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The importance of thiamine availability in the thermogenic competency of human adipocytes

Boglárka Ágnes Vinnai [a,b,1], Rini Arianti [a,c,1], Pamela Fischer-Posovszky [d,e], Martin Wabitsch [d,f], László Fésüs [a], Endre Kristóf [a,*]

[a] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, H-4032, Debrecen, Hungary
[b] Doctoral School of Molecular Cell and Immune Biology, University of Debrecen, H-4032, Debrecen, Hungary
[c] Universitas Muhammadiyah Bangka Belitung, 33134, Pangkalpinang, Indonesia
[d] German Center for Child and Adolescent Health (DZKJ), Partner Site Ulm, Ulm, Germany
[e] Department of Pediatrics and Adolescent Medicine, University Medical Center Ulm, Ulm, Germany
[f] Division of Pediatric Endocrinology and Diabetes, Department of Pediatrics and Adolescent Medicine, University Medical Center Ulm, Ulm, Germany

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ABSTRACT

Brown and beige adipocytes express uncoupling protein 1 (UCP1), which is located in the inner mitochondrial membrane and facilitates the dissipation of excess energy as heat. The activation of thermogenic adipocytes is a potential therapeutic target for treating type 2 diabetes mellitus, obesity, and related co-morbidities. Therefore, identifying novel approaches to stimulate the function of these adipocytes is crucial for advancing therapeutic strategies. Currently, there are limited amount of human adipocyte cell line models available to study the regulatory mechanisms of browning and key players in thermogenesis. The Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell line has been proven as a valuable model to investigate human adipocyte biology. In this study, we investigated how excess thiamine (vitamin B1), and the inhibition of thiamine transporters affect the expression of thermogenic markers and functional parameters during adrenergic stimulation in SGBS adipocytes. We found that limiting thiamine availability by pharmacological inhibitors impeded the dibutyryl-cAMP (db-cAMP)-dependent induction of thiamine transporter 1 and 2 (encoded by *SLC19A2* and *SLC19A3*), UCP1, PGC1a, and other browning markers, as well as proton leak respiration which is associated with UCP1-dependent heat generation. Contrarily, excess thiamine enhanced the db-cAMP-dependent induction of thiamine transporters, while UCP1, PGC1a, and other browning markers were upregulated. In addition, abundant amounts of thiamine increased the basal, unstimulated coupled and uncoupled respiration, and the expression of mitochondrial complex subunits. Our study highlights the critical role of excess thiamine in the thermogenic activation of SGBS adipocytes and its potential to enhance thermogenesis.

1. Introduction

Obesity has emerged as a global health issue and its prevalence has been steadily increasing in many countries. A recent study by NCD Risk Factor Collaboration highlights a global trend that shows a transition from underweight to obesity in adults in 1990. This trend is apparently emerging in school-aged children and adolescents, indicating a shift in body weight across age group [NCD Risk Factor Collaboration (NCD-RisC), 2024]. Excess fat accumulation, in particular central obesity, has been linked to several metabolic disorders, such as insulin resistance, hypertriglyceridemia, and hypertension. Several approaches have been developed to treat obesity, such as bariatric surgery and drugs targeting the central nervous system or gastrointestinal tract, which play a crucial role in regulating the appetite and energy homeostasis [Jackson et al., 2015; Perdomo et al., 2023].

Adipose tissue is important in physiological regulation including energy homeostasis, insulin sensitivity, and immune system. The high plasticity of adipose tissue allows it to respond to particular cues by altering its structure and phenotypes, thereby meeting the body's physiological demands [Sakers et al., 2022]. There are two types of adipose tissues that perform distinct physiological roles: white adipose tissue (WAT), which is primarily responsible in storing excess energy as

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[*] Corresponding author.
E-mail address: kristof.endre@med.unideb.hu (E. Kristóf).

[1] Authors contributed equally and shared first authorship.

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triglycerides, and brown adipose tissue (BAT) that can dissipate energy in the form of heat by a mechanism known as thermogenesis which may serve as a defense against hypothermic conditions and obesity [Kajimura et al., 2015]. Morphologically, white adipocytes possess one, large unilocular lipid droplet that accounts for more than 90% of the cell volume and variable amount of thin and elongated mitochondria. In contrast, brown adipocytes contain multilocular lipid droplets and abundant mitochondria which are large, spherical, and rich in cristae. Thermogenic adipocytes highly express uncoupling protein 1 (UCP1), a proton transporter located in the inner mitochondrial membrane. It uncouples the activity of the electron transport chain from ATP synthesis, therefore allowing the chemical energy to be dissipated in the form of heat [Farmer, 2008]. Another, inducible form of thermogenic adipocytes is the beige (also known as brite) adipocytes, which reside in WAT [Cheng et al., 2021]. The differentiation of beige adipocytes can be induced by various external cues and environmental factors, such as chronic cold exposure, exercise, long-term treatment with peroxisome proliferator-activated receptor γ (PPARγ), or β-adrenergic receptor agonists [Ikeda et al., 2018]. In recent decades, the study of brown and beige adipocytes has gained significant attention due to their potential as a therapeutic target for treating obesity and various metabolic disorders [Kajimura et al., 2015; Harms and Seale, 2013]. Enhancing the thermogenic activity of BAT or promoting the recruitment of new beige adipocytes through the browning process offer promising strategies to combat obesity and to improve metabolic health [Nedergaard and Cannon, 2010; Bartelt and Heeren, 2014].

Thiamine, or vitamin B1, is a water-soluble essential vitamin as it must be obtained from diet, although some types of bacteria in the gut can produce slight quantities of thiamine [Teran et al., 2021]. Thiamine plays a crucial role in cellular metabolism, mainly involved in glucose metabolism [Manzetti et al., 2014]. Thiamine has a notably short half-life of 1–12 h with limited storage capacity in the body. Therefore, continuous dietary supplementation is essential for maintaining constant tissue thiamine levels [Whitfield et al., 2018]. In our previous studies, we demonstrated that the abundant amount of thiamine is crucial for efficient thermogenic activation and competency in human primary adipocytes derived from human subcutaneous (SC) and deep cervical fat [Arianti et al., 2023; Vinnai et al., 2023]. Inhibition of thiamine transporter (ThTr) 1 and 2, which are encoded by *SLC19A2* and *SLC19A3*, respectively, led to the reduced expression of UCP1 and other thermogenic markers, as well as abrogated the UCP1-dependent heat generation reflected by proton leak respiration [Arianti et al., 2023]. We also found that the absence of thiamine during adipocyte differentiation [Vinnai et al., 2023] or dibutyryl-cAMP (db-cAMP) stimulation [Arianti et al., 2023] decreased the thermogenic competency of human SC and deep cervical-derived adipocytes. In the present study, we further investigated the importance of thiamine in a human adipocyte cell line isolated from the SC WAT of a subject with Simpson-Golabi-Behmel syndrome (SGBS). SGBS adipocytes closely resemble human white adipocytes in both morphology and function, allowing it to be a valuable cellular model to study adipocyte biology [Wabitsch et al., 2001; Allott et al., 2012; Fischer-Posovszky et al., 2008]. Analysis of single-nuclei RNA-sequencing (snRNA-seq) datasets [Sun et al., 2020] identified adipocytes as the major ThTr expressing cell type in human WAT. Our presented data revealed a pattern similar to our previous findings in human SC and deep cervical-derived adipocytes, highlighting the crucial roles of thiamine for thermogenic activation. These findings provide additional insights into the metabolic properties of SGBS adipocytes, and further emphasize the importance of thiamine availability in adipocyte thermogenesis.

2. Materials and methods

2.1. Materials

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

2.2. Differentiation and treatment of SGBS adipocytes

Cells were isolated from the SC adipose tissue specimen of an infant with SGBS. The cells then were neither transformed nor immortalized. The unlimited source of cells can be sustained due to their ability to proliferate for up to 50 passage numbers with retained capacity for adipogenic differentiation [Wabitsch et al., 2001; Fischer-Posovszky et al., 2008; Tews et al., 2022]. Mycoplasma-free SGBS preadipocytes were seeded in 6-well plates or XF96 assay plates (Seahorse Biosciences, North Billerica, MA, USA) and cultured in Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham (DMEM-F12) medium containing 33 μM biotin, 17 μM pantothenic acid, 100 U/ml penicillin/streptomycin, and 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C in 5% CO_2 until they reached complete confluence. They were differentiated by regular adipogenic differentiation medium to adipocytes (ADIP) induced for three days with serum-free DMEM-F12 medium supplemented with 33 μM biotin, 17 μM pantothenic acid, 100 U/ml penicillin/streptomycin, 2 μM rosiglitazone (Cayman Chemicals, Ann Arbor, MI, USA), 25 nM dexamethasone, 0.25 mM 3-isobutyl-1-methylxantine, 0.1 μM cortisol, 0.01 mg/ml human apo-transferrin, 0.2 nM triiodothyronine, and 20 nM human insulin. After the third day, rosiglitazone, dexamethasone, and 3-isobutyl-1-methylxanthine were removed from the medium for the remaining 11 days of the differentiation [Fischer-Posovszky et al., 2008; Kristóf et al., 2015].

The differentiation of adipocytes to higher browning capacity (B-ADIP) was induced for three days with serum-free DMEM-F12 medium supplemented with 33 μM biotin, 17 μM pantothenic acid, 100 U/ml penicillin/streptomycin, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 0.01 mg/ml human apo-transferrin, 0.2 nM triiodothyronine, and 0.85 μM human insulin. After the third day, dexamethasone and 3-isobutyl-1-methylxanthine were removed and 500 nM rosiglitazone was added to the medium for the remaining 11 days of differentiation [Elabd et al., 2009; Tóth et al., 2020]. Our previous study has reported that B-ADIP exerted higher thermogenic capacity as compared to ADIP marked by higher expression of thermogenic markers and cellular respiration [Klusóczki et al., 2019].

At the end of the adipocyte differentiation in DMEM-F12-HAM medium, ADIP and B-ADIP were treated with a single bolus of 500 μM db-cAMP (cat#D0627) for 10 h to mimic *in vivo* cold-induced thermogenesis. Fedratinib (Selleck Chemicals LLC cat#S2736, Houston, TX, USA) at 1 μM or amprolium (cat#137- 88-2) at 300 μM was administered also for 10 h to inhibit ThTr activity [Arianti et al., 2023]. Db-cAMP was also administered with the combination with fedratinib or amprolium for 10 h. After the 10 h treatment, cells were lysed in TRIzol or SDS-lysis buffer for further experiments.

In the experiments with excess thiamine, SGBS ADIPs and B-ADIPs were treated using DMEM-F12-HAM medium with or without the addition of excess (25 μM or 50 μM) thiamine hydrochloride (cat# T1270) for 10 h. In these experiments, the control cells were differentiated only in the presence of thiamine hydrochloride found in the DMEM-F12-HAM, which was 6.4 μmol/L. Cells were incubated at 5% CO_2 and 37°C. Media were changed every 3 days and cells were lysed or analyzed after 14 days of differentiation.

2.3. RNA isolation

Cells were collected in TRIzol and total RNA was isolated by chloroform-isopropanol extraction as described previously [Arianti et al., 2023; Klusóczki et al., 2019]. The concentration and purity of the isolated RNA was checked by using Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific).

2.4. Quantitative real time PCR (RT-qPCR)

RNA was diluted to 100 ng/μL for all samples and was reverse transcribed to cDNA by using reverse transcription kit (Thermo Fisher Scientific, cat#4368814) following the manufacturer's instructions. Validated TaqMan assays used in qPCR were designed and supplied by Thermo Fisher Scientific as listed in Supplementary Table 1, qPCR was performed in Light Cycler® 480 II (Roche). The following conditions were set to perform the reactions: initial denaturation step at 95 °C for 1 min followed by 50 cycles of 95 °C for 12 s and 60 °C for 30 s. Gene expression values were calculated by the comparative threshold cycle (Ct) method as described in the previous publication [Arianti et al., 2023; Huang et al., 2023]. Gene expressions were normalized to the geometric mean of *ACTB* and *GAPDH*. Normalized gene expression levels equal $2^{-\Delta Ct}$.

2.5. Immunoblotting and densitometry

Immunoblotting was carried out as described previously [Arianti et al., 2024]. Antibodies and working dilutions are listed in Supplementary Table 2. FIJI ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for densitometry analysis, where the density value of the target protein of interest was divided by the density value of the tubulin household protein, thus obtaining the normalized optical density.

2.6. Oxygen consumption (OCR) and extracellular acidification rate (ECAR) measurement

OCR and ECAR of adipocytes, which were differentiated as described in 2.2, were measured using an XF96 oximeter (Seahorse Biosciences) according to previously optimized protocols [Arianti et al., 2023]. After recording the baseline OCR, 500 μM db-cAMP, 1 μM fedratinib, 300 μM amprolium or combination of db-cAMP and one of the ThTr inhibitors were injected to the cells. Then, stimulated OCR was recorded every 30 min. Proton leak respiration was determined after injecting oligomycin (cat#495455) at 2 μM concentration. Cells received a single bolus of Antimycin A (cat#A8674) at 10 μM concentration for baseline correction (measuring non-mitochondrial respiration). The OCR was normalized to protein content.

For thiamine excess experiments, OCRs were detected for 10 h following the injection of 500 μM db-cAMP to mimic adrenergic stimuli, in the presence or absence of excess thiamine hydrochloride (25 μM or 50 μM). Further treatments and recordings followed the aforementioned protocol.

2.7. Single nuclei RNA-sequencing (snRNA-seq) analysis

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

2.8. Statistical analysis

The results are expressed as mean ± SD. The normality of the distribution of the data was tested by Shapiro–Wilk test. For multiple comparisons of each group, one-way ANOVA followed by Tukey's post hoc test was used. The data were visualized and analyzed by using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). A p value of less than 0.05 was considered statistically significant.

3. Results

3.1. The expression of thiamine transporters is mainly enriched in the adipocytes within human WAT

Primarily, we investigated the expression pattern of both ThTrs in human WAT by exploring the available snRNA-seq data [Sun et al., 2020]. We found that ThTr1 (encoded by *SLC19A2*) was mainly expressed in preadipocytes and adipocytes (Fig. 1A), while the expression of ThTr2 (encoded by *SLC19A3*) was detectable exclusively in mature adipocytes (Fig. 1B). The expression of both ThTr encoding genes was not detectable in other cell types, such as fibroblasts, endothelial cells, or $CD4^+$ T lymphocytes (Fig. 1). The strong clustering of ThTr expression to adipocytes suggests the important role of thiamine in WAT functions, especially adipocyte browning and heat production, in which processes vitamin B1 availability was critical for the thermogenically prone human cervical-derived adipocytes [Arianti et al., 2023; Vinnai et al., 2023]. Since SGBS adipocytes were isolated from human SC WAT [Wabitsch et al., 2001] and possess a significant thermogenic potential [Klusóczki et al., 2019], we decided to systematically investigate how thiamine availability affects their thermogenic capacity.

3.2. Adrenergic activation and excess thiamine treatment following SGBS adipocyte differentiation increase the expression of thiamine transporters

In our previous study, we found that the presence of excess thiamine during the adipogenesis of cervical area-derived adipocytes increased the expression of ThTrs [Vinnai et al., 2023]. Therefore, we investigated how treatment with an excess (25 μM and 50 μM) amount of thiamine after adipocyte differentiation affects the basal or db-cAMP-induced expression of ThTrs in SGBS adipocytes. After differentiation under normal culture conditions, cells were treated with 500 μM db-cAMP (cell-permeable cAMP analog to mimic adrenergic stimulation) in the presence or absence of excess thiamine. First, we found that db-cAMP-driven activation significantly increased the mRNA expression of *SLC19A2* in both ADIPs and B-ADIPs (Fig. 2A). At protein level, db-cAMP treatment significantly elevated the expression of ThTr1 in ADIPs (Fig. 2C). At different applied thiamine concentrations, the presence of 25 μM thiamine further increased the db-cAMP-induced upregulation of the transporter mRNA in B-ADIPs (Fig. 2A). In ADIPs, 50 μM thiamine significantly increased the basal and db-cAMP-induced expression of ThTr1 protein. However, only the basal expression of the protein was significantly affected in B-ADIPs (Fig. 2C). The thermogenic induction by db-cAMP also significantly increased the mRNA expression of *SLC19A3* in both ADIPs and B-ADIPs. The basal mRNA expression of the transporter was significantly elevated when cells were treated with 50 μM of thiamine in ADIPs. We also observed that the db-cAMP-induced upregulation was potentiated in the presence of 25 μM and 50 μM of thiamine in B-ADIPs (Fig. 2B). At protein level, db-cAMP treatment significantly increased the expression of ThTr2 in ADIPs but not in B-ADIPs. Treatment with 50 μM of thiamine, significantly elevated the basal or db-cAMP-induced expression of the transporter protein in ADIPs or B-ADIPs, respectively (Fig. 2C). In summary, abundant amounts of thiamine after the differentiation of SGBS adipocytes exert a potentiating effect on the basal and db-cAMP-stimulated expression of ThTrs.

3.3. Thiamine transporter inhibitors attenuate the induction of thiamine transporters by db-cAMP

Next, we aimed to investigate whether treatment with ThTr inhibitors, such as fedratinib or amprolium, after adipogenesis affects the basal or db-cAMP-stimulated expression of ThTrs in SGBS ADIPs and B-ADIPs. After the preadipocytes were differentiated into ADIPs and B-ADIPs under normal culture conditions, cells were treated with 500 μM db-cAMP in the presence or absence of ThTr inhibitor, fedratinib or amprolium. Fedratinib has a more prominent inhibitory effect on ThTr2 ($IC_{50} = 1.36$ μM), while a lesser one on ThTr1 ($IC_{50} = 7.10$ μM) [Zhang et al., 2014; Giacomini et al., 2017]. Amprolium is known as a thiamine structural analogue that acts as a competitive inhibitor of thiamine transport, effectively inhibiting its absorption and depleting intracellular thiamine content [Bettendorff et al., 1995; Park et al., 2000].

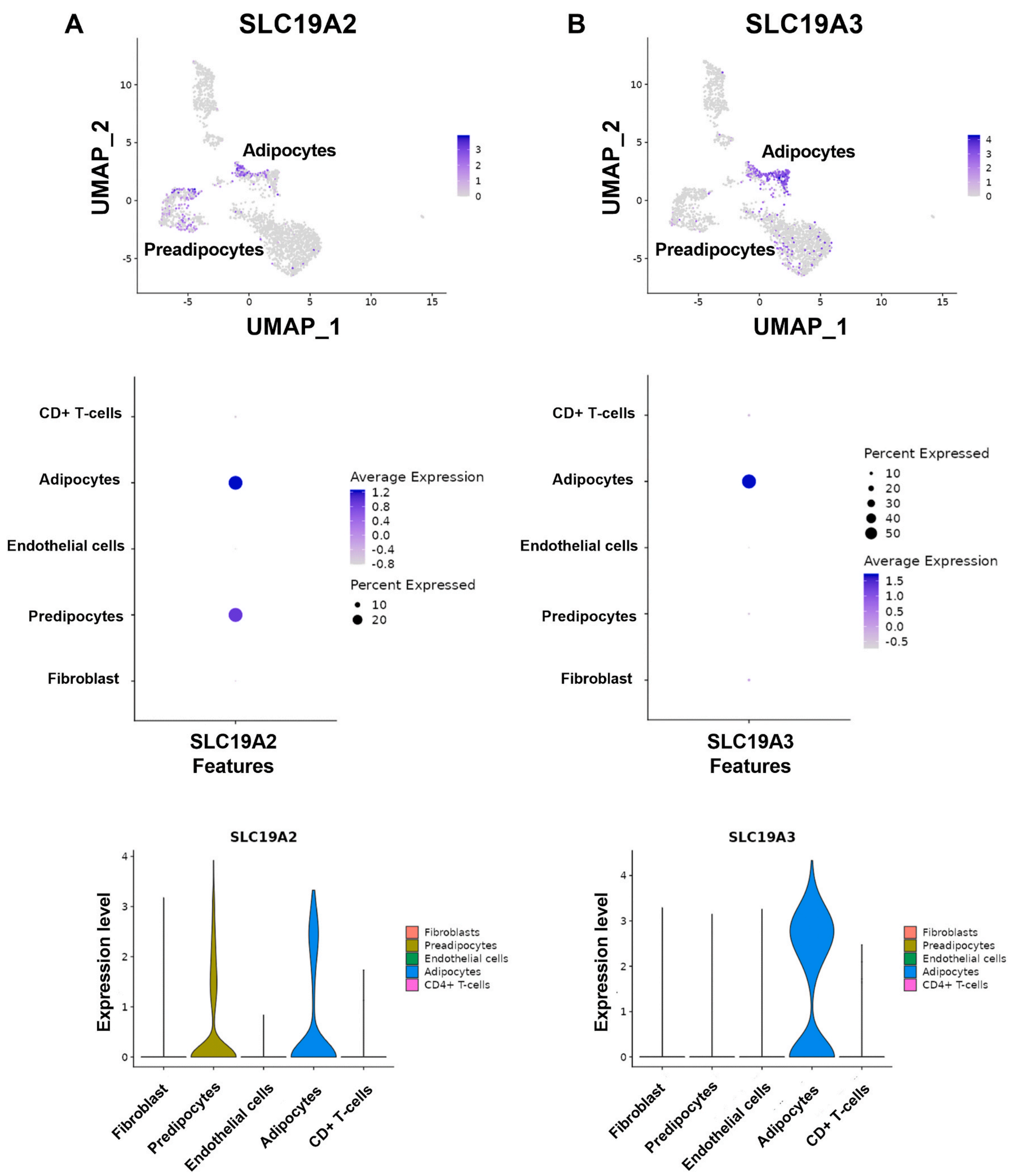

Fig. 1. The expression of thiamine transporter (ThTr) 1 (encoded by *SLC19A2*) and ThTr2 (encoded by *SLC19A3*) in 2.438 nuclei from human subcutaneous white adipose tissue (SC WAT) [Sun et al., 2020]. Embedding, dot, and violin plots showing the expression of *SLC19A2* (A) and *SLC19A3* (B) of single-nuclei RNA-sequencing for human SC WAT.

We observed that the mRNA and protein expression of both ThTrs was significantly increased upon db-cAMP-stimulation in ADIPs, while this effect was seen only at mRNA levels in B-ADIPs (Fig. 2D–F). Fedratinib significantly decreased db-cAMP-induced upregulation of *SLC19A2* and *SLC19A3* mRNA expression in both ADIPs and B-ADIPs (Fig. 2D and E). At protein level, this effect was only observed in case of db-cAMP-stimulated B-ADIPs for both transporters but not in ADIPs in which only ThTr2/SLC19A3 was affected (Fig. 2F). Amprolium treatment significantly decreased the mRNA expression of both transporters in db-cAMP-treated B-ADIPs (Fig. 2D and E), however, at protein level, this effect was only observed with respect to ThTr2/SLC19A3 (Fig. 2F). These results show that the adrenergic-driven upregulation of ThTrs is regulated by their access to thiamine in SGBS adipocytes.

3.4. Inhibition of thiamine transporters hinders coupled and uncoupled respiration in differentiated SGBS adipocytes

Next, we investigated the potential role of ThTrs during the thermogenic activation in differentiated SGBS adipocytes. During the measurement, ADIPs and B-ADIPs were treated with db-cAMP in the presence of ThTr inhibitors, fedratinib or amprolium. First the basal OCR was recorded, then after the injection of the mentioned compounds, the maximal stimulated respiration was measured. After the administration of oligomycin, which is known to inhibit ATP synthase, the proton leak respiration (that associates with UCP1-dependent thermogenesis) was registered. As expected, OCR was significantly elevated after db-cAMP injection in both ADIPs and B-ADIPs (Fig. 3A–D, left

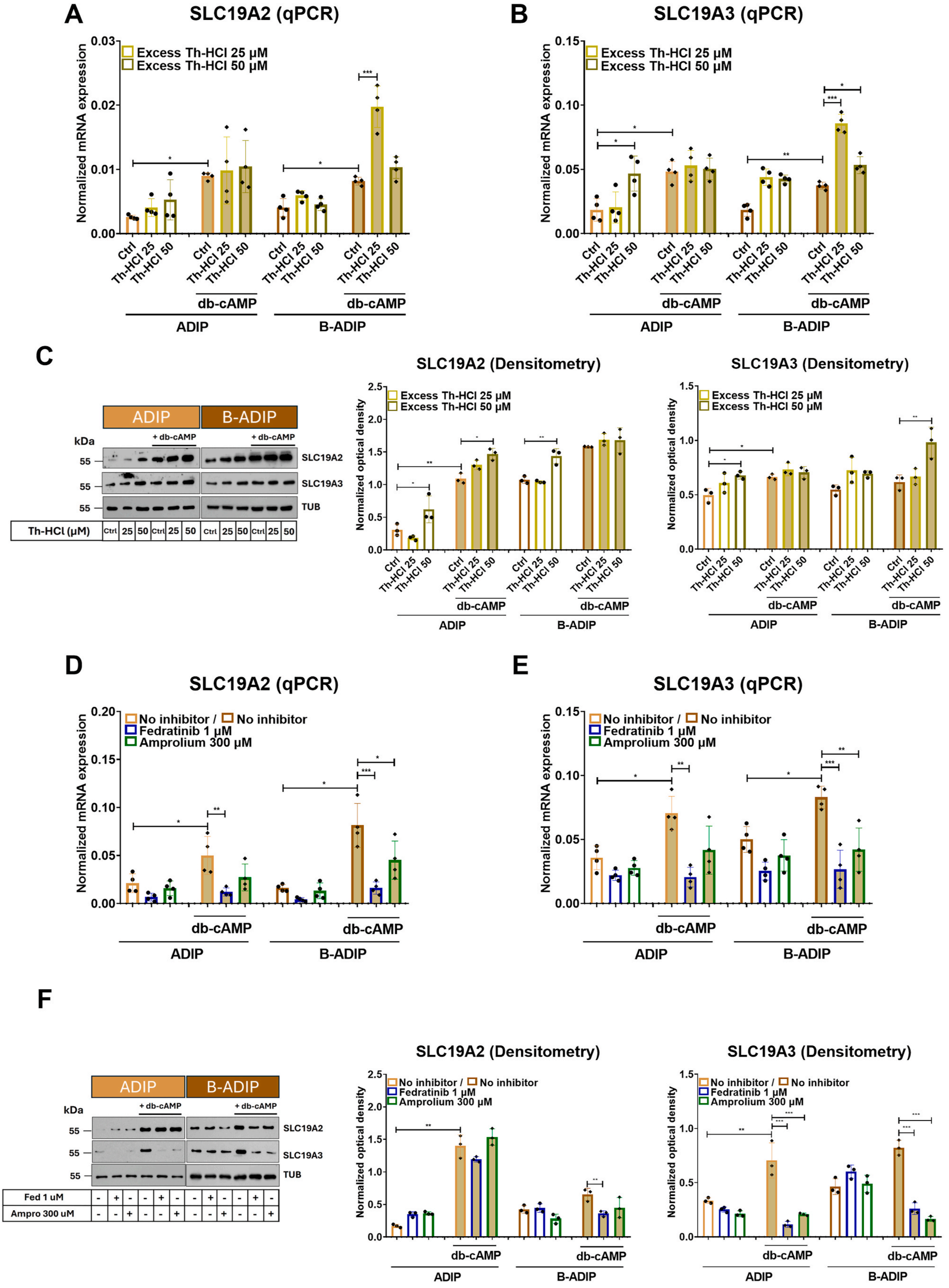

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Fig. 2. Effect of excess (25 μM and 50 μM) thiamine hydrochloride (Th-HCl) and Th transporter (ThTr) inhibitors (fedratinib or amprolium), on the basal and dibutyryl-cAMP (db-cAMP)-induced expression of ThTrs in SGBS adipocytes (ADIPs) and brown differentiated adipocytes (B-ADIPs). After differentiation at regular culture conditions (6.4 μM Th-HCl), ADIPs and B-ADIPs were treated with 500 μM db-cAMP (brown bars) in the presence or absence of excess Th-HCl or ThTr inhibitors for 10 h. (A and B) mRNA expression of *SLC19A2* and *SLC19A3* assessed by RT-qPCR, n = 4. (C) Protein expression of ThTr1/SLC19A2 and ThTr2/SLC19A3 detected by immunoblotting, n = 3. (D and E) mRNA expression of *SLC19A2* and *SLC19A3* assessed by RT-qPCR, n = 4. (F) Protein expression of ThTr1/SLC19A2 and ThTr2/SLC19A3 detected by immunoblotting, n = 3. The original pictures of the full-length blots are displayed in Supplementary Figs. 1–2. Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test, *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

panels). We observed that the db-cAMP-stimulated elevation of OCR and proton leak respiration was significantly decreased by fedratinib and amprolium in both ADIPs and B-ADIPs (Fig. 3A–D, left panels). The db-cAMP-stimulated ECAR was not affected significantly by the inhibition of ThTrs in both ADIPs and B-ADIPs (Fig. 3A–D, right panels).

Next, we evaluated the effect of ThTr inhibitors on the expression of mitochondrial complex subunits. We observed that db-cAMP-driven activation significantly increased the expression of complex I, II, III, and IV subunits, but not complex V, in SGBS ADIPs. Both fedratinib and amprolium significantly prevented the db-cAMP-stimulated upregulation of complex I subunit. Amprolium reduced the basal expression of both complex III and IV, while fedratinib decreased only the basal expression of complex IV subunit (Fig. 4). Fedratinib and amprolium administration also tended to downregulate complex II subunit expression but not to a statistically significant extent. These results demonstrate that inhibition of ThTrs after adipocyte differentiation decreases the thermogenic competency and mitochondrial biogenesis of SGBS ADIPs and B-ADIPs.

3.5. *Inhibition of thiamine transporters decreases the expression of thermogenic markers in differentiated SGBS adipocytes*

After observing decreased thermogenic capacity in the presence of ThTr inhibitors in SGBS adipocytes, we investigated how the inhibition of the transporters, therefore the limitation of the thiamine availability could affect the expression of thermogenic markers. Our results showed that db-cAMP elevated both the mRNA and protein expression of UCP1 in ADIPs and B-ADIPs, however, this upregulation was hampered in the presence of fedratinib except for *UCP1* mRNA in B-ADIPs. Fedratinib also decreased basal UCP1 expression at both mRNA and protein levels in B-ADIPs. Amprolium prevented the adrenergic-driven upregulation of *UCP1* gene in ADIPs and that of the encoded protein in B-ADIPs. It also decreased basal UCP1 expression at mRNA level in B-ADIPs and at protein level in ADIPs, respectively (Fig. 5A and C). At mRNA level, the db-cAMP-stimulated upregulation of *PGC1a* was hindered by fedratinib in both types of adipocytes and by amprolium in B-ADIPs (Fig. 5B). At protein level, both inhibitors treatment led to decreased expression of PGC1a in db-cAMP-stimulated conditions in both ADIPs and B-ADIPs (Fig. 5C). We also investigated the effect of ThTr inhibitors on the mRNA expression of several additional thermogenesis-related genes in SGBS ADIPs and B-ADIPs (Fig. 5D–H). Treatment with db-cAMP significantly increased the expression of *CITED1*, *DIO2* and *TBX1* in both SGBS ADIPs and B-ADIPs, while in case of *CIDEA* and *CKMT2* this happened only in B-ADIPs. Fedratinib significantly decreased the db-cAMP-stimulated upregulation of these markers in both SGBS ADIPs and B-ADIPs, where stimulation was observed. Taken together, these results show that inhibition of ThTrs after adipogenesis, leads to lower expression of thermogenic genes in adrenergic stimulated SGBS adipocytes.

3.6. *Excess thiamine treatment after adipocyte differentiation elevates coupled and uncoupled respiration*

We previously reported that excess thiamine (25 μM and 50 μM, which were higher than thiamine concentration in regular culture medium) during adipocyte differentiation elevated thermogenic potential of human adipocytes [Vinnai et al., 2023]. Therefore, we were tempted to investigate whether short-term excess thiamine treatment in differentiated SGBS ADIPs and B-ADIPs affected the thermogenic potential at basal conditions and during adrenergic stimulation by db-cAMP. We observed that excess thiamine at 50 μM concentration significantly increased the OCR and ECAR at unstimulated condition in both ADIPs and B-ADIPs (Fig. 6A and B). In accordance with previous findings, we recorded that db-cAMP increased maximal stimulated OCR, proton leak respiration, and ECAR in both ADIPs and B-ADIPs as compared to untreated ones, however excess thiamine did not increase further their db-cAMP-stimulated elevation (Fig. 6A and B). Next, we investigated the effect of excess thiamine on the expression of mitochondrial complex subunits in ADIPs. In accordance with the results shown in Fig. 4, we observed that db-cAMP treatment significantly elevated the protein expression of complex I, II, III, and IV subunits, but not complex V, in SGBS ADIPs. Treatment with 25 μM of thiamine significantly increased the protein expression of complex I and IV, but not complex II, III, and V subunits. Our data also showed that 50 μM of thiamine significantly elevated the protein expression of complex I, II, III, and IV subunits (Fig. 7), and a further increase was observed when the cells were activated by db-cAMP in case of complex II. We did not observe any effect of db-cAMP or thiamine on the expression of complex V subunit (Fig. 7). Altogether, our data suggest that abundant amounts of thiamine after adipocyte differentiation elevates the thermogenic potential and mitochondrial biogenesis of SGBS ADIPs and B-ADIPs.

3.7. *Abundant amounts of thiamine after adipocyte differentiation elevates thermogenic gene expression*

After observing that high levels of thiamine increased the cellular respiration, we assumed that abundant thiamine (25 μM and 50 μM), following SGBS adipocyte differentiation may further increase the expression of genes linked to mitochondrial biogenesis and thermogenesis. Our data showed that excess thiamine increased further the db-cAMP-stimulated elevation of mRNA expression of *UCP1* in B-ADIPs and *PGC1a* in ADIPs (Fig. 8A and B). Excess thiamine at 25 μM concentration elevated the PGC1a protein expression of unstimulated ADIPs. Thiamine at 50 μM concentration also elevated the expression of PGC1a mRNA in B-ADIPs and the protein expression in unstimulated ADIPs (Fig. 8B and C). The protein expression of UCP1 was increased by excess 50 μM thiamine in ADIPs during stimulation by db-cAMP and in B-ADIPs at unstimulated conditions (Fig. 8C). In addition, we also investigated the effect of excess thiamine on the expression of additional thermogenic markers. Our data showed that excess thiamine increased further the mRNA expression of *CIDEA, DIO2*, and *TBX1* in ADIPs during db-cAMP stimulation (Fig. 8D, G, H). While in B-ADIPs, the mRNA expression of *CMKT2*, *CITED1*, *DIO2,* and *TBX1* was potentiated by excess thiamine during thermogenic activation (Fig. 8E–H). Thiamine alone without db-cAMP stimulation was able to increase the expression of *CITED1* and *DIO2* in ADIPs, and *CKMT2* and *CITED1* in B-ADIPs, respectively (Fig. 8E–G). These results suggest that excess thiamine provided after differentiation of SGBS adipocytes potentiates the expression of thermogenesis-linked and brown/beige marker genes.

4. Discussion

Heat generation via UCP1 activity requires higher amount of metabolic substrates derived from glucose, fatty acids, and amino acids [McNeill et al., 2020; Townsend and Tseng, 2014]. In addition to these

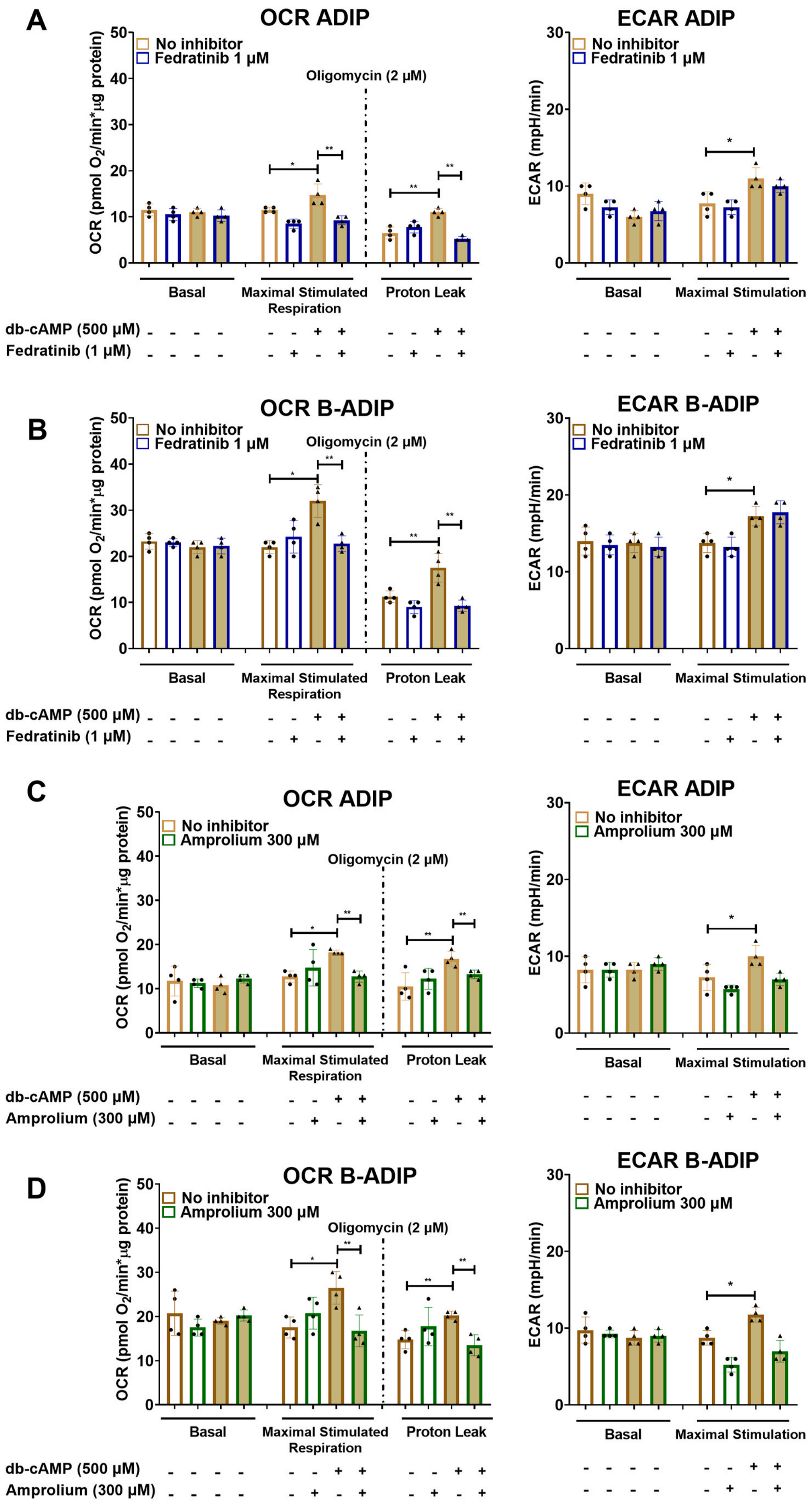

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Fig. 3. Effect of thiamine transporter (ThTr) inhibitors (fedratinib and amprolium) on the dibutyryl-cAMP (db-cAMP)-induced oxygen consumption (OCR) and extracellular acidification (ECAR) rates in SGBS adipocytes (ADIPs) and brown differentiated adipocytes (B-ADIPs). SGBS preadipocytes were differentiated into ADIP and B-ADIP at regular culture conditions, then OCRs were detected for 10 h following the injection of 500 μM db-cAMP in the presence or absence of ThTr inhibitors, fedratinib (A and B) or amprolium (C and D). OCR at basal, maximal db-cAMP-stimulated, and after oligomycin addition (left panels), and ECAR (right panels) were quantified in SGBS ADIPs and B-ADIPs of four independent measurements. Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test, *p < 0.05, **p < 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

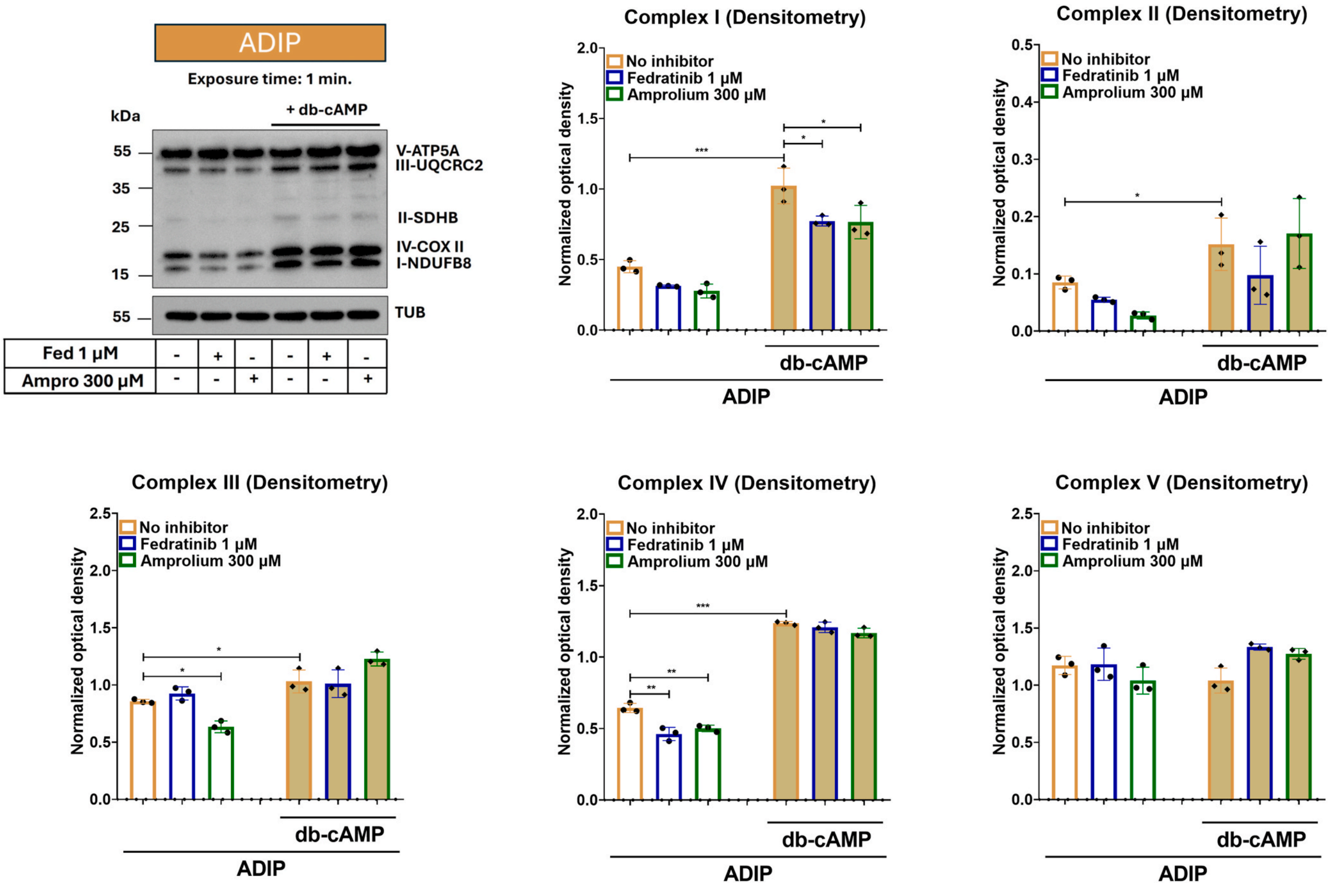

Fig. 4. Effect of thiamine transporter (ThTr) inhibitors (fedratinib and amprolium) on the dibutyryl-cAMP (db-cAMP)-induced expression of mitochondrial complex subunits in SGBS adipocytes (ADIPs). SGBS preadipocytes were differentiated into ADIP at regular culture conditions, then cells were treated with 500 μM db-cAMP (brown bars) in the presence or absence of ThTr inhibitors for 10 h. Protein expression of mitochondrial complex subunits detected by immunoblotting. The original pictures of the full-length blots with different exposure times are displayed in Supplementary Fig. 3A and Supplementary Fig. 4A, n = 3. Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test, *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

macronutrients, adipocytes also utilize micronutrients to support their functions and metabolism, such as vitamin A, D, E, and C [Imessaoudene et al., 2022; Nimitphong et al., 2020; Zhao and Qin, 2022]. Global transcriptomic analysis showed that the expression of ThTr1 and mitochondrial thiamine pyrophosphate (TPP) transporter (*SLC25A19*) was upregulated upon adrenergic stimulation in human primary brown/beige adipocytes [Arianti et al., 2024]. We previously reported that abundant thiamine, which is utilized by adipocytes via ThTr1 and 2, is required for the efficient thermogenic response upon db-cAMP treatment in human primary adipocytes derived from SC and deep cervical regions [Arianti et al., 2023]. The inhibition of ThTrs by pharmacological inhibitors led to the reduced expression of thermogenic marker genes, such as *UCP1* and *PGC1a*, and UCP1-mediated heat generation reflected by proton leak respiration. Furthermore, we also reported that supplementation of surplus thiamine elevates the thermogenic competency of human cervical-derived adipocytes [Vinnai et al., 2023]. A recent publication showed that thiamine, pantothenic acid, and riboflavin are important for the adipogenic program of human dermal fibroblasts towards brown adipocytes [Takeda and Dai, 2024].

SGBS cell line is a versatile *in vitro* model which is comparable to human SC white adipocytes [Wabitsch et al., 2001; Tews et al., 2022; Yeo et al., 2017; Halbgebauer et al., 2020]. After differentiation, it exhibits the characteristics of human primary adipocytes with browning properties, such as UCP1 expression [Klusóczki et al., 2019; Colitti et al., 2022]. SGBS cells have been frequently used as a cellular model to study adipocyte metabolism to obtain a better understanding of obesity and type 2 diabetes pathophysiology under chemically defined conditions [Wabitsch et al., 2001; Tews et al., 2022]. It is crucial to confirm experimental findings obtained from primary human adipocytes in cell line models, such as SGBS adipocytes, in order to ensure reproducibility and address limitations associated with donor variability in primary cells. Even though, primary human adipose-derived stromal cells, which are isolated from fat tissue, are commonly used to study adipose tissue function, their properties are often affected by donor-related factors, such as lifestyle, metabolism, and genetic background, all of which can exert an unexpected impact on the obtained results. By investigating the

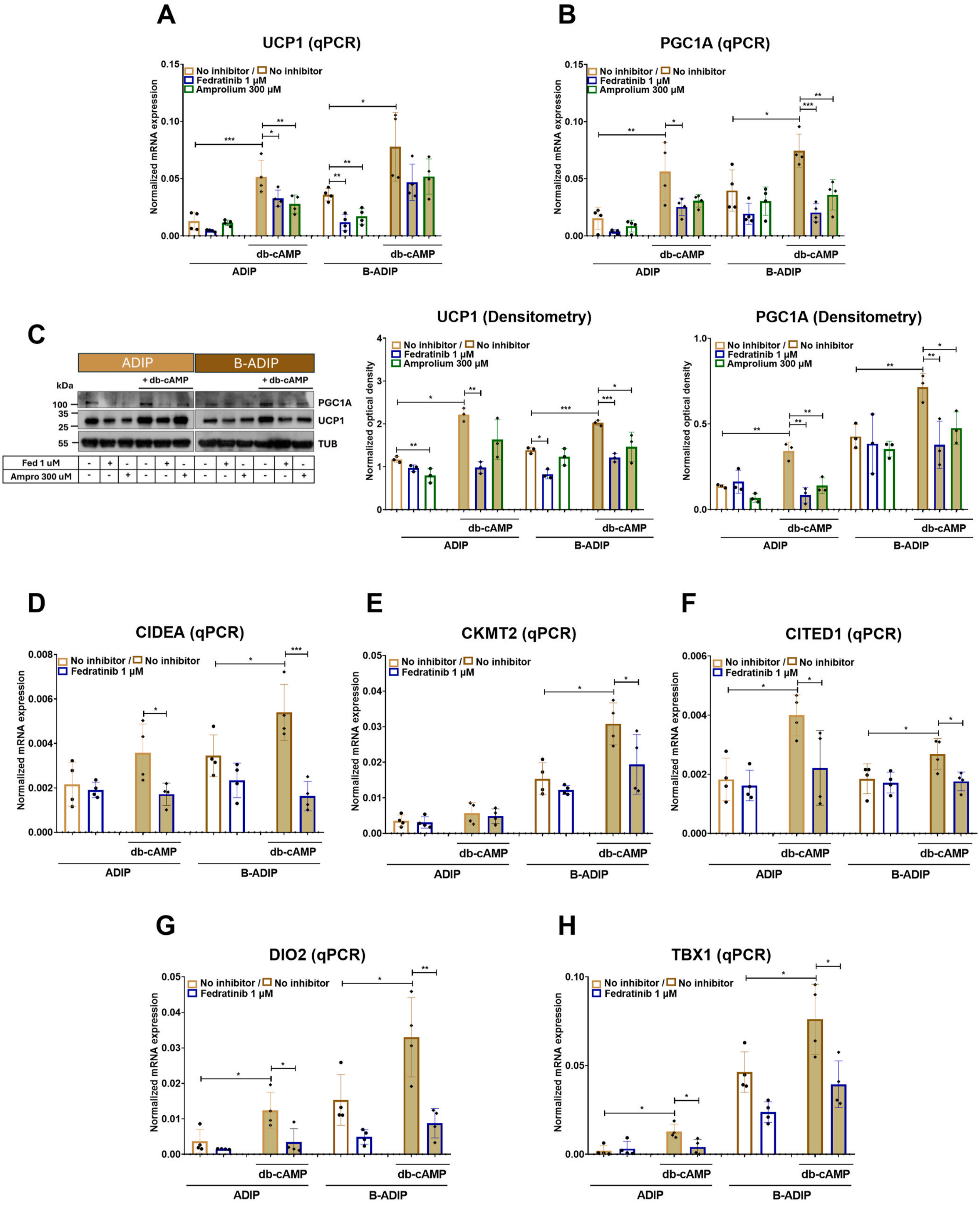

Fig. 5. Effect of thiamine transporter (ThTr) inhibitors (fedratinib and amprolium) on the basal and dibutyryl-cAMP (db-cAMP)-induced expression of thermogenic markers in SGBS adipocytes (ADIPs) and brown differentiated adipocytes (B-ADIPs). After differentiation at regular culture conditions, ADIPs and B-ADIPs were treated with 500 µM db-cAMP (brown bars) in the presence or absence of ThTr inhibitors for 10 h. (A and B) mRNA expression of *UCP1* and *PGC1a* assessed by RT-qPCR, n = 4. (C) Protein expression of UCP1 and PCG1a detected by immunoblotting, n = 3. The original pictures of the full-length blots are displayed in Supplementary Fig. 5A and Supplementary Fig. 6A. (D–H) mRNA expression of *CIDEA*, *CKMT2*, *CITED1*, *DIO2*, and *TBX1* assessed by RT-qPCR, n = 4. Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test, *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

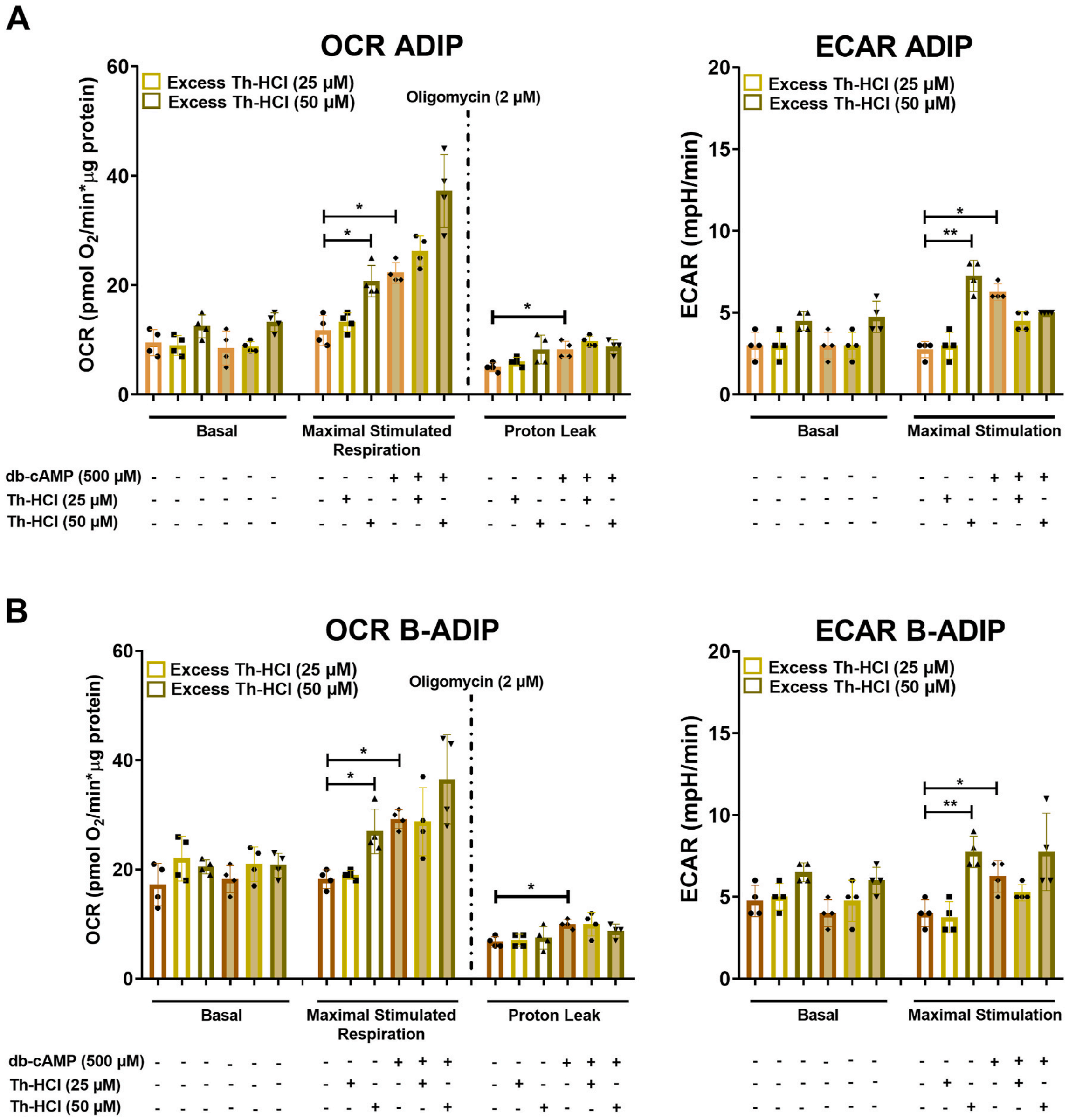

Fig. 6. Effect of excess (25 μM and 50 μM) thiamine hydrochloride (Th-HCl) on the dibutyryl-cAMP (db-cAMP)-induced oxygen consumption (OCR) and extracellular acidification (ECAR) rates in SGBS adipocytes (ADIPs) and brown differentiated adipocytes (B-ADIPs). SGBS preadipocytes were differentiated into ADIP and B-ADIP at regular culture conditions, then OCRs were detected for 10 h following the injection of 500 μM db-cAMP (brown bars) in the presence or absence of excess Th-HCl (A and B). OCR at basal, maximal db-cAMP-stimulated, and after oligomycin addition (left panels), and ECAR (right panels) were quantified in SGBS ADIPs and B-ADIPs of four independent measurements. Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test, $^*p < 0.05$, $^{**}p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

snRNA-seq data from human SC WAT, we found that ThTrs are exclusively expressed in the adipocyte cluster [Sun et al., 2020] suggesting its crucial role for the proper function of adipocytes residing in WAT. The SGBS cell line can be used to overcome these existing problems, as it allows researchers to bypass issues of limited biopsy material and donor variability [Claussnitzer et al., 2015; Ali et al., 2023]. Similarly to our previous observations in human primary cervical-derived adipocytes, we also found that ThTr inhibition led to decreased expression of thermogenic markers and cellular respiration in SGBS adipocytes. Thiamine supplementation during db-cAMP stimulation modestly increased the expression of UCP1 in white-differentiated SGBS adipocytes (ADIPs). WAT comprise 3–70% of total body weight in adults [Hausman et al., 2001; Parlee et al., 2014] and its composition is positively correlated with obesity. The expression of ThTr2 was lower in SC WAT from donors with obesity as compared to lean ones [Pereira et al., 2021]. A transcriptomic analysis reported that thermogenic adipocytes isolated from abdominal SC WAT had higher expression of both ThTr1 and 2 upon adrenergic stimulation by forskolin [Min et al., 2019]. Our present study also showed that adrenergic stimulation by db-cAMP elevated the expression of ThTr1 in SGBS adipocytes suggesting that thiamine uptake via ThTrs is crucial for thermogenic response. The active form of thiamine, TPP serves as a coenzyme for pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (α-KGDH). Inhibition of thiamine uptake can lead to decreased PDH activity, thereby restricting the formation of the high energy electron carrier, NADH. Recent publications reported that branched-chain amino acids (BCAAs) uptake was higher in the murine BAT mitochondria [Verkerke et al., 2024; Park et al., 2023]. The catabolism of BCAAs is catalyzed by a TPP-requiring enzyme complex, branched-chain alpha-keto acid dehydrogenase (BCKDH), therefore, sufficient thiamine uptake is required to support the high activity of

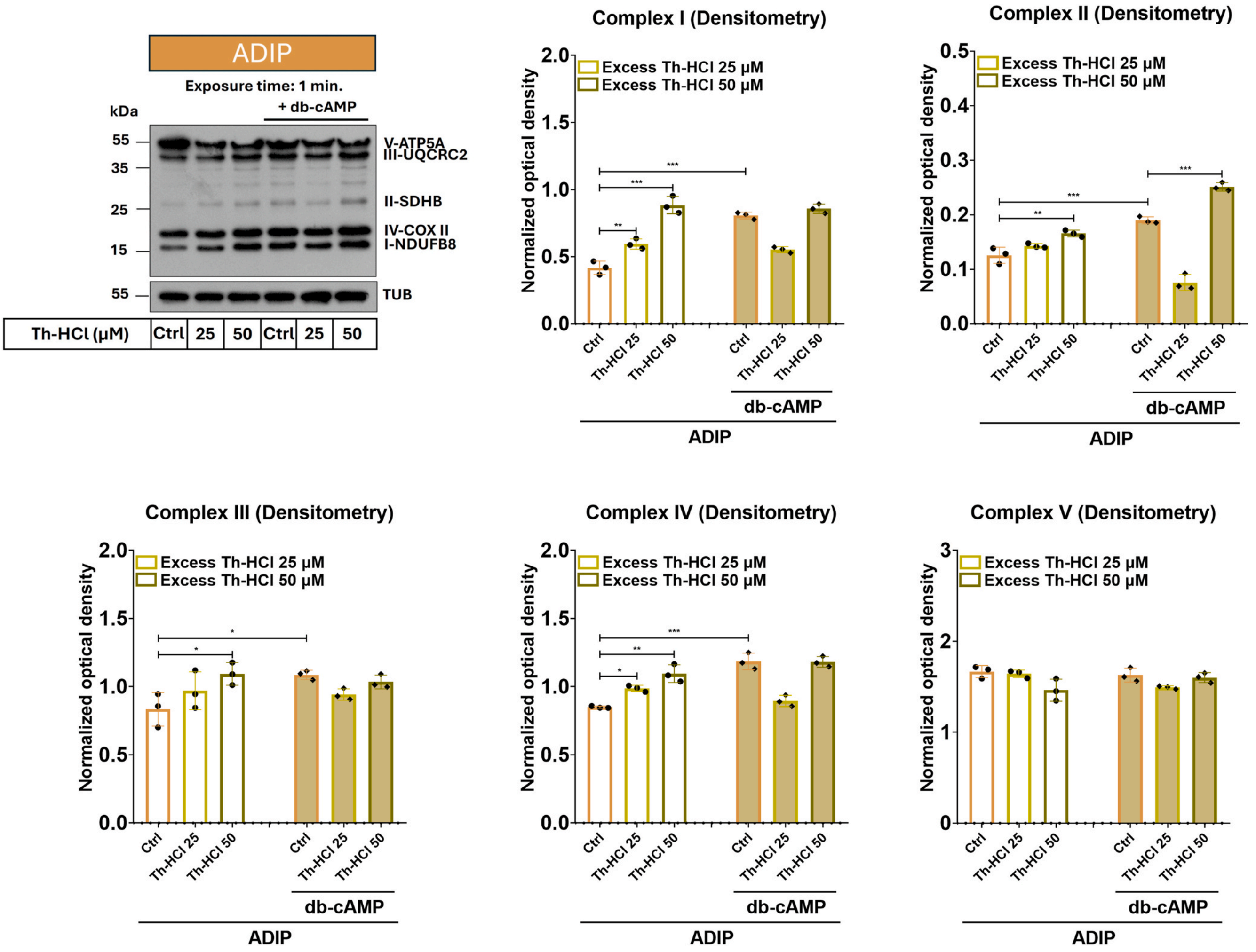

Fig. 7. Effect of excess (25 μM and 50 μM) thiamine hydrochloride (Th-HCl) on the dibutyryl-cAMP (db-cAMP)-induced expression of mitochondrial complex subunits in SGBS adipocytes (ADIPs). After differentiation at regular culture conditions (6.4 μM Th-HCl), ADIPs were treated with 500 μM db-cAMP (brown bars) in the presence or absence of excess Th-HCl for 10 h. (A) Protein expression of mitochondrial complex subunits detected by immunoblotting. The original pictures of the full-length blots with different exposure times are displayed in Supplementary Fig. 3B and Supplementary Fig. 4B, n = 3. Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test, *p < 0.05, **p < 0.01, ***p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

BCKDH.

Previous studies reported that patients with morbid obesity exhibited thiamine deficiency before bariatric surgery [Flancbaum et al., 2006; Lee, 2023]. Thiamine deficiency was also found in Thai children with obesity [Densupsoontorn et al., 2019], and has been linked to damage in the hypothalamus, which is the main regulatory center for appetite and body temperature [Tanev et al., 2008]. In addition, pediatric thiamine deficiency was also reported in high income countries including United States of America with diabetes, obesity, and high consumption of sweetened beverages as predisposing factors [Rakotoambinina et al., 2021]. A case study showed that thiamine administration for 2 days ameliorated the hypothermia in patients with Wernicke's encephalopathy [Hansen et al., 1984]. Thiamine excretion was elevated in patients with diabetes as compared to healthy subjects, with 24- and 16-fold in type 1 or 2 diabetes, respectively [Thornalley et al., 2007]. Thiamine administration at high dose (100 mg, 3 times a day) significantly reduced the level of plasma glucose in hyperglycemic subjects [Alaei Shahmiri et al., 2013]. In a randomized controlled trial, thiamine treatment decreased the level of blood glucose and leptin in 24 drug-naïve patients with type 2 diabetes [González-Ortiz et al., 2011]. Thiamine also improved the endothelial cell dysfunction, which may lead to vascular complications in patients with diabetes [Ascher et al., 2001; Wong et al., 2008]. These findings suggest that thiamine deficiency may play a role in metabolic dysfunctions associated with obesity and diabetes, highlighting the potential benefits of thiamine supplementation in managing these conditions.

Despite the valuable insights and confirmatory results gained from this study, several limitations should be acknowledged. We used pharmacological inhibitors of ThTrs which may cause off-target effects. To further explore the importance of thiamine or ThTrs in thermogenesis or adipocyte browning, utilizing gene-editing techniques, such as CRISPR-Cas9 or siRNA-mediated silencing should be considered for future studies. These approaches would allow a more specific modification and precise investigation regarding the functional role of thiamine and its transporters in adipocyte biology. Nevertheless, our results raise the possibility of using thiamine as a safe, cost-effective food supplement to prevent or combat obesity and its comorbidities by augmenting the heat generating mechanism of thermogenic adipocytes.

CRediT authorship contribution statement

Boglárka Ágnes Vinnai: Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation. **Rini Arianti:** Writing – original draft, Methodology, Investigation, Conceptualization.

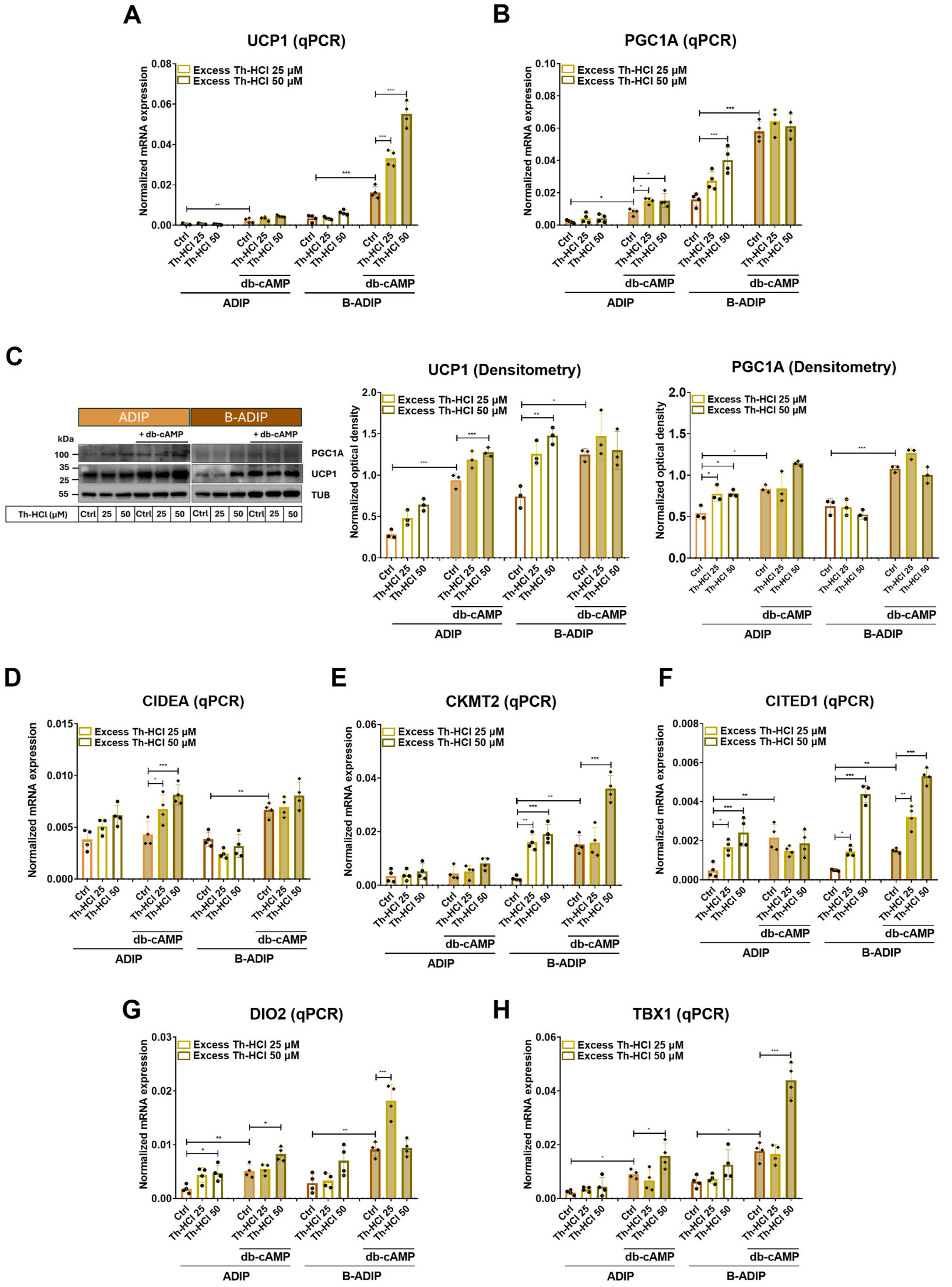

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Fig. 8. Effect of excess (25 μM and 50 μM) thiamine hydrochloride (Th-HCl) on the basal and dibutyryl-cAMP (db-cAMP)-induced expression of thermogenic markers in SGBS adipocytes (ADIPs) and brown differentiated adipocytes (B-ADIPs). After differentiation at regular culture conditions, ADIPs and B-ADIPs were treated with 500 μM db-cAMP (brown bars) in the presence or absence of excess Th-HCl for 10 h. (A and B) mRNA expression of *UCP1* and *PGC1a* assessed by RT-qPCR, n = 4. (C) Protein expression of UCP1 and PCG1a detected by immunoblotting, n = 3. The original pictures of the full-length blots are displayed in Supplementary Fig. 5B and Supplementary Fig. 6B. (D–H) mRNA expression of *CIDEA*, *CKMT2*, *CITED1*, *DIO2*, and *TBX1* assessed by RT-qPCR, n = 4. Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Pamela Fischer-Posovszky: Resources. **Martin Wabitsch:** Resources. **László Fésüs:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Endre Kristóf:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Endre Kristof reports financial support was provided by National Research, Development and Innovation Office of Hungary. Rini Arianti reports financial support was provided by National Research, Development and Innovation Office of Hungary. Endre Kristof reports a relationship with National Research, Development and Innovation Office of Hungary that includes: funding grants. Rini Arianti reports a relationship with National Research, Development and Innovation Office of Hungary that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2025.112483.

Data availability

Data will be made available on request.

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