THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 13 Issue 7

RESEARCH ARTICLE

Cellular Reprogramming of Human Primary Adipocytes into Brown Adipose Tissue-Like Cells Preferentially Utilize Glucose

Taylor SR[1], Cartwright KM[1], Sferrella AM[1], and Harding PA[1*]

[1]Department of Biology, Cell Molecular and Structural Biology Program, Miami University, Oxford, OH 45056, United States

OPEN ACCESS

PUBLISHED
31 July 2025

CITATION
Taylor, SR., et al., 2025. Cellular Reprogramming of Human Primary Adipocytes into Brown Adipose Tissue-Like Cells Preferentially Utilize Glucose. Medical Research Archives, [online] 13(7). https://doi.org/10.18103/mra.v13i7.6709

DOI
https://doi.org/10.18103/mra.v13i7.6709

ISSN
2375-1924

ABSTRACT

Brown adipose tissue has emerged as a potential therapeutic target for the treatment of 2 diabetes and obesity. This aim of this study is to investigate the role of a disintegrin and metalloprotease 12S expression alone in stimulating cellular reprogramming of a variety of human primary adipocytes into BAT-like cells and if the reprogrammed cells function as brown adipose cells. ADAM 12S-infected cells exhibited significant lipid droplet accumulation, as confirmed by Oil Red O staining and up-regulate PGC-1α, a key regulator of mitochondrial biogenesis, while uncoupling protein-1 resulted in an increased level, but not statistically significant. PR domain-containing 16, a marker of brown fat differentiation, was downregulated in a disintegrin and metalloprotease 12S-infected cells. Metabolic analysis using the Seahorse XF24 platform demonstrated a significant increase in glycolysis and extracellular acidification rates following stress induced by carbonyl cyanide-p-trifluoromethoxyphenylhydrazone and oligomycin compared to basal conditions. Our results suggest human primary adipocytes may be reprogrammed into brown adipose tissue-like cells as possible therapeutic application for type 2 diabetes.

Keywords: brown adipose tissue, a disintegrase and metalloproteinase, heparin-binding EGF-like growth factor, cellular reprogramming

Introduction

Obesity remains one of the most significant public health challenges globally, especially in developed nations where the increased availability of calorie-dense foods and a sedentary lifestyle have contributed to its rising prevalence[1]. Defined by a body mass index (BMI) of 30 kg/m² or higher, obesity is strongly associated with serious health conditions such as type 2 diabetes, cardiovascular diseases, and certain cancers[1]. This growing epidemic not only impacts individual health but also places a tremendous burden on healthcare systems worldwide, resulting in escalating healthcare costs. Current treatment strategies focusing on dietary modifications, increased physical activity, and lifestyle interventions often demonstrate limited long-term efficacy, underscoring the urgent need for innovative therapeutic approaches.

In recent years, brown adipose tissue (BAT) has garnered considerable interest as a potential target for obesity treatment and related metabolic disorders. Unlike white adipose tissue (WAT), which primarily stores energy, BAT dissipates energy through non-shivering thermogenesis by oxidizing glucose and fatty acids. This process is mediated by uncoupling protein-1 (UCP-1), which enables BAT to uncouple oxidative phosphorylation from ATP production, thereby generating heat[2]. BAT's capacity to burn energy and improve glucose metabolism, reduce insulin resistance, and promote lipid catabolism makes it a promising candidate for therapeutic strategies aimed at increasing energy expenditure and reducing obesity-related comorbidities[3,4]. Additionally, the role of BAT in thermogenesis has been supported by studies demonstrating its activation by various stimuli, including cold exposure and β-adrenergic agonists, which promote mitochondrial biogenesis and the expression of thermogenic genes[5,6].

Although BAT is abundant in newborns, where it plays a key role in thermoregulation, its presence in adults was historically thought to be negligible. However, recent discoveries have demonstrated that functional BAT depots persist in adults, particularly in regions such as the neck and upper thorax[2]. The identification of beige or brite cells, which are inducible within WAT in response to stimuli like cold exposure or β-adrenergic activation, has expanded the therapeutic potential of BAT. These cells exhibit a BAT-like phenotype, suggesting that the reprogramming of WAT into metabolically active BAT could serve as an innovative approach to treating obesity and metabolic diseases[7].

Our laboratory has previously demonstrated that the co-expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF) and the soluble form of a disintegrin and metalloproteinase 12 (ADAM 12S) induces the reprogramming of cells into a BAT-like phenotype[8]. HB-EGF, a transmembrane protein that undergoes proteolytic cleavage, generates both a soluble form (sHB-EGF) and a carboxy-terminal fragment (HB-EGF C), each with mitogenic activity[9]. HB-EGF is involved in numerous physiological processes, including wound healing, cell proliferation, and tissue remodeling, and has been linked to the expansion of WAT and enhanced adipogenesis in obesity[10,11].

ADAM 12, a member of the ADAM family of metalloproteinases, plays a crucial role in the proteolytic processing of HB-EGF and is involved in extracellular matrix remodeling, myogenesis, and adipogenesis[12]. ADAM 12 exists in both a membrane-bound form (ADAM 12L) and an alternatively spliced soluble form (ADAM 12S), with ADAM 12S shown to induce adipogenesis in transgenic mice[13]. Previous work from our lab demonstrated that the co-expression of HB-EGF and ADAM 12S in murine fibroblasts, human epidermoid carcinoma cells, and preadipocytes resulted in lipid accumulation, upregulation of BAT-specific genes such as PGC-1α, and increased mitochondrial density, all characteristic features of BAT-like cells[14].

Given the metabolic potential of brown and beige adipose tissues to enhance energy expenditure and improve metabolic health, this study seeks to expand on our previous findings by examining the effects of ADAM 12S expression in human primary

adipocytes that are representative of a wide variety of patients from all levels of adiposity and age including both male and female donors. We hypothesize that ADAM 12S expression alone, independent of HB-EGF co-expression, can drive the reprogramming of white adipocytes into metabolically active BAT-like cells through endogenous HB-EGF signaling. Such reprogramming could lay the groundwork for novel therapeutic strategies aimed at increasing energy expenditure, reducing fat mass, and addressing the metabolic dysfunctions associated with obesity and type 2 diabetes.

Materials and Methods

GENERATION OF ADAM12S ADENOVIRUS

The ADAM12S adenovirus used in this study was generated using the AdEasy adenoviral system (Agilent Technologies) as previously described[14]. Briefly, the ADAM12S cDNA (2.1 kb) was cloned into the pShuttle-IRES-hrGFP-1 plasmid for co-expression with a humanized recombinant green fluorescent protein (hrGFP), allowing visual confirmation of infection. The recombinant plasmid was electroporated into BJ5183-AD-1 cells, followed by recombination with the pAdEasy-1 backbone. High-titer viral stocks were produced in AD-293 cells, and viral titers were determined by serial dilution as described in our prior work[14].

GENERATION OF ADAM 12S EXPRESSING HUMAN PRIMARY PREADIPOCYTES

Human primary subcutaneous preadipocytes (ZenBio Inc.) were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 µg/mL), streptomycin (100 µg/mL), and amphotericin B (2.5 µg/mL) until they reached 40% confluence. Cells were then infected with either 15 µL of Ad-ADAM 12S or Ad-MOCK viral particles in the same media, as previously described[15]. The primary human cultured preadipocytes and adipocytes are pooled lots of cells derived from a wide variety of patients with representation from all levels of adiposity and age including both male and female donors appropriate for basic research in the field of adipogenesis. Successful infection was confirmed by observing hrGFP fluorescence 48 hours post-infection using an Olympus fluorescence microscope (Figure 1 panels C and D). To ensure the cells remained healthy and viable for subsequent experiments, the media was replaced with fresh growth media every 72 hours.

Lipid accumulation in infected cells was observed under brightfield microscopy three weeks post-infection. To quantitatively assess lipid content, Oil Red O staining was performed. Cells were washed with PBS, fixed with 10% formalin for 60 minutes at room temperature, and stained with freshly prepared Oil Red O solution for five minutes. Hematoxylin was used to counterstain the nuclei, and images were captured to compare lipid droplet formation between Ad-ADAM 12S and Ad-MOCK infected cells.

RT-PCR IN HUMAN PRIMARY PREADIPOCYTES

Total RNA was extracted from Ad-ADAM 12S and Ad-MOCK infected human primary subcutaneous preadipocytes using TriReagent (Sigma-Aldrich) following the manufacturer's instructions. RNA was treated with DNase I to remove any contaminating genomic DNA, and concentrations were measured using a NanoDrop spectrophotometer. RT-PCR was performed using the Superscript One-Step RT-PCR kit (Invitrogen) with 1.5 µg of total RNA. The specific primers for human HB-EGF were designed based on previous work[5] and used to amplify a 627 bp product with and without reverse transcriptase. Reactions were subjected to 30 cycles of denaturation (94°C for 30 seconds), annealing (59°C for one minute), and extension (72°C for 45 seconds), followed by a final extension at 72°C for 15 minutes. PCR products were visualized on a 0.8% agarose gel stained with ethidium bromide.

ADAM 12S INFECTION OF HUMAN PRIMARY PREADIPOCYTES

Adenovirus expressing ADAM 12S or empty (Mock) were prepared as previously described[15]. Western blot analysis of ADAM 12S was performed as previously described[8] using cell extracts of Ad-ADAM 12s infected mouse fibroblasts (MLC), human A431epidermoid carcinoma cells, and human

embryonic kidney cells (HEK 293), and resulted in immunoreactive proteins of 68kDa while no ADAM 12S immunoreactive proteins were observed in Ad-mock infected cells.

qPCR IN HUMAN PRIMARY PREADIPOCYTES

For quantitative PCR (qPCR), total RNA from Ad-ADAM 12S and Ad-MOCK infected cells was reverse-transcribed into cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems). qPCR was conducted using a SYBR Green qRT-PCR kit (Quantabio) on a CFX Connect Real-Time PCR Detection System (BioRad). The relative expression of brown adipose tissue (BAT) marker genes—PRDM16, PGC-1α, and UCP-1—was determined using the 2^-ΔΔCt method, normalizing against the housekeeping genes β-actin and GAPDH[16]. Each reaction was performed in triplicate, and data were analyzed using CFX Manager software.

SEAHORSE METABOLIC ANALYSIS

The metabolic activity of Ad-ADAM 12S and Ad-MOCK infected human primary subcutaneous preadipocytes was assessed using the Seahorse XF24 Analyzer (Seahorse Bioscience). Cells were seeded into XF24 plates at a density of 100,000 cells per well and incubated overnight. The following day, the sensor cartridge was hydrated, and assay medium (1 mM pyruvate, 2 mM glutamine, 10 mM glucose, pH 7.4) was prepared. After basal metabolic measurements, cells were exposed to catecholamines (4 µg/mL) to stimulate thermogenesis, followed by a stressor mix containing FCCP (a mitochondrial uncoupler) and Oligomycin (an ATP synthase inhibitor). The extracellular acidification rate (ECAR), which reflects the rate of glycolysis, and oxygen consumption rate (OCR), indicative of mitochondrial respiration, were measured under both basal and stressed conditions.

Results

RT-PCR OF HUMAN PRIMARY PREADIPOCYTES

To confirm the expression of endogenous HB-EGF in human primary preadipocytes, RT-PCR was conducted using primers specific to human HB-EGF (**Table 1**). A 627 bp product was observed in reverse-transcriptase-positive (+RT) samples, indicating the presence of HB-EGF (**Figure 1 panel A)** mRNA in these cells. In contrast, no product was detected in reverse-transcriptase-negative (-RT) samples (**Figure 1 panel A)**, confirming the specificity of the amplification. These results suggest that HB-EGF is endogenously expressed in human primary preadipocytes, potentially playing a role in the cellular reprogramming observed in this study.

LIPID ACCUMULATION AND OIL RED O STAINING IN INFECTED HUMAN PRIMARY PREADIPOCYTES

Human primary preadipocytes infected with Ad-ADAM 12S exhibited significant lipid accumulation three weeks post-infection, as visualized by Oil Red O staining (**Figure 1 Panel G**). The stained lipid droplets appeared as bright red structures under the microscope, confirming the presence of stored lipids in these cells. Ad-MOCK infected cells, in contrast, showed no visible lipid accumulation (**Figure 1 panel D)**, indicating that the presence of ADAM 12S promotes a BAT-like phenotype characterized by triglyceride storage. These findings are consistent with previous studies that have shown ADAM 12S's role in adipogenesis and lipid metabolism[17].

DIFFERENTIAL GENE EXPRESSION IN HUMAN PRIMARY PREADIPOCYTES

qPCR analysis was performed to assess the expression of BAT-specific genes in Ad-ADAM 12S and Ad-MOCK infected cells (**Table 2**). ADAM 12S infection resulted in a significant upregulation of PGC-1α ($p = 0.0018$), a master regulator of mitochondrial biogenesis and thermogenesis[18]. Although UCP-1, the hallmark gene of BAT thermogenesis, was upregulated, the increase did not reach statistical significance ($p = 0.2294$). Interestingly, PRDM16, a gene critical for brown fat differentiation, was significantly downregulated in ADAM 12S-infected cells ($p = 0.0008$). This may suggest that PRDM16 expression is just a molecular switch and not required for maintaining the BAT program[19]. In order to assess differential gene expression due to ADAM

12S expression, PPARg levels were compared to Ad-mock infected human primary preadipocytes and resulted in no significant difference.

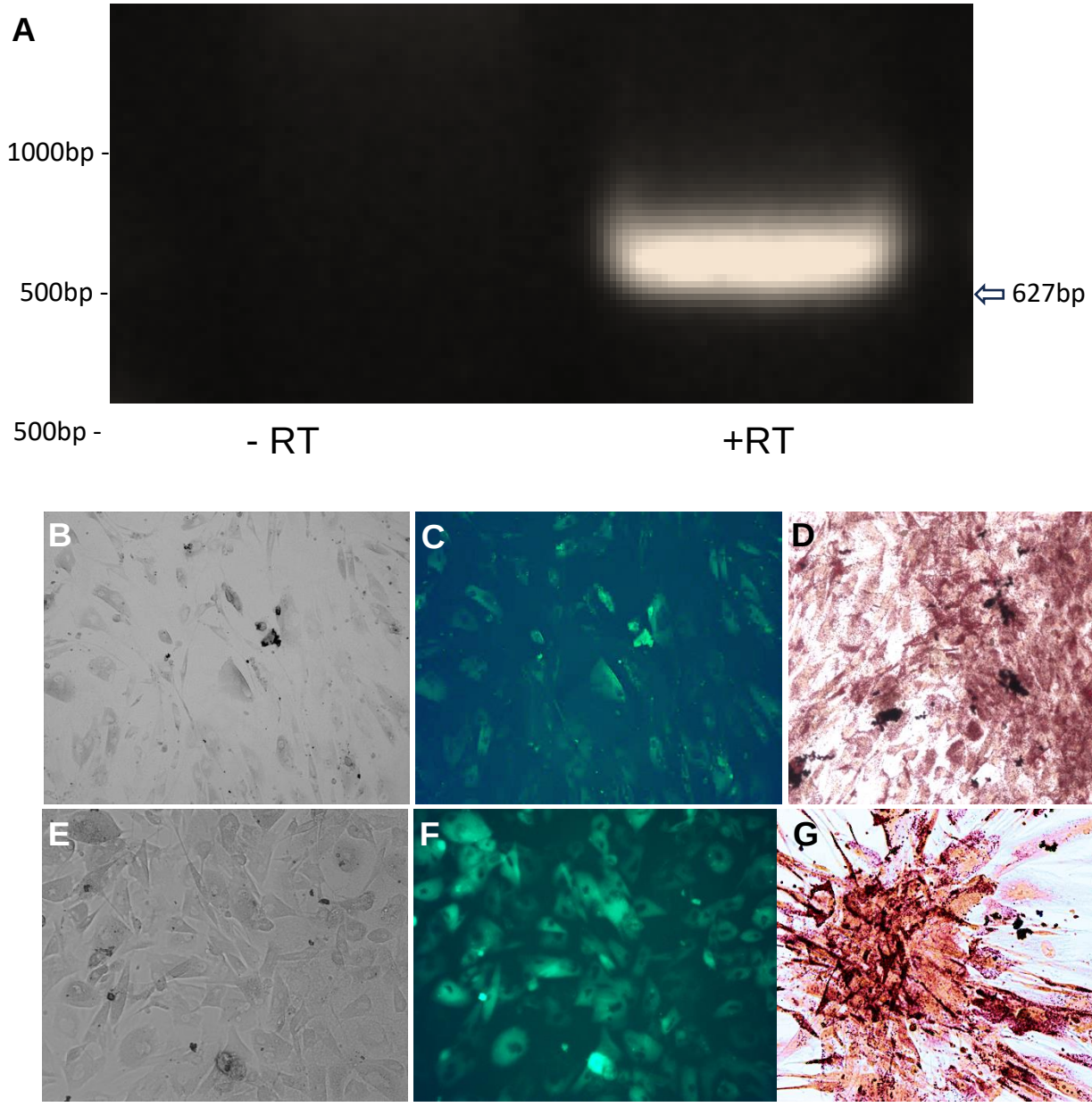

Figure 1. ADAM 12S Infection Induces Lipid Accumulation and HB-EGF Expression in Human Primary Preadipocytes. (A) RT-PCR detection of endogenous HB-EGF in primary human preadipocytes resulting in 627bp DNA product from RNA isolated from human primary preadipocytes in + reverse transcriptase (+RT) and no detection in – reverse transcriptase (-RT). **(B-D)** Microscopy images of primary human preadipocytes infected with mock adenovirus and **(E-G)** ADAM 12S adenovirus. **(B,E)** Bright field microscopy, **(C,F)** Fluorescent microscopy to determine mock adenovirus infection, **(D,G)** Oil Red O staining for lipid accumulation. (20x magnification).

Table 1. Human primer sequences for all PCR and qPCR products. All primers are from 5′ to 3′

Gene Name	Forward Primers	Reverse Primers
PRDM16	GAGCATTTCACCCCATCAAC	GGAGTCTGGAGCATTTCACC
UCP-1	TCTACGACACGGTCCAGG	GTCTGACTTTCACGACCTCTG
PGC-1α	GCCAAACCAACAACTTTATCTCTTC	CACACTTAAGCGTTCAATAGTC
β-actin	TCCCTGGAGAAGAGCTACGA	AGCACTGTGTTGGCGTACAG
GAPDH	AATCCCATCACCATCTTCCA	TGGACTCCACGACGTACTCA
PPARg	CCACAGTTGATTTCTCCAGC	GCAGGTTCTACTTTGATCGC
HB-EGF	ATGAAGCTGCTGCCGTCGG	AGTGGGAATTAGTCATGCCC

Table 2. Differential Gene Expression in Human Primary Preadipocytes. Analysis of total RNA from human primary preadipocytes infected with either Ad-MOCK (n = 3) or Ad-ADAM12S (n = 3) adenoviral vectors. Analysis of brown adipose tissue (BAT) marker genes normalized to β-actin and GAPDH. Each gene expression value represents the mean of three biological replicates, with RNA isolated from a single well of a 6-well plate.

Gene Name	Fold Change	p-value
PRDM16	-2.401	0.0008
UCP-1	3.74	0.2294
PGC-1α	21.4	0.0018

SEAHORSE METABOLIC ASSAY

The metabolic profile of Ad-ADAM 12S-infected cells was assessed using Seahorse XF24 analysis, which measures both glycolysis and oxidative phosphorylation (**Figure 2**). Under basal conditions, there was no significant difference in the extracellular acidification rate (ECAR) between ADAM 12S and MOCK-infected cells. However, upon stimulation with catecholamines, ADAM 12S-infected cells exhibited a significant increase in ECAR compared to MOCK cells ($p < 0.05$), indicating a higher rate of glycolysis in response to thermogenic activation. Furthermore, when stressed with a mix of FCCP and Oligomycin, ADAM 12S-infected cells displayed a dramatic increase in ECAR, significantly surpassing both their basal rates and those of MOCK-infected cells ($p < 0.05$). This suggests that ADAM 12S-infected cells possess enhanced metabolic flexibility and an increased capacity for glycolytic energy production, key features of thermogenically active BAT.

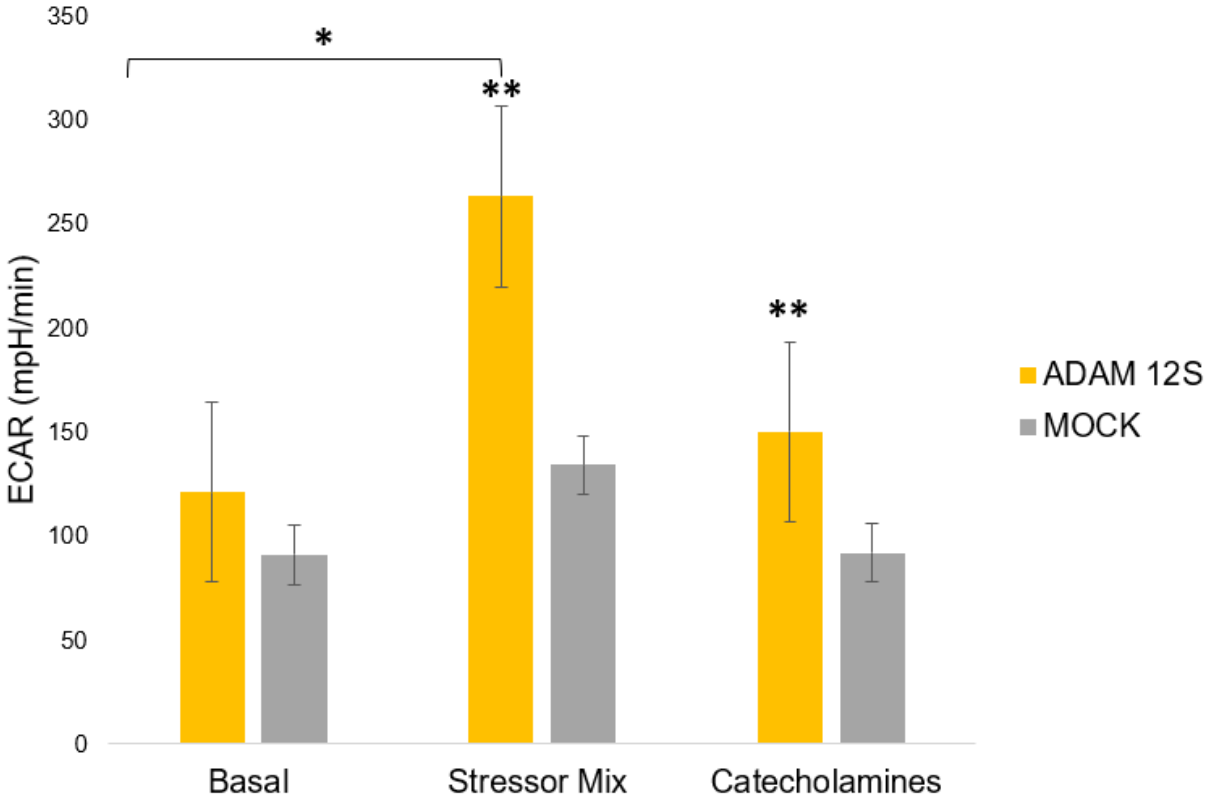

Figure 2. Metabolic Output of Human Primary Preadipocytes. Metabolic analysis of extracellular acidification rate (ECAR) of human primary adipocytes infected with Ad-ADAM12S (n = 10) or Ad-MOCK (n = 10) under basal conditions, stressor mix of FCCP and oligomycin, and catecholamine exposure. * indicates comparisons between the basal and stressed states of ADAM12S-infected cells. ** denotes comparisons between ADAM 12S- and mock-infected cells for each condition, $p < 0.05$.

Discussion

According to the World Health Organization, 1 in 8 people in the world were living with obesity in 2022. Obesity and its related metabolic complications, including but not limited to type 2 diabetes and heart disease, represent a significant and growing global health concern, with traditional lifestyle interventions often proving insufficient for long-term management[1]. The potential for brown adipose tissue (BAT) to increase energy expenditure and promote weight loss has generated considerable interest in its therapeutic applications. Unlike white adipose tissue (WAT), which stores energy, BAT dissipates energy as heat through non-shivering thermogenesis. This process is mediated by uncoupling protein-1 (UCP-1), which uncouples oxidative phosphorylation, thereby converting chemical energy into heat instead of ATP[3]. As a result, BAT activation can reduce fat mass, improve insulin sensitivity, and lower blood glucose levels, positioning it as a promising target for the treatment of obesity and metabolic diseases.

In this study, we extend from our previous findings by investigating the effects of ADAM 12S expression in human primary preadipocytes. Our laboratory had previously demonstrated that co-expression of HB-EGF and ADAM 12S could induce a BAT-like phenotype in both murine and human cell lines[8] and display increased metabolic activity[15]. Here, we hypothesized that infection with ADAM 12S alone, leveraging endogenous HB-EGF abundantly expressed in human adipose tissue[20], would similarly result in the reprogramming of human primary adipocytes into metabolically active BAT-like cells. Our findings support this hypothesis, as ADAM 12S-infected cells exhibited hallmark features of BAT, including lipid droplet accumulation, upregulation of key BAT markers such as PGC-1α, and increased glycolytic activity.

One of the key observations in this study was the significant lipid accumulation in ADAM 12S-infected cells, which became prominent three weeks post-infection. Lipid accumulation is a characteristic feature of brown adipocytes, which store fuel for thermogenesis in the form of multilocular triglyceride droplets. Oil Red O staining confirmed the presence of lipids in ADAM 12S-infected cells, while mock-infected cells showed no such accumulation, consistent with previous findings in transgenic mice expressing ADAM 12S[17]. This phenotypic shift towards BAT-like cells underscores the role of ADAM 12S in adipocyte reprogramming, likely through its involvement in extracellular matrix remodeling and the promotion of adipogenesis[12].

To further characterize the BAT-like properties of ADAM 12S-infected cells, we analyzed the expression of key thermogenic and mitochondrial biogenesis genes. Notably, we observed a statistically significant upregulation of PGC-1α, a critical regulator of mitochondrial biogenesis and thermogenesis[18]. PGC-1α plays a pivotal role in coordinating the transcriptional programs required for the development of BAT and its ability to generate heat. It also induces the expression of UCP-1, which facilitates the uncoupling of oxidative phosphorylation in brown fat mitochondria, a hallmark of BAT's thermogenic function[18]. Although UCP-1 expression was upregulated in ADAM 12S-infected cells, the increase did not reach statistical significance. This could be due to the timing of the experiment or variations in the cellular environment. Nonetheless, the increase in PGC-1α supports the notion that these cells are acquiring BAT-like metabolic properties.

Interestingly, PRDM16, a transcription factor known to govern the differentiation of preadipocytes into brown fat cells, was found to be significantly downregulated in ADAM 12S-infected cells. PRDM16 is essential for the commitment of precursor cells to the brown fat lineage, driving the expression of BAT-specific genes and suppressing muscle differentiation[19]. The observed downregulation of PRDM16 in this study may suggest that its role is limited to the early stages of BAT differentiation, after which its expression diminishes once cells have committed to the brown adipocyte fate. This aligns with findings that PRDM16 is more critical during the differentiation phase, whereas genes like PGC-1α and UCP-1 are involved in maintaining the thermogenic function of mature brown adipocytes[19]. Moreover, the increased expression of FGF2, KLF3, and the decreased expression of C/EBPα and GLUT4 in HB-EGF/ADAM 12S co-expressing cells has been identified as key components in BAT cellular reprogramming, further suggesting that HB-EGF/ADAM 12S directs cellular reprogramming into BAT rather than WAT[15].

The metabolic activity of ADAM 12S-infected cells was assessed using Seahorse XF24 analysis, which provided insights into the cells' glycolytic and oxidative capacity. The extracellular acidification rate (ECAR), a measure of glycolysis, was significantly increased in ADAM 12S-infected cells compared to mock-infected controls following exposure to catecholamines and a stressor mix containing FCCP and Oligomycin. This increase in ECAR indicates that ADAM 12S-infected cells are more metabolically active and rely heavily on glycolysis to meet their energy demands. Non-shivering thermogenesis is a highly energetic process and relies on a readily available fuel supply of circulating glucose or free fatty acids from white adipose tissue[21]. BAT is known for its high metabolic activity, particularly its ability to oxidize glucose and lipids for thermogenesis[4]. The increased glycolytic activity observed in ADAM 12S-infected cells is consistent with this metabolic profile, suggesting that these cells are functionally similar to brown adipocytes.

Moreover, the response of ADAM 12S-infected cells to catecholamines, which stimulate BAT thermogenesis, further supports their functional reprogramming. Catecholamines activate β-adrenergic receptors, leading to the activation of PKA and the induction of lipolysis and UCP-1 expression in BAT[22]. In this study, ADAM 12S-infected cells showed a significant increase in ECAR following

catecholamine exposure, indicating that these cells not only exhibit a BAT-like phenotype but also respond to thermogenic stimuli in a manner akin to native brown adipocytes. The subsequent stress response induced by FCCP and Oligomycin, which disrupts mitochondrial membrane potential and inhibits ATP synthase, respectively, further demonstrated the metabolic flexibility of these reprogrammed cells. The significant increase in ECAR in ADAM 12S-infected cells following this stress exposure underscores their enhanced metabolic capacity.

The ability of ADAM 12S to reprogram human primary preadipocytes into metabolically active BAT-like cells holds significant therapeutic potential. BAT activation and the promotion of brown adipocyte development have been linked to improvements in metabolic health, including increased energy expenditure, enhanced glucose metabolism, and reduced fat mass[23]. By increasing the amount of active BAT or promoting the conversion of WAT into BAT-like cells (a process referred to as "browning"), it may be possible to harness BAT's thermogenic properties to combat obesity and type 2 diabetes. Additionally, the ability of BAT to reduce circulating glucose levels and improve insulin sensitivity presents a promising avenue for addressing metabolic diseases associated with obesity[4].

Conclusion

This study demonstrates that ADAM 12S expression in human primary adipocytes leads to the reprogramming of these cells into BAT-like cells by interacting with the endogenous HB-EGF and is characterized by increased lipid accumulation, upregulation of PGC-1α, and enhanced glycolytic. The glucose uptake by the reprogrammed BAT-like human primary cells may be the result of an AMP activated protein kinase-dependent mechanism, indicating that glucose is likely used to replenish ATP through aerobic glycolysis to compensate for the decreased ATP production in mitochondria due to active UCP1 that uncouples ATP synthase[24,25]. These findings provide further evidence of the molecular role of ADAM 12S role in promoting adipocyte plasticity and support its potential as a therapeutic tool for metabolic disorders. Future research will further elucidate the precise mechanisms underlying ADAM 12S-mediated reprogramming and exploring the therapeutic efficacy of these reprogrammed cells *in vivo*.

Acknowledgements:

We would like to acknowledge Dr. Debra Mayes and her staff from Wright State University for their help on the Seahorse experiments.

References:

1. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. *Arterioscler Thromb Vasc Biol*. 2006;26(5):968-976. doi:10.1161/01.ATV.0000216787.85457.

2. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. *Nat Med*. 2013;19(10):1252-1263. doi:10.1038/nm.3361

3. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab*. 2007;293(2):E444-E452. doi:10.1152/ajpendo.00691.2006

4. Peirce V, Vidal-Puig A. Regulation of glucose homoeostasis by brown adipose tissue. *Lancet Diabetes Endocrinol*. 2013;1(4):353-360. doi:10.1016/S2213-8587(13)70055-X

5. no M, Raab G, Lau K, Abraham JA, Klagsbrun M. Purification and characterization of transmembrane forms of heparin-binding EGF-like growth factor. *J Biol Chem*. 1994;269(49):31315-31321

6. Suzuki M, Raab G, Moses MA, Fernandez CA, Klagsbrun M. Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site. *J Biol Chem*. 1997;272(50):31730-31737. doi:10.1074/jbc.272.50.31730

7. Ghorbani M, Claus TH, Himms-Hagen J. Hypertrophy of brown adipocytes in brown and white adipose tissues and reversal of diet-induced obesity in rats treated with a beta3-adrenoceptor agonist. *Biochem Pharmacol*. 1997;54(1):121-131. doi:10.1016/s0006-2952(97)00162-7

8. Zhou Z, Darwal MA, Cheng EA, Taylor SR, Duan E, Harding PA. Cellular reprogramming into a brown adipose tissue-like phenotype by co-expression of HB-EGF and ADAM 12S. *Growth Factors*. 2013;31(6):185-198. doi:10.3109/08977194.2013.84029

9. Higashiyama S, Nanba D. ADAM-mediated ectodomain shedding of HB-EGF in receptor cross-talk. *Biochim Biophys Acta*. 2005;1751(1):110-117. doi:10.1016/j.bbapap.2004.11.009

10. Nanba D, Mammoto A, Hashimoto K, Higashiyama S. Proteolytic release of the carboxy-terminal fragment of proHB-EGF causes nuclear export of PLZF. *J Cell Biol*. 2003;163(3):489-502. doi:10.1083/jcb.200303017

11. Asakura M, Kitakaze M, Takashima S, et al. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: metalloproteinase inhibitors as a new therapy. *Nat Med*. 2002;8(1):35-40. doi:10.1038/nm0102-35

12. Kurisaki T, Masuda A, Sudo K, et al. Phenotypic analysis of Meltrin alpha (ADAM12)-deficient mice: involvement of Meltrin alpha in adipogenesis and myogenesis. *Mol Cell Biol*. 2003;23(1):55-61. doi:10.1128/MCB.23.1.55-61.2003

13. Kawaguchi N, Xu X, Tajima R, et al. ADAM 12 protease induces adipogenesis in transgenic mice. *Am J Pathol*. 2002;160(5):1895-1903. doi:10.1016/S0002-9440(10)61136-4

14. Taylor, S.R., Klements, J.R., Markesbery, M.G., Johnson, K.D., & Harding, P.A. (2014). Cellular transdifferentiation into brown adipose-like cells by adenoviral-directed expression or stable transfection of HB-EGF and ADAM 12S. *J Cell Mol Biol*, 10(2): 185-198.

15. Taylor SR, Gemma CA, Cartwright KM, et al. HB-EGF and ADAM 12S directed cellular reprogramming results in metabolically active brown adipose tissue-like cells[J]. *AIMS Cell and Tissue Engineering*, 2018, 2(4): 203-219. doi: 10.3934/celltissue.2018.4.203

16. Ahfeldt T, Schinzel RT, Lee YK, et al. Programming human pluripotent stem cells into white and brown adipocytes. *Nat Cell Biol*. 2012;14(2):209-219. Published 2012 Jan 15. doi:10.1038/ncb2411

17. Kawaguchi N, Xu X, Tajima R, et al. ADAM 12 protease induces adipogenesis in transgenic mice. *Am J Pathol*. 2002;160(5):1895-1903. doi:10.1016/S0002-9440(10)61136-4

18. Uldry M, Yang W, St-Pierre J, Lin J, Seale P, Spiegelman BM. Complementary action of the

PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab*. 2006;3(5):333-341. doi:10.1016/j.cmet.2006.04.002

19. Seale P, Bjork B, Yang W, et al. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. 2008; 454(7207):961-967. doi:10.1038/nature07182

20. Matsumoto S, Kishida K, Shimomura I, et al. Increased plasma HB-EGF associated with obesity and coronary artery disease [published correction appears in Biochem Biophys Res Commun 2002 Jul 12;295(2):571]. *Biochem Biophys Res Commun*. 2002; 292(3):781-786. doi:10.1006/bbrc.2002.6720

21. Jeong JH, Chang JS, Jo YH. Intracellular glycolysis in brown adipose tissue is essential for optogenetically induced nonshivering thermogenesis in mice. *Sci Rep*. 2018;8(1):6672. Published 2018 Apr 27. doi:10.1038/s41598-018-25265-3

22. Barclay JL, Agada H, Jang C, Ward M, Wetzig N, Ho KK. Effects of glucocorticoids on human brown adipocytes. *J Endocrinol*. 2015;224(2):139-147. doi:10.1530/JOE-14-0538

23. Winther S, Isidor MS, Basse AL, et al. Restricting glycolysis impairs brown adipocyte glucose and oxygen consumption. *Am J Physiol Endocrinol Metab*. 2018;314(3):E214-E223. doi:10.1152/ajpendo.00218.2017

24. Inokuma K, Ogura-Okamatsu Y, Toda C, Kimura K, Yamashita H, Saito M. Uncoupling protein 1 is necessary for norepinephrine-induced glucose utilization in brown adipose tissue. *Diabetes*. 2005; 54(5):1385-1391. doi:10.2337/diabetes.54.5.1385

25. Teperino R, Amann S, Bayer M, et al. Hedgehog partial agonism drives Warburg-like metabolism in muscle and brown fat. *Cell*. 2012;151(2):414-426. doi:10.1016/j.cell.2012.09.021