC19A3 mRNA levels were quantified by RT-qPCR, $n = 4$. (C) ThT protein levels were analyzed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

7.2.4. Effect of thiamine transporter inhibition on coupled and uncoupled respiration in differentiated SGBS adipocytes

We next assessed the potential involvement of ThTs in the activation of NST in mature SGBS adipocytes. ADCYs and B-ADCYs were stimulated with db-cAMP in the presence of the ThTIs, fedratinib or amprolium. Basal OCR was quantified first, followed by maximal

respiration after compound injection. Oligomycin, an ATP-synthase inhibitor, was then injected to determine PrL OCR, which reflects UCP1-mediated NST. As expected, db-cAMP markedly increased OCR in both ADCYs and B-ADCYs. Db-cAMP-induced elevations in OCR and PrL respiration were significantly blunted by both fedratinib and amprolium in each cell type (Figure 31A, B and Figure 32A, B left panels). In contrast, db-cAMP-stimulated ECAR was influenced by ThT inhibition in neither ADCYs nor B-ADCYs (Figure 31A, B and Figure 32A, B right panels).

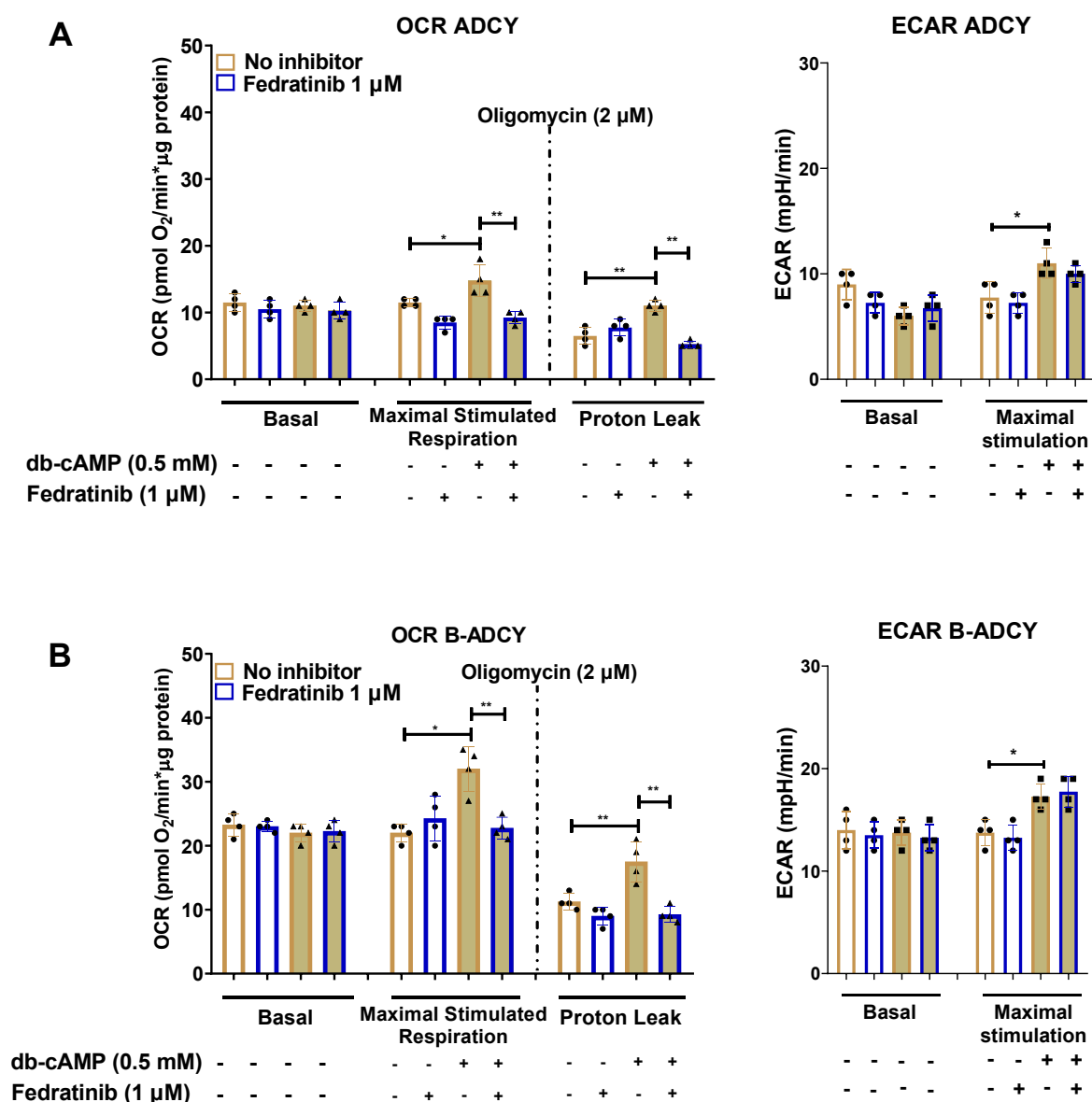


Figure 31. Effect of the SLC19A3 inhibitor fedratinib on dibutyryl-cAMP (db-cAMP)–stimulated oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). SGBS preadipocytes were differentiated to ADCYs or B-ADCYs under standard culture conditions, after which OCR was monitored for 10 h following addition of 0.5 μ M db-cAMP (brown bars) with or without 1 μ M fedratinib (blue outline). Basal OCR, maximal db-cAMP-stimulated OCR, and OCR after

oligomycin treatment (left panels), together with ECAR (right panels), were quantified in SGBS ADCYs (A) and B-ADCYs (B) from four independent experiments. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$.

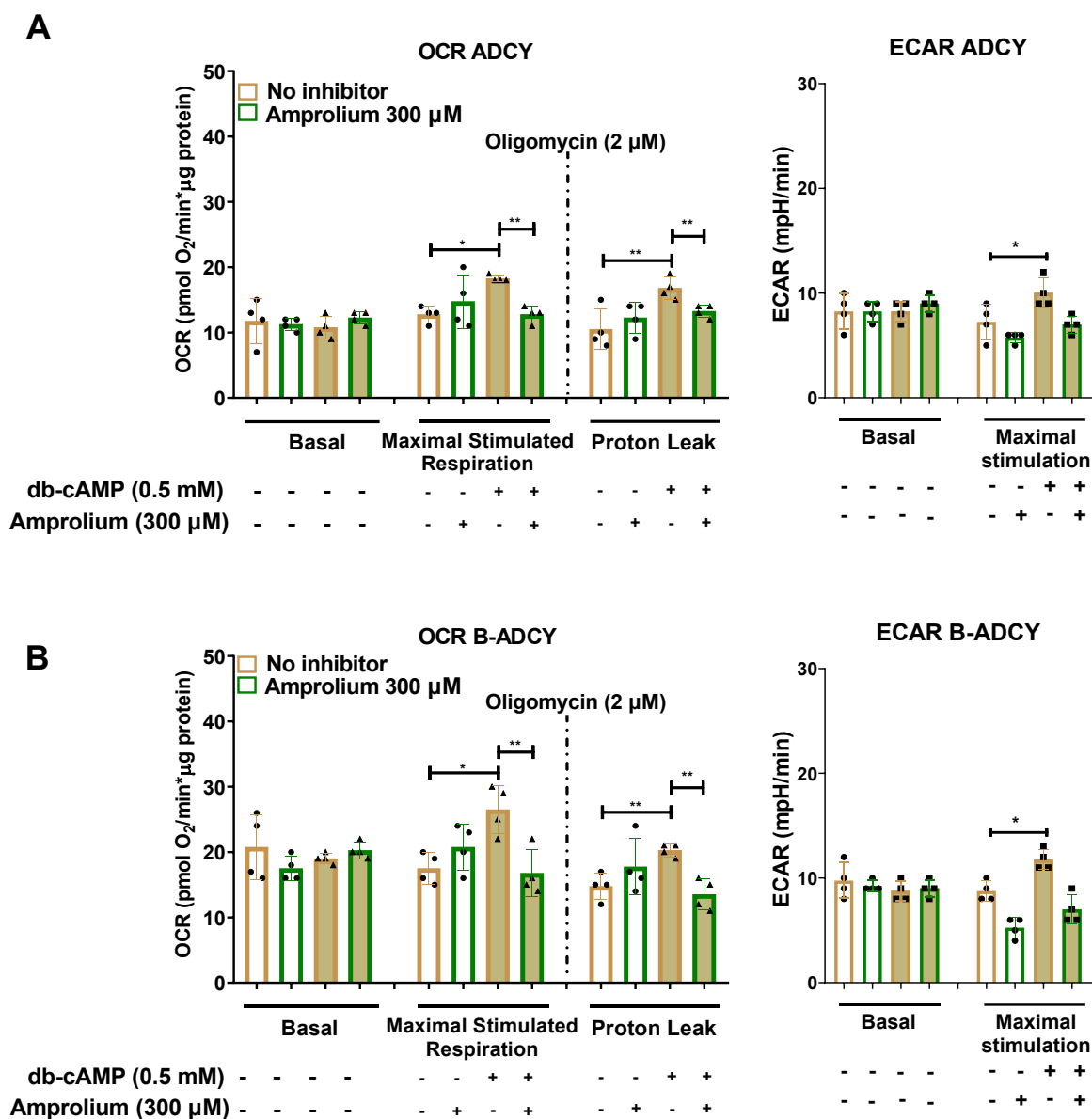


Figure 32. Effect of the thiamine transporter inhibitor amprolium on dibutyryl-cAMP (db-cAMP)-stimulated oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in SGBS white-like (ADCYs) and “beige”-like adipocytes (B-ADCYs). SGBS preadipocytes were differentiated and treated as in Figure 31 with or without 300 μM amprolium (green outline). Basal OCR, maximal db-cAMP-stimulated OCR, and OCR after oligomycin treatment (left panels), as well as ECAR (right panels), were quantified in SGBS ADCYs (A) and B-ADCYs (B) from four independent experiments. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$.

7.2.5. Post-differentiation inhibition of thiamine transporters impairs mitochondrial biogenesis in SGBS adipocytes

We next evaluated how blocking ThTs influences the abundance of mitochondrial complex subunits. In SGBS ADCYs, adrenergic stimulation strongly elevated the protein levels of subunits of complexes I–IV, whereas that of the complex V was unaffected. The db-cAMP-driven rise in complex I subunit expression was significantly hampered by both fedratinib and amprolium. Amprolium also lowered basal levels of the subunits of complexes III and IV, while fedratinib selectively reduced basal complex IV expression (Figure 33). Both ThTIs showed a tendency to decrease complex II subunit levels, but these changes were not statistically significant.

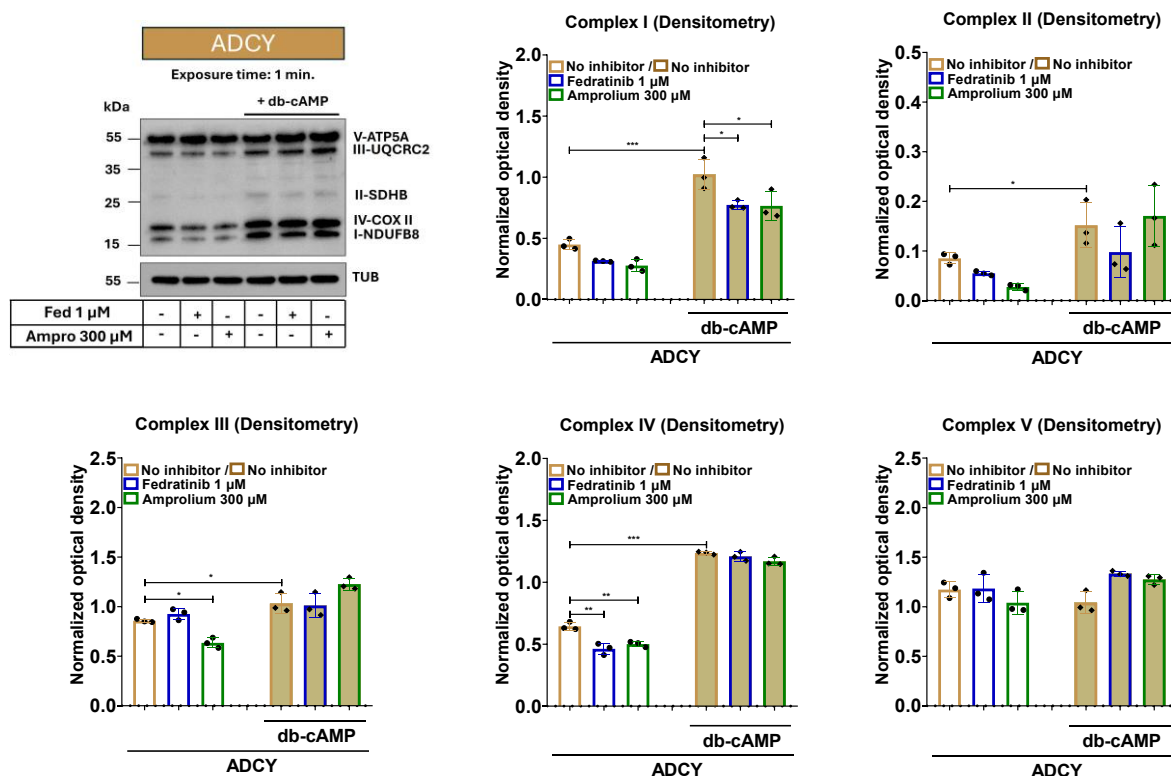


Figure 33. Effect of thiamine transporter inhibitors (ThTIs) on basal and dibutyryl-cAMP (db-cAMP)-stimulated expression of mitochondrial complex subunits in differentiated SGBS white-like adipocytes (ADCYs). SGBS preadipocytes were differentiated to ADCYs under standard culture conditions and then exposed to 0.5 mM db-cAMP (brown bars) for 10 h in the presence or absence of the ThTIs, fedratinib (1 μ M, blue outline) or amprolium (300 μ M, green outline). Mitochondrial complex subunit protein levels were analyzed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.2.6. Effect of thiamine transporter inhibition on thermogenic marker expression in differentiated SGBS adipocytes

Following the observation of reduced thermogenic capability in SGBS adipocytes when ThTIs were present, we looked into the potential effects of transporter inhibition, and therefore, thiamine availability limitation on thermogenic marker expression. According to our findings and in line with the literature, db-cAMP increased UCP1 expression at both mRNA and protein levels in both adipocyte types. This induction was largely blunted by fedratinib, except for *UCP1* gene expression in B-ADCYs. Fedratinib also lowered basal UCP1 expression in B-ADCYs at both the transcript and protein levels. Amprolium suppressed db-cAMP-driven induction of UCP1 protein expression in B-ADCYs and that of the *UCP1* mRNA in ADCYs. Additionally, the competitive ThTI reduced basal UCP1 expression in B-ADCYs at mRNA level and in ADCYs at protein level, respectively (**Figure 34A and C**). For *PGC1A*, fedratinib prevented the db-cAMP-stimulated increase in both adipocyte types at mRNA level, while amprolium resulted in a similar inhibition in B-ADCYs (**Figure 34B**). Under db-cAMP activation, both ThTIs decreased PGC1A protein abundance in ADCYs and B-ADCYs (**Figure 34C**).

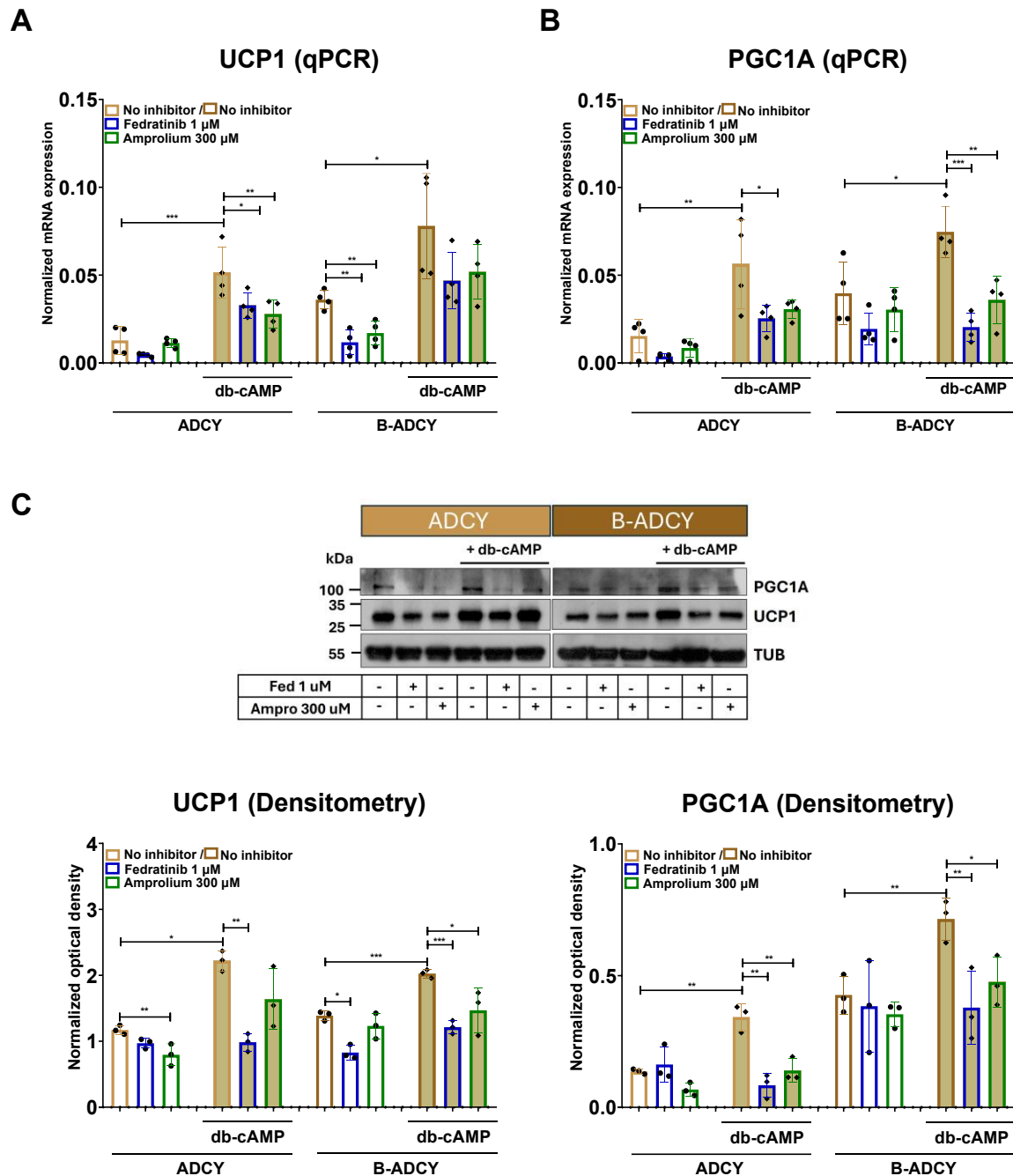


Figure 34. Effect of thiamine transporter inhibitors (ThTIs) on basal and dibutyryl-cAMP (db-cAMP)–stimulated expression of thermogenic markers, UCP1 and PGC1A, in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). Following differentiation under standard conditions, ADCYs or B-ADCYs were exposed to 0.5 mM db-cAMP (brown bars) for 10 h in the presence or absence of the ThTIs, fedratinib (1 μM, blue outline) or amprolium (300 μM, green outline). (A, B) UCP1 and PGC1A mRNA levels were quantified by RT-qPCR, $n = 4$. (C) Protein levels were analyzed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.2.7. Inhibition of thiamine transporters lowers thermogenic marker expression in mature SGBS adipocytes

Additionally, we analyzed the impact of ThTIs on the mRNA levels of several other NST-related genes in SGBS ADCYs and B-ADCYs (**Figure 35**). Db-cAMP treatment robustly upregulated *CITED1*, *DIO2*, and *TBX1* in both cell types, whereas induction of *CIDEA* and *CKMT2* was confined to B-ADCYs. In both SGBS ADCYs and B-ADCYs, fedratinib strongly blunted the db-cAMP-driven induction of these markers.

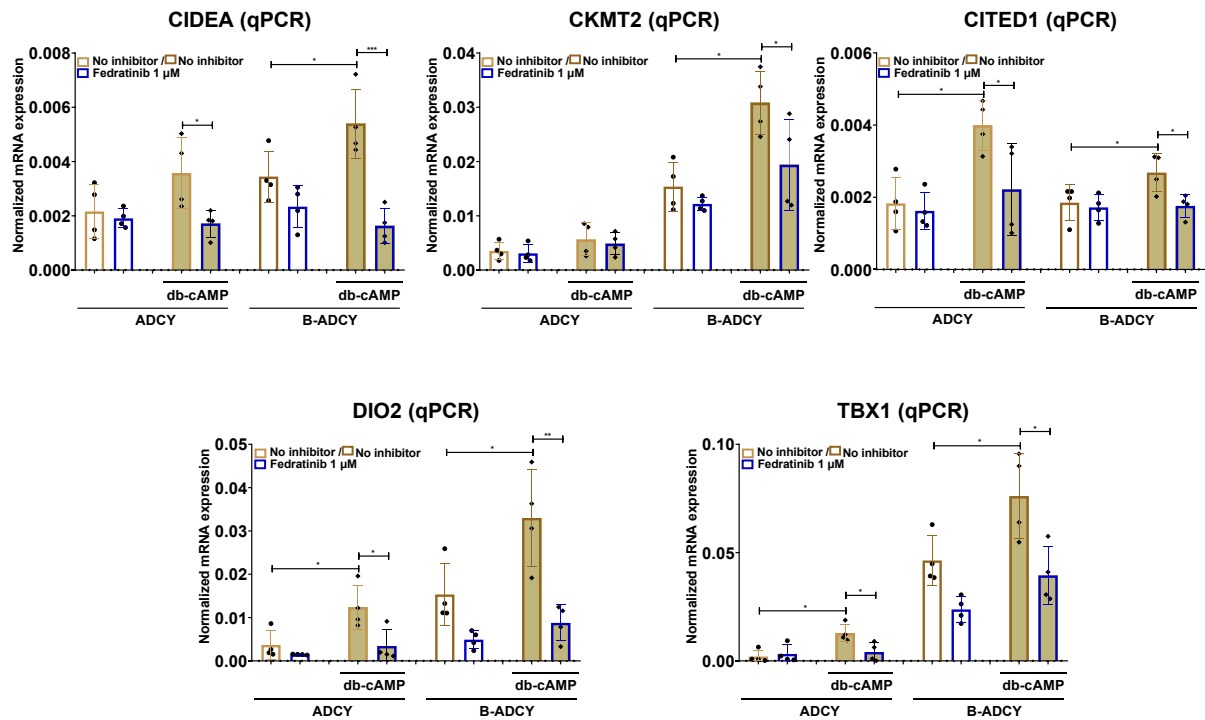


Figure 35. Effect of the *SLC19A3* inhibitor fedratinib on dibutyryl-cAMP (db-cAMP)–induced expression of thermogenic markers in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). After differentiation under standard conditions, ADCYs and B-ADCYs were exposed to 0.5 mM db-cAMP (brown bars) with or without 1 μ M fedratinib (blue outline) for 10 h. mRNA levels were measured by RT-qPCR, $n = 4$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.2.8. Effect of excessive thiamine administration on coupled and uncoupled respiration following adipogenesis

We showed that providing abundant vitamin B1 — 25000 or 50000 nM — above the level present in standard medium) during adipogenesis, enhanced the thermogenic capacity of human adipocytes (**Figure 23**). Based on our findings, we examined whether short-term exposure to high thiamine concentrations affects the thermogenic activity of differentiated SGBS ADCYs and B-ADCYs at basal conditions and following db-cAMP–mediated adrenergic stimulation. In both cell types, acute treatment with 50000 nM thiamine significantly enhanced OCR and ECAR in the absence of stimulation (**Figure 36A, B**). Additional vitamin

B1 did not further augment db-cAMP-induced changes; however, db-cAMP alone elevated maximal OCR, PrL respiration, and ECAR in both ADCYs and B-ADCYs compared with untreated cells, which is in line with our previous observations (Figure 36A, B).

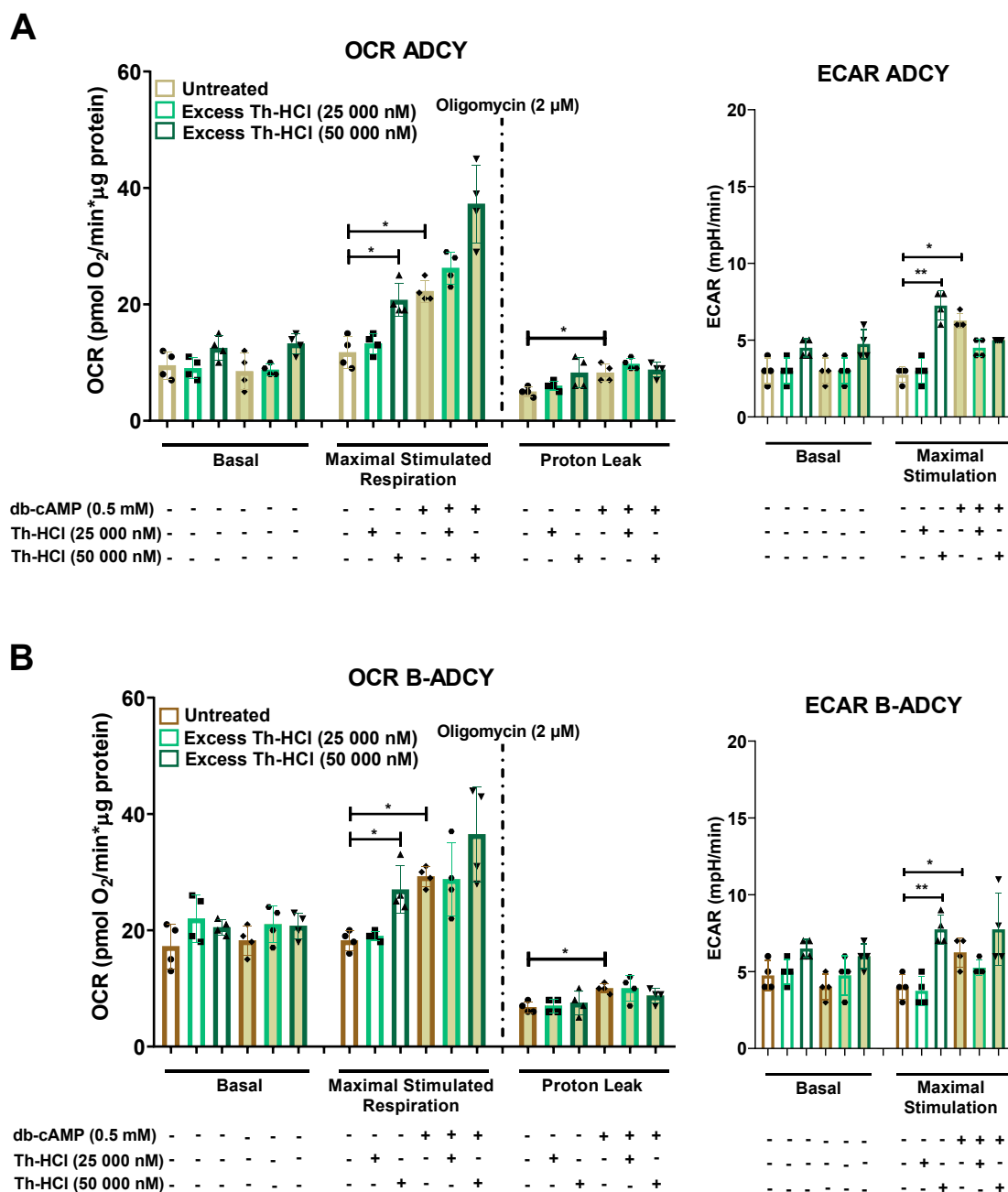


Figure 36. Effect of high thiamine hydrochloride (Th-HCl) concentrations (25000 and 50000 nM) on dibutyl-cAMP (db-cAMP)–stimulated oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). SGBS preadipocytes were differentiated to ADCYs or B-ADCYs under standard conditions, then OCR was monitored for 10 h after addition of 0.5 mM db-cAMP (brown bars) with or without excess Th-HCl (A, B). Basal OCR, maximal db-cAMP-stimulated OCR, and OCR after oligomycin treatment are shown in the left panels, and ECAR in the right panels, for SGBS ADCYs and B-ADCYs from four independent experiments. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$.

7.2.9. Enhanced mitochondrial biogenesis in SGBS adipocytes induced by high thiamine levels during differentiation

We next analyzed how abundant vitamin B1 affects the expression of the subunits of mitochondrial respiratory-chain components in ADCYs. Consistent with the results in **Figure 33**, db-cAMP-driven activation strongly augmented the protein levels of complexes I–IV, but not complex V, in SGBS ADCYs. Exposure to 25000 nM thiamine significantly elevated complex I and IV subunit levels, while that of the complexes II, III, and V remained unchanged. In contrast, 50000 nM thiamine produced a marked increase with regard to complexes I–IV (**Figure 37**). Complex II subunit showed even stronger upregulation when cells were additionally stimulated with db-cAMP. Neither thiamine nor db-cAMP altered the expression of the ATP synthase subunit (**Figure 37**).

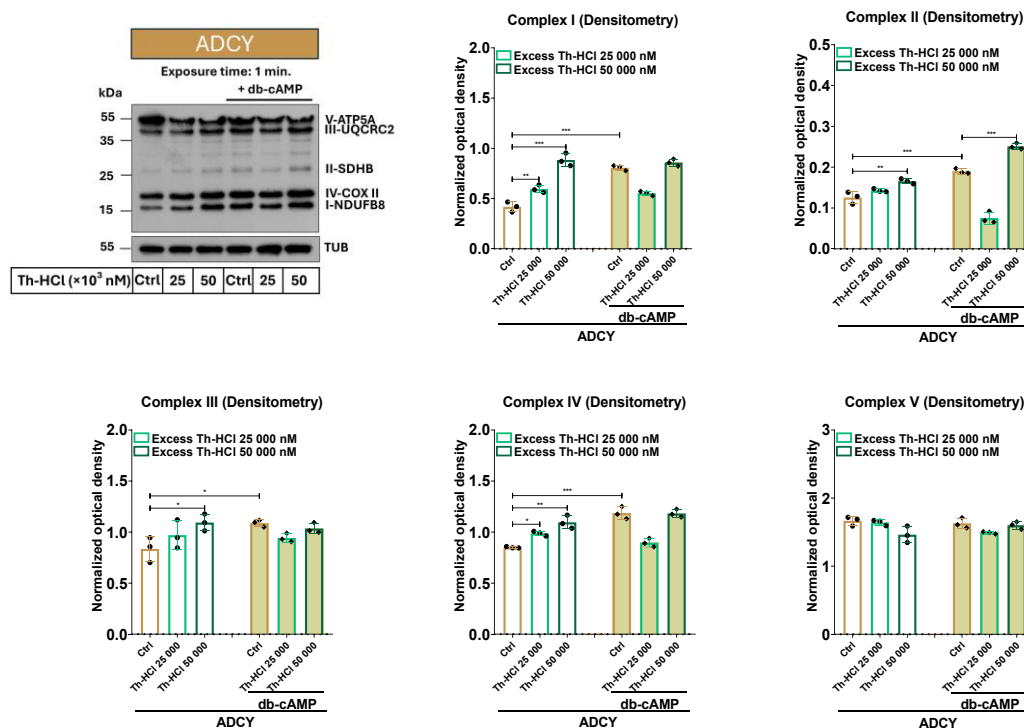


*Figure 37. Effect of excess thiamine hydrochloride (Th-HCl; 25000 and 50000 nM) on dibutyryl-cAMP (db-cAMP)–stimulated expression of mitochondrial complex subunits in differentiated SGBS white-like adipocytes (ADCYs). After differentiation under standard conditions, ADCYs were exposed to 0.5 mM db-cAMP (brown bars) for 10 h in the presence or absence of additional Th-HCl. Protein levels were assessed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.*

7.2.10. Following adipocyte development, high levels of thiamine increase the expression of thermogenic markers

After observing that high vitamin B1 levels enhance mitochondrial catabolism, we postulated that excess thiamine (25000 and 50000 nM) applied after SGBS adipocyte differentiation might further promote the expression of genes governing mitochondrial biogenesis and NST. Our findings demonstrated that the db-cAMP-stimulated increase in mRNA levels of *PGC1A* in ADCYs and *UCPI* in B-ADCYs was further enhanced by extra thiamine (**Figure 38A, B**). PGC1A protein expression of unstimulated ADCYs was elevated by surplus vitamin B1 at a concentration of 25000 nM. 50000 nM thiamine likewise upregulated *PGC1A* mRNA in B-ADCYs and PGC1A protein levels in unstimulated ADCYs (**Figure 38B, C**). Furthermore, 50000 nM thiamine elevated UCP1 protein levels in ADCYs under db-cAMP stimulation and in B-ADCYs at basal conditions (**Figure 38C**).

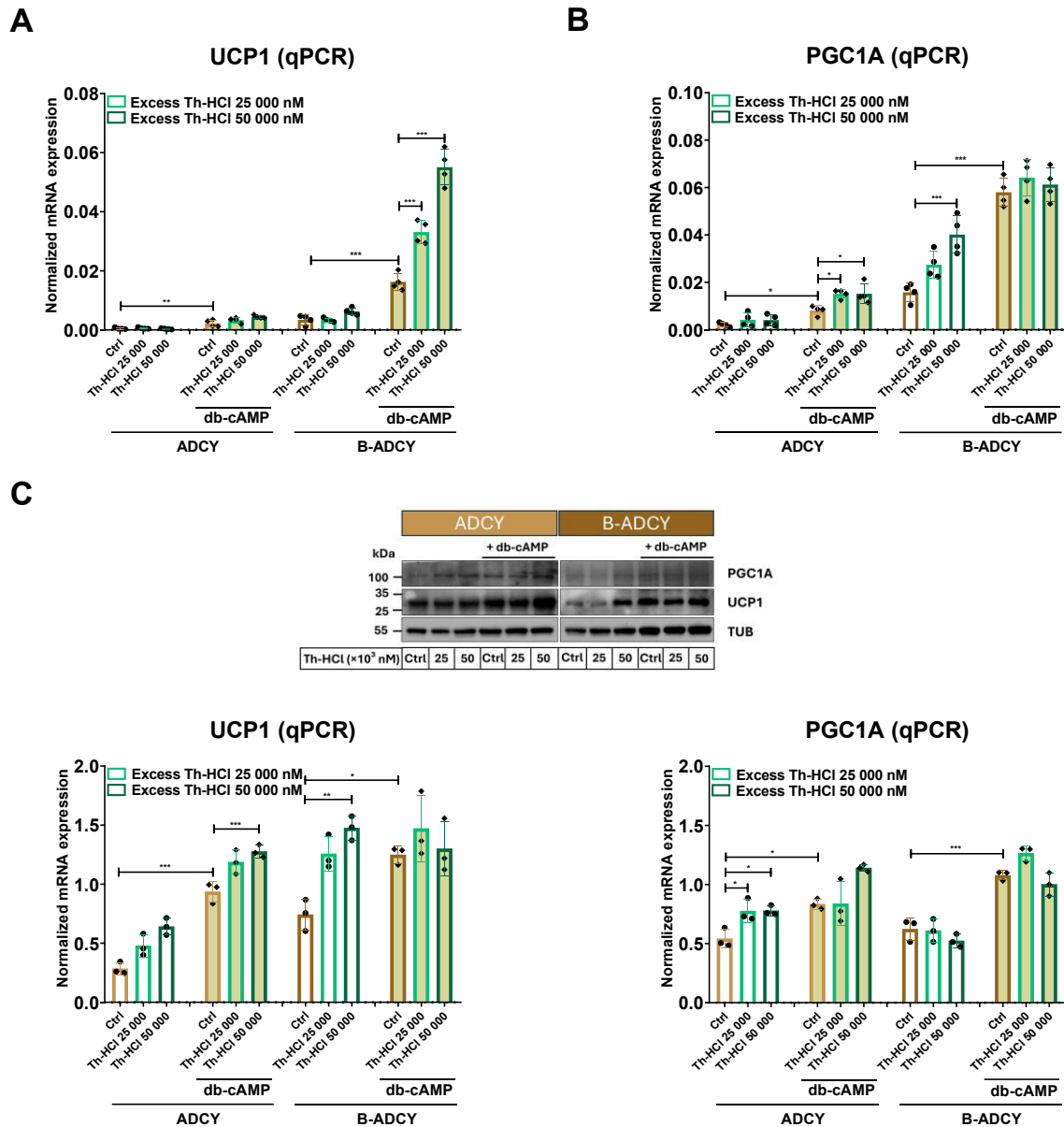


Figure 38. Effect of excess thiamine hydrochloride (Th-HCl; 25000 and 50000 nM) on basal and dibutyryl-cAMP (db-cAMP)–stimulated expression of thermogenic markers in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). Following differentiation under standard culture conditions, ADCYs and B-ADCYs were exposed to 0.5 mM db-cAMP (brown bars) for 10 h in the presence or absence of additional Th-HCl. (A, B) UCP1 and PGC1A mRNA levels were quantified by RT-qPCR, $n = 4$. (C) Protein levels were analyzed by western blotting, $n = 3$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

7.2.11. Effect of high thiamine levels on thermogenic marker expression following adipocyte development

We further examined the impact of high thiamine levels on additional NST-related genes. Our findings demonstrated that during adrenergic stimulation, excess thiamine further elevated the mRNA expression of *TBX1*, *DIO2*, and *CIDEA* in ADCYs (Figure 39A, D, E). In contrast,

increased thiamine during thermogenic activation in B-ADCYs enhanced the mRNA levels of *CMKT2*, *CITED1*, *DIO2*, and *TBX1* (**Figure 39B-E**). Thiamine alone, in the absence of db-cAMP, also upregulated *CITED1* and *DIO2* in ADCYs, and *CKMT2* and *CITED1* in B-ADCYs (**Figure 39B, C, D**).

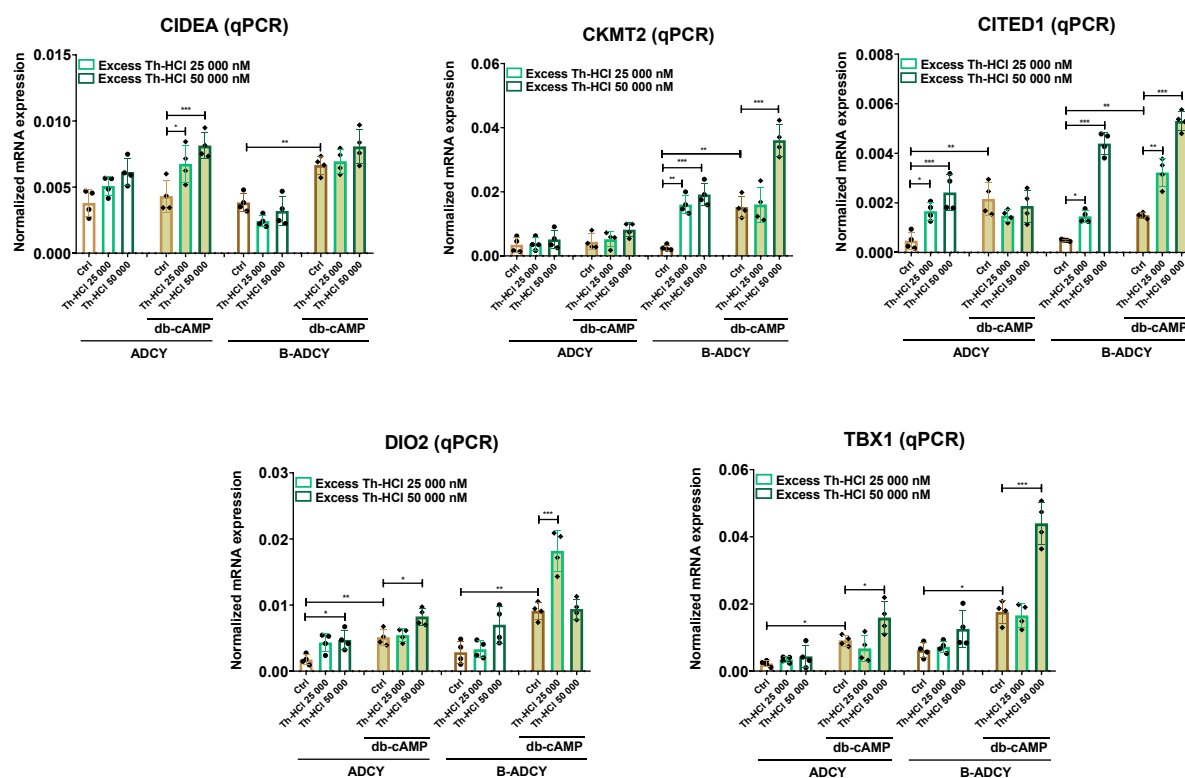


Figure 39. Effect of excess thiamine hydrochloride (Th-HCl; 25000 and 50000 nM) on basal and dibutyryl-cAMP (db-cAMP)–stimulated expression of thermogenic markers in white-like (ADCYs) and “beige”-like SGBS adipocytes (B-ADCYs). After differentiation under standard conditions, ADCYs and B-ADCYs were exposed to 0.5 mM db-cAMP (brown bars) for 10 h in the presence or absence of additional Th-HCl. (A–E) mRNA levels were quantified by RT-qPCR, $n = 4$. Statistical evaluation: one-way ANOVA; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

8. DISCUSSION

8.1. Modulation of thermogenesis in human neck-derived adipocytes: the role of SLC and vitamin B1 transporters in the differentiation and browning of adipocytes

Tóth *et al.* previously analyzed mature adipocyte populations originated from the SVFs of paired DeN and SuC fat depots from nine donors [Tóth *et al.*, 2020]. Using bulk RNA-seq, they characterized global gene expression profiles to uncover the heterogeneous molecular machineries involved in thermogenic regulation. Conventional ADCY or B-ADCY, which promoted adipocyte browning, differentiation protocols were applied to induce adipogenesis. Regardless of which protocol was used [Tóth *et al.*, 2020], DeN exhibited a higher composition of brown adipocytes and greater browning capacity compared with adipocytes originating from the SuC depot, as confirmed by the scores quantified by publicly accessible tools, BATLAS and ProFAT [Cheng *et al.*, 2018; Perdikari *et al.*, 2018; Tóth *et al.*, 2020].

The identified differentially expressed genes involved 21 genes that encode SLC transporters, which facilitate the uptake of nutrients and are recognized for their involvement in various biological functions. The high energy requirements of metabolically active brown/“beige” adipocytes likely necessitate increased expression of these transporters to secure sufficient uptake of key nutrients or co-factors for ATP production. Notably, ThT2— encoded by *SLC19A3* — was among the SLC transporters whose expression was elevated in DeN-derived adipocytes: its levels rose during adipocyte differentiation and were markedly higher in DeN cells than in adipocytes originating from the SuC area. We have also reported that elevated levels of thiamine, the substrate of SLC19A3, were necessary for the optimal adrenergic-mediated thermogenic response of adipocytes derived from neck adipose depots [Arianti *et al.*, 2023].

A recent study demonstrated that vitamins B1, B2, and B5 are critical for the adipogenic conversion of human dermal fibroblasts into brown adipocytes [Takeda and Dai, 2024]. Building on this, we examined how extracellular vitamin B1 accessibility during adipogenesis influences the thermogenesis potential of human adipocytes originating from the neck region. Previous studies have highlighted the physiological relevance of SLC19A3 in both intestinal thiamine absorption and systemic energy metabolism. For instance, reduced SLC19A3 expression has been reported in SuC WAT of individuals with obesity compared to lean subjects, with its expression positively correlating with weight loss, suggesting a potential role in metabolic regulation [Pereira *et al.*, 2021]. In murine models, SLC19A3 was shown to compensate for the absence of SLC19A2, as SLC19A3-deficient mice exhibited impaired

intestinal thiamine absorption, while SLC19A2 deficiency alone did not affect plasma thiamine levels [Reidling *et al.*, 2010; Marce-Grau *et al.*, 2019]. Our results showed that both surplus and gradually increased thiamine concentrations enhanced SLC19A2 and SLC19A3 mRNA and protein levels in ADCYs and B-ADCYs from both cervical depots (**Figure 14C, D, Figure 15, Figure 16C, D, Figure 17**).

8.2. Concentration-dependent control of thermogenesis by thiamine during adipocyte differentiation

Certain dietary components are notably high in thiamine, such as whole grains, brown rice, legumes, and several types of meat products [Sharma *et al.*, 2013]. The current recommended daily allowance for adults is 1.2 mg/day for men and 1.1 mg/day for women, with higher requirements during pregnancy [Turck *et al.*, 2016]. Despite the presence of thiamine in numerous food items, typical diets may lack sufficient micronutrients. Vitamin B1 deficiency primarily affects the nervous, cardiovascular, and gastrointestinal systems, and generally develops when the recommended daily intake is not met over time. This deficiency can arise from impaired intestinal absorption or from increased urinary loss of thiamine, for example in chronic alcoholism or malnutrition [Thomson *et al.*, 1970; Fouarge *et al.*, 2019]. The most frequent clinical syndromes linked to thiamine deficiency include WKS and Beriberi [Mateos-Díaz *et al.*, 2022]. WKS is characterized by ataxia, memory impairment, and hypothermia, the latter commonly occurring as a secondary manifestation. Experimental and clinical data indicate that thiamine deficiency can damage the hypothalamus, a key center controlling body temperature and feeding behavior [Tanev *et al.*, 2008]. Clinical observations further suggest that hypothermia can be reversed by thiamine supplementation even within 48 hours [Hansen *et al.*, 1984].

Our findings demonstrated that the omission of vitamin B1 from the differentiation cocktail that governed adipogenesis resulted in diminished thermogenic capabilities of adipocytes derived from the human neck area. This was evidenced by reduced mitochondrial respiration and PrL OCR that is indicative of heat generation via UCP1-mediated uncoupling (**Figure 18A, B**), and lowered expression of thermogenic markers (**Figure 20A, B, Figure 21, Figure 22A-F**). On the contrary, increasing thiamine concentrations throughout differentiation resulted in an enhancement of thermogenic capacity and thermogenic marker expression in a concentration-dependent manner (**Figure 18A, B, Figure 19, Figure 20A, B, Figure 21, Figure 22A-F**), thereby indicating the advantageous role of vitamin B1 in directly enhancing the browning potential of human neck area-derived adipocytes.

8.3. Supplementation of vitamin B1 enhances the thermogenic potential of neck-derived adipocytes

Thiamine levels in standard cell culture media are around 10000 nM, whereas circulating vitamin B1 in human plasma is typically in the nanomolar range. In our study, supplementing standard medium with 25000 nM thiamine during differentiation of neck-derived adipocytes significantly increased basal, maximal, and PrL respiration, consistent with enhanced heat production (**Figure 23A, B**). High thiamine availability during differentiation also upregulated several subunits of ETC complexes (**Figure 24**). Moreover, thiamine supplementation throughout *ex vivo* adipogenesis strengthened the expression of thermogenic markers in adipocytes from the thermogenically favorable cervical depot (**Figure 25A, B, Figure 26, Figure 27A-F**). Brown/“beige” adipocytes are attractive therapeutic targets for combatting obesity and DM, primarily due to their substantial capacity to act as sinks for glucose and FAs [Wu *et al.*, 2006; Cypess *et al.*, 2009]. Implantation of hASCs that had been differentiated into brown/“beige” adipocytes into host mice improved glucose disposal and increased EnE [Singh *et al.*, 2020]. However, even when hASCs are successfully converted into “beige” adipocytes, achieving a sufficiently strong thermogenic output *in vivo* remains difficult. Our data suggests that supplying additional extracellular thiamine during the differentiation period enhances PrL respiration, indicating higher heat production, even in the absence of acute stimulation.

8.4. Impact of thiamine transporter inhibition on the thermogenesis in SGBS adipocytes

The SGBS cell line provides a highly adaptable differentiation model that shows resemblance to white adipogenesis of human SuC origin [Wabitsch *et al.*, 2001; Yeo *et al.*, 2017; Halbgebauer *et al.*, 2020; Tews *et al.*, 2022]. Mature, differentiated SGBS adipocytes show traits of primary human fat cells, such as significant browning properties that include the expression of UCP1 [Klusóczycki *et al.*, 2019; Colitti *et al.*, 2022]. Because of these characteristics, SGBS cells are widely employed as a controlled *in vitro* system to investigate adipocyte metabolism and to elucidate mechanisms underlying obesity and type 2 DM [Wabitsch *et al.*, 2001; Tews *et al.*, 2022]. Verifying experimental results derived from primary human adipocytes is essential using cell line models, such as SGBS, to assure reproducibility and to address challenges linked to donor variability in primary cells. Although primary hASCs isolated from WAT are widely studied as a model of adipose functions, their characteristics may be strongly influenced by donor-related parameters — such as lifestyle, metabolic state, and genetic background — which can introduce considerable variability and unpredictably affect experimental outcomes.

Analyzing publicly available snRNA-seq results from human SuC WAT, we discovered that ThT expression is confined to the adipocyte subpopulation [Sun *et al.*, 2020], emphasizing the importance of these transporters for proper adipocyte function within WAT (**Figure 28A, B**). By allowing researchers to get beyond the limitations that result from donor variability and restricted biopsy materials, the SGBS cell line offers an alternative solution to cope with these problems [Claussnitzer *et al.*, 2015; Ali *et al.*, 2023]. In our earlier work, we showed that pharmacological blockade of ThTs attenuates activation of NST in human neck-derived adipocytes [Arianti *et al.*, 2023]. In line with this, we now observe that inhibiting ThTs with either fedratinib or amprolium causes a moderate but significant repression in the expression of several thermogenic markers, although their expression is not completely abolished (**Figure 34A-C, Figure 35A-E**), and reduced cellular respiration in SGBS adipocytes (**Figure 31A,B, Figure 32A,B, Figure 33**).

Fedratinib preferentially targets SLC19A3, displaying a much lower IC₅₀ for SLC19A3 (1.36 μ M) than for SLC19A2 (7.10 μ M) [Zhang *et al.*, 2014; Giacomini *et al.*, 2017]. Amprolium, a close structural analogue of thiamine, acts as a competitive inhibitor of vitamin B1 uptake and thereby substantially limits cellular thiamine accumulation [Bettendorff *et al.*, 1995; Park *et al.*, 2000]. While fedratinib is mainly used as a SLC19A3 inhibitor, it is also known to inhibit JAK2, which raises the possibility that some of its effects on NST might be related to JAK2 inhibition. However, previous studies showed that JAK2 is only required for BAT activation when other cell types, like immune cells, are present, and it's not essential for UCP1 induction in “beige” adipocytes [Shi *et al.*, 2016]. Since our experiments were done under *in vitro* conditions using the SGBS adipocyte cell line, which does not contain other cell types, it is unlikely that JAK2 contributed primarily to the observed effects. To eliminate any doubts, we conducted all experiments using amprolium as well, which had similar effects to those of fedratinib, confirming the reliability of our findings. Similar results were observed by using human primary neck area-derived adipocytes differentiated *ex vivo* [Arianti *et al.*, 2023].

The metabolically active derivative of thiamine, TPP functions as an essential coenzyme for both PDH and α -KGDH. Decrease in vitamin B1 influx may result in blunted PDH activity, consequently limiting the production of the high-energy reduced equivalent, NADH. Recent studies have indicated that the uptake of BCAAs is elevated in murine BAT mitochondria, compared to WAT and systemic levels, particularly during cold exposure or β -adrenergic stimulation [Park *et al.*, 2023; Verkerke *et al.*, 2024]. The breakdown of BCAAs is mediated by a TPP-dependent enzyme complex known as BCKDH; thus, adequate thiamine uptake is essential for maintaining the high enzymatic activity of BCKDH.

8.5. Thiamine supplementation enhances thermogenic gene expression in SGBS adipocytes

In adult humans, WAT can account for up to 70% of total body mass [Hausman *et al.*, 2001; Parlee *et al.*, 2014]. Higher WAT content closely associates with the occurrence of obesity. Notably, *SLC19A3* expression is markedly reduced in SuC WAT from individuals with obesity compared with lean subjects [Pereira *et al.*, 2021]. Transcriptomic profiling has shown that thermogenic adipocytes derived from abdominal SuC WAT upregulate both *SLC19A2* and *SLC19A3* in response to forskolin-induced adrenergic stimulation [Min *et al.*, 2019]. Consistent with this, we found that db-cAMP-mediated adrenergic signaling elevated *SLC19A2* expression in SGBS adipocytes, indicating that vitamin B1 import via ThTs is important for an appropriate thermogenic response (**Figure 29A–C**). Furthermore, our data show that adding thiamine during db-cAMP treatment augments the expression of UCP1 and other NST-related markers in differentiated SGBS adipocytes (**Figures 38C and 39A–F**).

The exact mechanism by which thiamine contributes to thermogenic gene induction during adrenergic stimulation in adipocytes remains unclear. Thiamine may affect *UCP1* gene transcription, which is regulated by the 5' non-coding region containing elements that control tissue specificity and differentiation. These elements interact with transcription factors like cAMP-responsive proteins and the PGC-1 α co-activator [Villaroya *et al.*, 2017]. *UCP1* transcription is further modulated by cAMP signaling and rapid phosphorylation of key transcription factors such as CREB and ATF2 [Robidoux *et al.*, 2005].

8.6. The clinical importance of thiamine

Although thiamine deficiency is relatively rare in industrialized nations, it has become increasingly prevalent among individuals with chronic alcohol use in Europe, North America, and Australia. [DiNicolantonio *et al.*, 2018]. Additionally, in bariatric interventions for the treatment of obesity, such as gastric bypass surgery, 15.5–29% of patients develop thiamine deficiency [Carrodeguas *et al.*, 2005; Kerns *et al.*, 2015; Flancbaum *et al.*, 2006; Lee, 2023]. Patients with DM also experience the consequences of thiamine deficiency, which results in increased oxidative stress, endothelial dysfunction, and cellular damage brought on by hyperglycemia [DiNicolantonio *et al.*, 2018]. 17–79% of DM patients had reduced erythrocyte transketolase activity [Jermendy, 2006; Page *et al.*, 2011]. In a cohort of 120 individuals with type 2 DM, including 46 with microalbuminuria, vitamin B1 deficiency (plasma thiamine < 70 nM) was detected in 98% of those with microalbuminuria and in 100% of those without, respectively. Notably, 100% of the healthy control group also exhibited low plasma thiamine levels, and the concentration of TPP was even higher in the diabetic group compared to controls

[Nix *et al.*, 2015]. Moreover, obesity, DM, and high intake of sugar-sweetened beverages have been associated with thiamine deficiency in children, even in high-income countries such as the USA [Rakotoambinina *et al.*, 2021].

Vitamin B1 supplementation has also been shown to ameliorate endothelial dysfunction, a key contributor to vascular damage in DM [Ascher *et al.*, 2001; Wong *et al.*, 2008]. Collectively, these data indicate that the lack of thiamine may worsen the metabolic disturbances associated with obesity and DM, and that thiamine supplementation could offer therapeutic benefits in these conditions. Thiamine supplemented for six weeks at 100 mg three times daily (100 times higher than the recommended daily allowance) alleviated glucose intolerance in individuals with hyperglycemia [Thornalley *et al.*, 2007]. In another trial, a daily dose of 150 mg vitamin B1 for four weeks significantly lowered fasting plasma glucose in drug-naïve individuals with type 2 DM [Alaei Shahmiri *et al.*, 2013]. Thiamine treatment has also been reported to improve lipid parameters and serum creatinine levels in individuals with type 2 DM [González-Ortiz *et al.*, 2011]. Additional conditions that increase the risk of vitamin B1 deficiency include immune disorders, cardiac failure, hyperthyroidism, and dialysis-dependent renal failure [DiNicolantonio *et al.*, 2018].

TPP, which is produced from thiamine intracellularly, is pivotal for the optimal functioning of enzyme complexes, such as PDH, in the mitochondrial matrix. The importance of this vitamin in male Otsuka Long-Evans rats was highlighted by Tanaka *et al.*; however, how micronutrient availability influences the differentiation of human adipocytes is still largely unrevealed [Tanaka *et al.*, 2010]. Hirata *et al.* [Hirata *et al.*, 2020] described that thiamine promoted T-lymphocyte maturation by inducing production of TGF- β family members in thymic stromal cells, pointing to vitamin B1 as a critical nutritional regulator in immune cell development.

High-protein diet combined with vitamin B1 supplementation (2.8 mg/day) resulted in weight losses of up to 11 kg over 16 weeks, without altering red blood cell thiamine levels in a randomized controlled trial that involved 100 participants with obesity or overweight status [Keogh *et al.*, 2012]. Our findings suggest that optimizing extracellular thiamine availability may improve the differentiation of thermogenic adipocytes, which is crucial for potential transplantation strategies. Regular thiamine intake may therefore represent a simple, low-cost, and safe strategy for the prevention or management of obesity and associated metabolic diseases. Overall, our findings highlight thiamine as a promising nutritional supplement for boosting thermogenic adipocyte activity and contributing to body weight management.

9. SUMMARY

9.1. How extracellular thiamine levels shape thermogenic programming in human neck-derived adipocytes

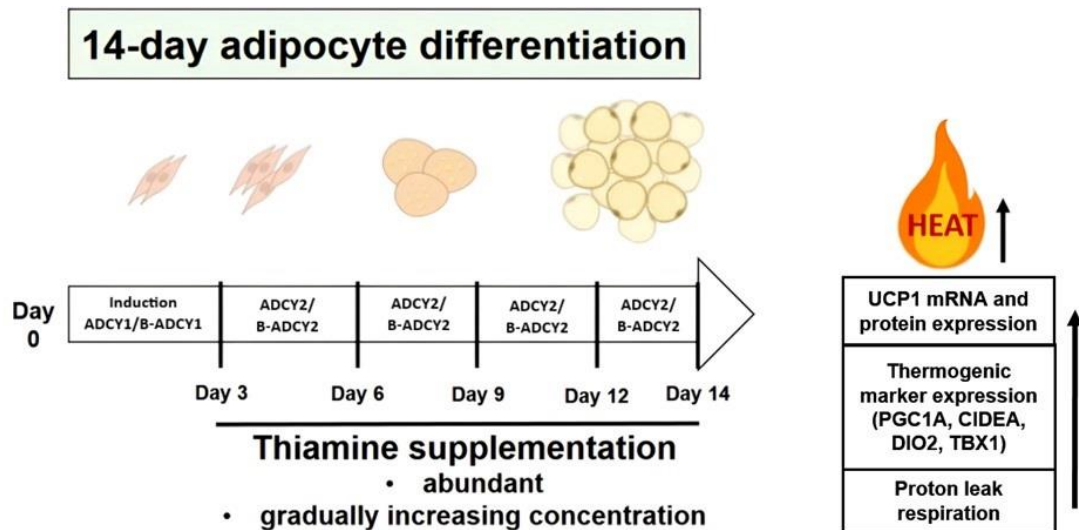


Figure 42 - Schematic illustration of the impact of thiamine supplementation during adipocyte differentiation on thermogenesis (created with BioRender)

- BAT is a primary site of NST, where UCP1 dissipates stored energy as heat. In humans, particularly in the cervical region, BAT comprises both classical brown and recruitable “beige” adipocytes, which makes this depot a promising target for anti-obesity interventions. Because of its contribution to whole-body EnE, approaches that promote the development and metabolic activity of brown/“beige” adipocytes are gaining therapeutic relevance. In the present work, we examined how extracellular thiamine influences human adipocyte differentiation and their thermogenic function.
- We conducted *ex vivo* differentiation of primary human adipogenic progenitor cells isolated from SuC and DeN fat depots. During the 14-day differentiation process, cells were exposed to gradually increasing availability of thiamine, allowing us to assess dose-dependent effects.
- Elevated thiamine availability during differentiation markedly increased the mRNA and protein expression of SLC19A2 and SLC19A3 ThTs in DeN-derived adipocytes.
- Functionally, increasing thiamine levels enhanced several key features of mitochondrial respiration, including basal oxygen consumption, cAMP-stimulated respiration, and PrL, indicating elevated thermogenic potential.

- Thiamine supplementation also stimulated mitochondrial biogenesis, with dose-dependent increases in main thermogenic markers — including UCP1, PGC1 α and CKMT2 — in adipocytes obtained from both SuC and DeN areas.
- Together, these data highlight micronutrient supply, and thiamine in particular, as an important determinant of the molecular and functional characteristics of thermogenic adipocytes. By modulating transcriptional programmes and mitochondrial function, thiamine promotes adipocyte browning and strengthens thermogenic capacity.
- Considering that vitamin B1 deficiency is frequently reported in individuals with obesity and type 2 DM, our findings suggest that long-term thiamine supplementation could serve as a novel and accessible strategy to promote adipocyte browning and counteract metabolic disorders.

9.2. Thiamine-dependent regulation of thermogenesis in human adipocytes

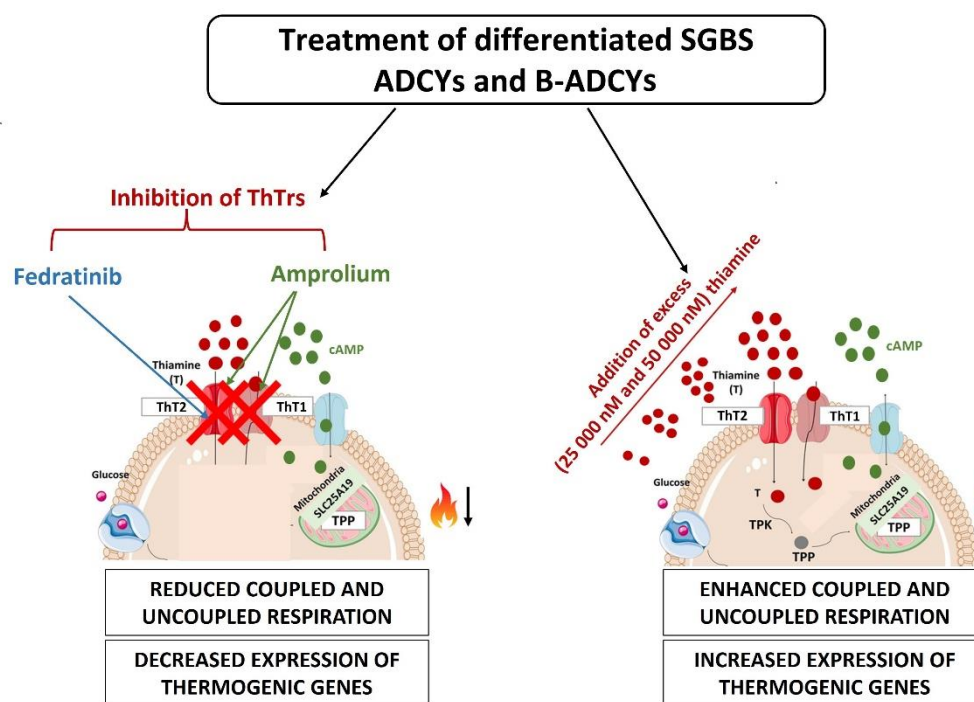


Figure 43 – Summarizing illustration of the impact of thiamine accessibility on the thermogenic function of human adipocytes (created with BioRender)

- Thermogenic adipocytes constitute a distinct fat cell subtype that dissipates energy as heat via UCP1 in the inner membrane of mitochondria. Because of this heat-producing capacity, they are considered attractive therapeutic targets for obesity, type 2 DM, and associated metabolic disturbances.

- The activation and enhancement of thermogenic pathways in human adipocytes could provide a powerful tool for combating energy imbalance and insulin resistance. However, a significant limitation in advancing this research is the lack of reliable human adipocyte models that model thermogenic behavior and response under controlled experimental conditions.
- The SGBS cell line offers a well-characterized, non-immortalized human adipocyte model capable of differentiating into adipocytes. This cell line serves as an excellent tool to explore regulatory mechanisms of browning and mitochondrial function.
- In this work, we examined how altering thiamine availability — by supplementation or by blocking ThTs — modulates thermogenic activation in differentiated SGBS adipocytes, with a particular focus on responses to db-cAMP, which mimics sympathetic adrenergic stimulation.
- Our findings showed that inhibiting thiamine transport by targeting SLC19A2 and SLC19A3, attenuated the db-cAMP-induced upregulation of crucial thermogenic genes, including *UCP1* and *PGC1 α* , and significantly reduced PrL respiration, a hallmark of UCP1-dependent heat production.
- In contrast, excess extracellular thiamine during adrenergic stimulation potentiated the expression of thermogenic markers and further enhanced the db-cAMP-induced increase in ThT expression, suggesting a feed-forward mechanism that amplifies mitochondrial activity.
- We further found that high thiamine levels increased both coupled and uncoupled respiration under resting conditions, suggesting that thiamine availability supports basal mitochondrial activity.
- At the mitochondrial level, thiamine supplementation upregulated the expression of mitochondrial complex subunits, suggesting improved mitochondrial biogenesis and respiratory efficiency.
- In summary, these findings point to thiamine as a key determinant of the thermogenic programme in human adipocytes. Beyond its classical role as a coenzyme, thiamine availability appears to modulate transcriptional and functional responses to adrenergic cues, which may have implications for future nutritional or pharmacological strategies to enhance NST and EnE in humans.

10. ÖSSZEFOGLALÁS

- A barna zsírszövet kulcsszerepet játszik a nem-didergéses hőtermelésben, amelyet az UCP1 közvetít. Az UCP1 a mitokondrium belső membránjában helyezkedik el, és lehetővé teszi az energia hő formájában történő leadását azáltal, hogy szétkapcsolja az oxidatív foszforilációt az ATP-termeléstől. A humán adipociták termogenikus funkcióinak aktiválása és fokozása ígéretes stratégiát jelenthet az elhízás, a 2-es típusú cukorbetegség és egyéb metabolikus rendellenességek kezelésére. Ezen folyamatok tanulmányozását, valamint jobb megértését azonban jelentősen korlátozza a megbízható, humán eredetű adipocitamodellek hiánya, amelyek képesek pontosan és reprezentatíván tükrözni a termogén mechanizmusokat.
- Jelen vizsgálatunk célja az extracelluláris tiamin hatásának tanulmányozása volt a humán adipociták differenciálódása és termogén aktivitása során. *Ex vivo* körülmények között humán nyaki szubkután és mély nyaki zsírszöveti depókból izolált primer adipocita progenitor sejteket differenciáltunk. A 14 napos differenciációs folyamat során a sejteket különböző koncentrációjú tiaminnal kezeltük, lehetővé téve a koncentráció-függő hatások elemzését. Eredményeink alapján a tiamin-koncentráció növekedése szignifikánsan fokozta a tiamin transzporterek expresszióját mind mRNS-, mind fehérjeszinten, különösen a mély nyaki eredetű adipocitákban. A többlet tiamin emellett fokozta a mitokondriális aktivitást, beleértve a bazális oxigénfogyasztást, a db-cAMP-stimulált respirációt és a protoncsorgásos légzést is, amely utóbbi a termogén potenciál fokozódására utal. A többlet tiamin jelenléte növelte a mitokondriális biogenezist is, valamint koncentráció-függő módon fokozta számos termogenikus marker — mint például az UCP1, PGC1 α , CKMT2 — expresszióját mind szubkután, mind mély nyaki eredetű adipocitákban.
- A tiamin hatásainak vizsgálatára alkalmaztuk továbbá az SGBS sejtvonalat, amely egy jól ismert humán adipocita modell. E sejtvonal differenciáltatását majd kezelését követően megfigyeltük, hogy a tiamin transzporterek gátlása szignifikánsan mérsékelte a db-cAMP által indukált kulcsfontosságú termogén gének, köztük az *UCP1* és *PGC1 α* expresszióját, valamint csökkentette a protoncsorgásos respirációt. Ezzel szemben, a többlet tiaminnal történő kezelés az adrenerg stimuláció mellett fokozta a termogenikus markerek expresszióját, illetve növelte a db-cAMP által indukált tiamin transzporter-expressziót, ezzel fokozva a mitokondriális aktivitást. A tiaminnal történő kezelés önmagában, illetve db-cAMP-vel is fokozta több mitokondriális komplex alegység expresszióját, amely a mitokondriális biogenezis, illetve a respiráció fokozódására utal.

- Eredményeink arra utalnak, hogy a tiamin fontos szerepet játszik a humán adipociták hőtermelő folyamatainak szabályozásában. A többlet tiaminnal történő kezelés nemcsak a mitokondriális biogenezist és respirációt serkenti, hanem fokozza a termogenikus markerek expresszióját is, ezáltal hozzájárulva a „browning” folyamatának az aktiválásához. A tiamin hiány magas prevalenciája, különösen az elhízással és 2-es típusú diabétesszel élő egyének körében, arra enged következtetni, hogy a tiamin pótlás új, könnyen hozzáférhető megoldást kínálhat az adipociták hőtermelő aktivitásának fokozására, valamint a metabolikus rendellenességek kezelésére. Eredményeink elősegítik a tiamin szerepének jobb megértését a termogenezis serkentésében, és alapot adnak további táplálkozási vagy farmakológiai beavatkozások kifejlesztéséhez.

11. APPENDIX

Table 2. Compounds applied for cell culture and cell differentiation

Compounds	Vendor, catalog number
Apo-Transferrin Human	Sigma-Aldrich #T2252
Biotin	Sigma-Aldrich #B4639
Collagenase from Clostridium histolyticum	Sigma-Aldrich #C1639
Dexamethasone	Sigma-Aldrich #D1756
Dibutyryl-cAMP (Db-cAMP)	Sigma-Aldrich #D0627
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich #D2650
DMEM Nutrient Mixture F-12	Sigma-Aldrich #D8437
Thiamine-free DMEM F-12 Medium	Gibco #ME2107367
Fetal Bovine Serum (FBS)	Gibco #10270
Hydrocortisone	Sigma-Aldrich #H0888
3-Isobutyl-1-methylxanthine (IBMX)	Sigma-Aldrich #I5879
Insulin solution human	Sigma-Aldrich #I9278
Pantothenic acid	Sigma-Aldrich #P5155
Penicillin-Streptomycin	Sigma-Aldrich #P43333
Rosiglitazone	Cayman Chemicals #7174

Table 3. SLC inhibitors and compounds used in Seahorse measurement

Compounds	Company, catalog number
Thiamine hydrochloride	Sigma-Aldrich #T1270
Fedratinib	Selleck Chemicals LLC #S2736
Amprolium	Sigma-Aldrich #137-88-2
Oligomycin	Sigma-Aldrich #495455
Antimycin A	Sigma-Aldrich #A867430
Seahorse XF Medium	Agilent #103575-100

Table 4. Gene primers and probes

Genes	Assay ID
<i>ACTB</i>	Hs01060665_g1
<i>CIDEA</i>	Hs00154455_m1
<i>CITED1</i>	Hs00918445_g1
<i>CKMT2</i>	Hs00176502_m1
<i>DIO2</i>	Hs00255341_m1
<i>GAPDH</i>	Hs99999905_m1
<i>LEP</i>	Hs00174877_m1
<i>PPARGC1A</i>	Hs01016719_m1
<i>SLC19A2</i>	Hs00949693_m1
<i>SLC19A3</i>	Hs00228858_m1
<i>TBX1</i>	Hs00271949_m1
<i>UCP1</i>	Hs00222453_m1

Table 5. Antibodies used in immunoblotting

Antibody	Company	Catalog Number	Dilution
UCP1	R&D Systems, Minneapolis, MN, USA	MAB6158	1:750

SLC19A3	Novus Biologicals, Centennial, CO, USA	NBP1-69703	1:500
SLC19A2	Abcam, Cambridge, MA, USA	Ab229680	1:500
PGC1 α	Novus Biologicals, Centennial, CO, USA	NBP1-04676	1:1000
Total OXPHOS	Abcam, Cambridge, MA, USA	ab110411	1:1000
TUBULIN	Santa Cruz, CA, USA	sc-5274	1:10000
Horseradish- peroxidase- conjugated goat anti- rabbit IgG	Advansta, San Jose, CA, USA	R-05072-500	1:5000
Horseradish- peroxidase- conjugated goat anti- mouse IgG	Advansta, San Jose, CA, USA	R-05071-500	1:5000

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14. PUBLICATIONS



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DOI: <http://dx.doi.org/10.1016/j.mce.2025.112483>
IF: 3.8 (2023)
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3. Arianti, R., **Vinnai, B. Á.**, Alrifai, R., Karadsheh, G., Qais, A. K. Y., Póliska, S., Győry, 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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Total IF of journals (all publications): 29,364

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The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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