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REVIEW ARTICLE

An emerging role for epigenetic regulation of Pgc-1α expression in environmentally stimulated brown adipose thermogenesis

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Abstract

Metabolic disease is a leading cause of death worldwide, and obesity, a central risk factor, is reaching epidemic proportions. Energy expenditure and brown adipose tissue (BAT) thermogenesis are implicated in metabolic disease, and it is becoming evident that impaired BAT activity is regulated by gene/environment interactions. Peroxisome proliferator-activated receptor γ coactivator 1α (Pgc-1α) is a critical regulator of BAT thermogenesis, which is highly inducible by environmental stimuli such as cold and diet. This review focuses on the environmentally mediated epigenetic and transcriptional regulation of Pgc-1α gene expression during BAT thermogenesis. We illustrate interactions between histone modifications and transcription factors at the Pgc-1α promoter that cause BAT Pgc-1α transcription in response to cold. Histone modifications also modulate BAT Pgc-1α transcription in response to nutrients though diet has been less characterized than cold with respect to regulation of Pgc-1α transcription. Pgc-1α DNA methylation and RNA expression were also correlated to indicators of adiposity and glucose homeostasis across numerous human tissues. Although post-translational modification of Pgc-1α protein has been well-characterized across diverse tissues and environments, comparatively little is known of the epigenetic mechanisms regulating Pgc-1α transcription, particularly in BAT thermogenesis.

Key words: brown adipose tissue; epigenetics; gene regulation; peroxisome proliferator-activated receptor γ coactivator 1α; thermogenesis

Introduction

The prevalence of metabolic disease is rising rapidly across societies. Brown adipose tissue (BAT) is an important contributor to metabolism and its impaired activity is implicated in metabolic disease. Proliferator-activated receptor γ coactivator 1α (Pgc-1α) is a critical regulator of BAT activity in response to environmental stimuli such as cold temperature and diet. In this review we explore whether epigenetic modification of the Pgc-1α gene by covalent histone or DNA modifications could play a role in its regulation of environmentally stimulated-BAT activity.

This review examines the environmentally mediated epigenetic and transcriptional regulation of Pgc-1α gene expression involved in BAT thermogenesis. First we briefly summarizing

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the importance of BAT in thermogenesis and metabolic diseases. Then we introduce Pgc-1α, which acts as a critical regulator of thermogenesis through its integration of environmental signals with gene expression changes that result in thermogenic physiological responses. We next highlight some transcriptional co-activation events facilitated by Pgc-1α with a focus on those involving chromatin structural consequences. Last we survey two environments- cold and diet- that modulate BAT thermogenesis through epigenetic regulation of Pgc-1α expression. As a corollary we also provide human evidence that connects Pgc-1α DNA methylation to indicators of metabolism that are commonly associated with dietary factors. This review highlights opportunities to improve the mechanistic evidence for the role of environment in BAT thermogenesis mediated by epigenetic regulation of Pgc-1α expression.

What Is BAT

BAT is present in most mammals where it primarily functions in adaptive, non-shivering thermogenesis, and thus regulates energy expenditure in the form of heat dissipation. The exothermic capacity of this small tissue (<100 g) is remarkable given it has been estimated that BAT thermogenesis may account for 5% of basal metabolic rate in adult humans [1].

The characteristic brownish color of BAT cells is a result of intense vascularization and high concentrations of densely packed mitochondria, an organelle that converts nutrients to water, carbon dioxide and ATP [2]. BAT mitochondria are capable of converting much of their energy production from ATP to heat through a proton leak catalyzed by uncoupling protein-1 (UCP1) that uncouples ATP synthesis from oxidative phosphorylation [3–5]. BAT vascularization aids in heat dissipation, while its jacketing of major arteries and veins insulates the bloodstream [6, 7]. BAT thermogenic response to cold exposure, its primary stimulant, is regulated by the innervation of BAT from the sympathetic nervous system (SNS; further detailed in Section 'Cold-induced thermogenesis) [8, 9]. In altricial animals (e.g. mice, humans) BAT is derived from mesodermal tissue and activated immediately after birth to maintain heat in response to low ambient temperature [10]. Recently discovered in adult humans through functional and structural visualization (positron-emission tomography of glucose analog radiotracer uptake combined with computed tomography), the prevalence of detectable BAT in healthy adults during cold exposure ranges from 50 to 95% [11, 12]. However, these indirect estimates of prevalence based on glucose tracer uptake are likely underestimating true BAT prevalence in adult humans because BAT may be present but not active or may be taking up lipids, the favored substrate for BAT thermogenesis [13–15]. Indeed BAT not activated by cold is associated with lower prevalence estimates. Since the discovery of thermogenic AT in adult humans, there has been extensive discussion of whether this tissue is innate (BAT) or inducible white AT (brite or beige AT). As the clinical importance of this distinction is not yet clear, for the purpose of this review we refer to BAT as all thermogenic AT.

Given the prominent role of external temperature in stimulating BAT, it is intuitive that BAT activity is inversely associated with both outdoor temperature and the experimental manipulation of indoor temperature among studies of adult humans [12, 16, 17]. When one considers the importance of fatty acids and glucose as substrates for BAT thermogenesis, it is not surprising that human BAT activity has also been inversely associated with fasting glucose levels and body mass index (BMI) [13, 18]. Indeed glucose uptake by BAT can be stimulated by insulin in addition to cold-induced SNS signaling in humans [19]. BAT activity is also decreased in the pathological states associated with fasting glucose and BMI, namely type 2 diabetes (T2D) and obesity, respectively [20]. For example, BAT prevalence has been negatively associated with obesity in humans from the USA, Netherlands, Finland, Germany, Australia, Canada and Japan [11, 13, 21–25]. Furthermore, independent of measures of adiposity or obesity, BAT prevalence has also been negatively associated with T2D in Canadians [23].

BAT transplants in rodents support this human evidence for a role of BAT activity in pathologies related to obesity and T2D. BAT transplants from metabolically healthy mice to obese mice increased body temperature and whole body oxygen consumption, while improving glucose- and insulin-tolerance and reducing body- and liver- fat mass [26–28]. Remarkably, glucose tolerance and BAT glucose uptake are improved in direct proportion to the mass of transplanted BAT [29]. Complete coverage of the role of BAT in obesity and T2D is out of the scope of this review; readers are directed to recent reviews of this vast animal literature for further perspective [30, 31].

Pgc-1α: Critical Regulator of Thermogenesis

The function of Pgc-1α as a cold-inducible transcriptional coactivator of thermogenesis was discovered through its interaction with the nuclear receptor peroxisome proliferator activated receptor gamma (PPARγ) in BAT from mice [32]. Pgc-1α is considered the master regulator of mitochondrial biogenesis because it has a critical role in regulating mitochondrial content and respiration [32, 33]. Pgc-1α is also a critical regulator of BAT thermogenesis, and its absence in brown adipocytes is associated with a gross inability to activate the program of gene expression that generates thermogenesis in response to cold [33, 34].

Pgc-1α shares high (93%) sequence homology between humans and rodents (Fig. 1). Knockout mouse models reinforce the central role of Pgc-1α in thermogenesis. Mice lacking global Pgc-1α activity have reduced mitochondrial gene expression in mitochondrial dense tissue, including BAT, heart and skeletal muscle, and increased cold sensitivity [35, 36]. Although we know of no BAT specific Pgc-1α mutant mammal models, mice with a fat-specific knock-out of Pgc-1α have increased cold sensitivity, and decreased RNA expression of Ucp-1, mitochondrial genes, and substrate utilization genes [37]. Further, *in vitro* knock out of Pgc-1α in brown adipocytes in rodents demonstrates its central role in activating gene expression in response to sympathetic stimulation under both basal and uncoupled conditions [38]. Indeed, altered Pgc-1α expression appears to be a universal response to the environmental factors that alter BAT thermogenesis, namely cold and diet.

Pgc-1α as a Transcriptional Coactivator

At the molecular level, Pgc-1α is a critical regulator of BAT thermogenesis and is important in the differentiation of brown adipocytes in large part due to its function as a coactivator of transcription. Transcriptional coactivators do not bind to DNA directly. Instead, coactivators activate transcription by altering chromatin structure via histone-acetyltransferase (HAT), deacetylase (HDAC), methyltransferase and demethylase, or by altering pre-initiation complex formation through the mediation of DNA binding proteins and RNA polymerase II interactions. Pgc-1α utilizes both mechanisms to facilitate expression of its target genes and we focus on the former mechanism briefly here.

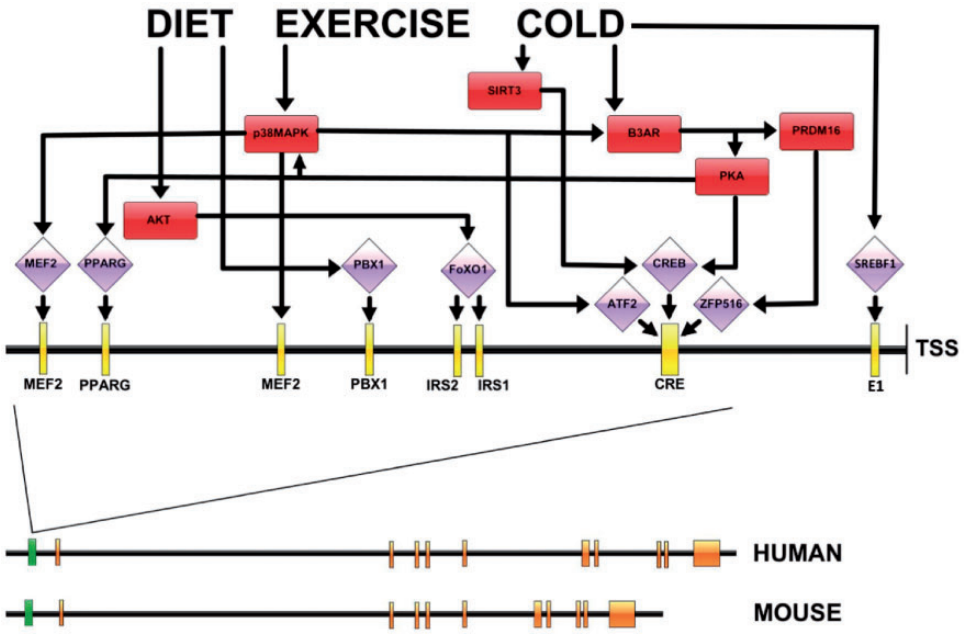

Figure 1: signaling factors influencing the Pgc-1α promoter activity and Pgc-1α gene structural comparison, representing 93% amino acid sequence identity between human and mouse. Promoters, green; exons, orange. The expanded inset is a graphical representation of the human Pgc-1α proximal promoter upstream of the transcription start site (TSS). Environmentally induced signaling pathways modulate transcription factors (purple diamonds) with binding sites (yellow) on the human Pgc-1α promoter. All transcription factor binding consensus sequences here are conserved between human and mouse. Signaling pathways include diet-induced protein kinase B (Akt) activity, which modulates cytoplasmic levels of FoxO1; diet may also regulate Pgc-1α activity through the transcription factor PBX1; and cold stimulates the β_3-adrenergic receptors, leading to PKA regulation of CREB, PPARγ, and p38MAPK; cold activates SIRT3, stimulating CREB phosphorylation; cold induced SNS stimulation also transcriptionally activates Pgc-1α through PRDM16 by interacting with the transcription factor ZFP516. ATF2, activating transcription factor 2; IRS, insulin response sequence; CRE, cAMP response element; MEF2, myocyte enhancer factor 2; SREBF1, sterol regulatory element binding transcription factor 1.

Although exhibiting little evidence of HAT domains along its sequence, Pgc-1α interacts with other HAT-specific coactivators, including steroid receptor coactivator 1 (SRC-1), the pleotropic coactivator cyclic adenosine monophosphate (cAMP) response element (CRE)-binding protein (CBP) and p300; all of these HAT-specific coactivators increase the ability of Pgc-1α to induce mRNAs of target genes in mouse fibroblasts [39]. The canonical nuclear receptor coactivator SRC-3 regulates the expression of lysine acetyltransferase 2A (Kat2a also known as GCN5), a Pgc-1α acetyltransferase, to inhibit target gene expression in mouse liver [40]. SRC-3 −/− mutant mice have increased fatty acid oxidation, oxidative phosphorylation, and energy expenditure phenotypes, with a notable upregulation of Pgc-1α in BAT [41]. The authors also observed increased body temperature in these mutants during cold exposure, suggesting enhanced adaptive thermogenesis.

The methylation state of histones is regulated by methyltransferases and demethylases. H3K9 lysine-specific demethylase 3A (Kdm3a commonly known as Jhdm2a) activates Ucp-1 by binding to its 5 prime enhancer (PPAR response element) to recruit transcription factors (e.g. Pparγ) and coactivators (e.g. Pgc-1α) while reducing H3K9me2 levels in human BAT. Loss of the Jhdm2a demethylase disrupts beta-adrenergic-stimulated lipolysis and oxygen consumption in the BAT of mice and leads to their obesity and hyperlipidemia [42].

Pgc-1α also contains activating domains in its carboxyl terminus, including a PPARγ-dependent thyroid hormone receptor-associated protein/vitamin D receptor-interacting protein (Mediator) domain, which facilitates DNA bound activator and transcriptional machinery cross-talk in mouse embryonic fibroblasts. In addition to transcriptional coactivation, several domains for mRNA splicing factors (i.e. RNA recognition motifs) also reside along the carboxyl terminus of Pgc-1α. See Finck and Kelly [43] for a thorough overview of Pgc-1α coactivators, and for a review of post-translation modifications mediating Pgc-1α activity [44].

Numerous repressors of Pgc-1α co-activation have also been described (recently reviewed in [45]). For example, Twist-1 directly binds Pgc-1α protein while recruiting HDAC5 to the UCP1 promoter to suppress histone H3 acetylation which is typically facilitated by Pgc-1α to induce *Ucp1* transcription in mouse brown adipose tissue [46]. As testament to the importance of Twist-1 in regulating thermogenesis via Pgc-1α, overexpression of rodent adipose Twist-1 caused decreased body temperature and oxidative respiration of brown adipocytes while promoting diet-induced obesity, and reduced oxidative respiration while its knock down caused the opposite.

Pgc-1α is a molecular nexus for the transcriptional control of metabolic activity in BAT. These studies highlight that the effects of post-translational histone modifications on Pgc-1α protein activity are coming into focus but their role in BAT in response to environment remains an outstanding research need.

Cold-Induced Thermogenesis

Cold exposure is the primary and best characterized environmental stimulus of BAT thermogenesis [47–50]. Seminal work established that BAT undergoes hyperplasia and hypertrophy in rats exposed to cold chronically over days to weeks [51–54]. This adaptive thermogenic phenotype of chronically cold-exposed

mice also includes increased mitochondrial biogenesis and vascularization of BAT [55, 56].

The route from environmental cold signal to physiological response is relatively well-elucidated in mammals. Cutaneous cold receptors (e.g. TRPM8, transient receptor potential cation channel M8) relay sensory information to the hypothalamus, which excites SNS pre-ganglionic neurons projecting from the spinal cord to the stellate ganglion and subsequently excites post-ganglionic SNS neurons extending to interscapular BAT [57]. Upon this stimulation, norepinephrine is released by sympathetic nerve efferent fibers to activate BAT beta-adrenergic receptors [32]. These receptors induce transcription of Pgc-1α and its transcriptional coactivation of Ucp-1, which uncouples oxidative phosphorylation from ATP production to convert chemical energy to heat [32]. Much of what is known about the regulation of Pgc-1α expression has been elucidated from beta-adrenergic stimuli, and is detailed further below.

Cold-induced beta-adrenergic stimulation activates BAT thermogenesis by recruiting a number of transcription factors that induce Ucp-1 and/or Pgc-1α expression. For example, beta-adrenergic stimulation recruits the binding of sterol regulatory element binding transcription factor 1 (SREBF1) to a conserved proximal promoter region of Pgc-1α, driving Pgc-1α expression in BAT (Fig. 1 [58]). Pgc-1α and Ucp-1 are also transcriptionally activated by Zfp516 in conjunction with PRDM16 following SNS stimulation (Fig. 1 [59]).

During cold exposure, beta-adrenergic activation increases intracellular cAMP which in turn leads to phosphorylation of activating transcription factor 2 (ATF2) and CREB [60]. Shi *et al.* [61] demonstrated that cold (and fasting) exposure induces sirtuin 3 (SIRT3), a class III HDAC in mouse BAT, while enforced SIRT3 expression in human BAT cells contributes to Pgc-1α upregulation by stimulating CREB phosphorylation (Fig. 1). Phosphorylation of ATF2 by p38 MAPK and of CREB by protein kinase A (PKA) facilitates their binding to the Pgc-1α promoter to activate Pgc-1α transcription (Fig. 1 [60]).

When brown adipocytes are activated by beta-adrenergic signaling, HDAC1 dissociates from the CRE region of the Pgc-1α promoter which increases activating H3K27 acetylation at the CRE region [62]. HDAC1 dissociation from the Pgc-1α promoter also leads to the recruitment of the H3K27 lysine (K)-specific demethylase 6A (UTX) and of CBP to the CRE, which decreases the repressive H3K27 trimethylation of Pgc-1α (Fig. 1 [62, 63]). These UTX-mediated events increase lipolysis and decrease mitochondrial membrane potential, consistent with thermogenically uncoupled oxidative respiration [63]. These data demonstrate that sympathetic activation of brown adipocytes leads to activating histone modifications that permit transcription factor binding to a Pgc-1α response element which induces Pgc-1α transcription and the thermogenic program.

Diet-Induced Thermogenesis

Early dietary studies in rodents expanded the known function of BAT thermogenesis beyond protecting against cold to protecting against obesity and insulin resistance [64]. Delicately tuned metabolic control of energy intake, utilization, and storage involve glucose and insulin as well as AMP-activated protein kinase (AMPK) and SIRT1 pathways [65]. Although rodents with diet-induced obesity generally exhibit decreased SNS activity and BAT thermogenesis [66], whether a thermogenic mechanism is responsible for whole body energy expenditure is questioned [67]. However, a study of healthy men at thermoneutral temperature demonstrated that those men with metabolically active BAT had significantly increased diet-induced thermogenesis and lipid utilization (fat burning) compared with individuals with metabolically inactive BAT, supporting a physiological role of BAT thermogenesis in diet-induced thermogenesis and overall energy metabolism [68]. Indeed, diet-induced thermogenesis has been shown in mice maintained at thermal neutrality to be completely contingent on Ucp-1 activity [69], indirectly suggestive of a regulatory role for Pgc-1α in diet-induced thermogenesis. This is further supported in mice engineered to express a mutant transcription factor forkhead box-containing protein O subfamily 1 (FoxO1, Fig. 1) in their BAT. These mice were protected from the adverse effects of high fat diet feeding on diet-induced thermogenesis, and instead exhibited improved glucose tolerance and insulin sensitivity, increased body temperature and oxygen consumption, and upregulation of Pgc-1α protein [70].

Several dietary studies implicate a role of dietary fat in modulating Pgc-1α DNA methylation and mRNA expression. Among men of low-birth weight, acute (3 days) high fat diet consumption was associated with increased DNA methylation of Pgc-1α in AT compared with those on a normal diet. Insulin stimulation of these men of low-birth weight who were fed a high fat diet resulted in increased Pgc-1α mRNA expression in their AT [71]. These results may reflect modulation of inducible thermogenic AT in humans. Similar hypermethylation of the Pgc-1α promoter occurred in primary human skeletal myocytes in response to exposure to excess saturated fatty acids such as palmitate, but not glucose or insulin [72]. This study also indicated a link between dietary fatty acids and epigenetic modification by showing that palmitate also reduced Pgc-1α mRNA expression and mitochondrial DNA abundance dependent on the activity of the DNA methyltransferase Dnmt3b in human skeletal muscle. The functional relevance of these changes was evidenced by decreased mitochondrial numbers, area, and respiratory chain proteins in the skeletal muscle of these T2DM patients. As further proof of concept that changes in Pgc-1α DNA methylation could be functionally relevant, palmitate exposure also increased Pgc-1α promoter methylation, decreased Pgc-1α mRNA expression, and reduced mitochondrial numbers in various mouse central nervous system cell types *in vitro* and in the brains of humanized mice [73]. These data suggest that nutrients can lead to signals that modulate Pgc-1α DNA methylation to influence Pgc-1α expression in several human tissues. Whether this can modulate the activity of thermogenic AT should be further evaluated in human subscapular BAT and rodent models.

There are several examples of nutrient-driven epigenetic regulation of Pgc-1α. For instance, flavin adenosine dinucleotide (FAD) is an essential co-factor in fatty acid oxidation and the respiratory chain. When FAD synthesis drops, lysine-specific demethylase-1 (LSD1) decreases while H3 acetylation, H3K4 dimethylation and trimethylation all increase at the Pgc-1α promoter, and both Pgc-1α expression and oxidative respiration increases in adipocytes [74]. LSD1 depletion in high fat diet fed mice also increases Pgc-1α expression [74]. These data support a role of the dietary environment in modulating adipose Pgc-1α expression and energy expenditure via activating histone modifications.

Pgc-1α DNA Methylation Related to Metabolic Indicators

We know of no human or experimental studies evaluating Pgc-1α DNA methylation in BAT, but a number of human studies

have described associations of Pgc-1α DNA methylation in numerous other tissues with glucose, insulin, and adiposity parameters. We include those studies in this section as a follow-up to Section "Diet-induced thermogenesis" given these glucose, insulin and adiposity parameters are commonly modulated by dietary factors.

Maternal fasting glucose and insulin levels during pregnancy were correlated with placental Pgc-1α DNA methylation at two of its cytosine guanine (CpG) dinucleotide sites, and cord blood glucose was also correlated with high Pgc-1α DNA methylation in humans [75]. This is consistent with a prior study demonstrating that maternal glucose levels during pregnancy were correlated with the DNA methylation of the Pgc-1α promoter of placenta and cord blood in humans [76]. The link between Pgc-1α methylation and metabolic disease risk factors are also in agreement with earlier findings of a correlation between DNA methylation of the Pgc-1α promoter and reduced insulin secretion in pancreatic islet cells from patients with T2D [77]. Three of these CpG sites on the Pgc-1α promoter had stable blood methylation status across childhood that was predictive of later adiposity [78]. One of these CpG sites resides within a predicted binding site of the transcription factor pre B cell leukemia homeobox 1 (PBX1) (Fig. 1), and its methylation strengthened the binding of a putative PBX1 complex there [78]. Further, in another human study, Pgc-1α promoter methylation in liver biopsies was positively associated with fasting insulin and insulin resistance while inversely associated with Pgc-1α mRNA and mitochondrial DNA levels in those biopsies [79]. Similarly the promoter of Pgc-1α has been shown to be hypermethylated in the skeletal muscle of type 2 diabetics compared to healthy people, and among those T2D patients, these methylation levels were inversely correlated with Pgc-1α mRNA and mitochondrial DNA abundance [72]. However, glucose tolerance of first degree relatives of T2D patients was not correlated to Pgc-1α promoter methylation in skeletal muscle [80]. This may reflect a larger role for environment over genetics in modulating observed relationships between Pgc-1α promoter methylation and metabolic indicators observed in other studies.

Excess Pgc-1α DNA methylation is consistent with a closed chromatin state and a silencing of the Pgc-1α thermogenic program however whether Pgc-1α DNA methylation causes a closed chromatin state that quiets the Pgc-1α thermogenic program in humans remains to be demonstrated empirically. Linking BAT phenotypes to DNA methylation of Pgc-1α in response to the environment is technically challenging but remains vital for establishing a causal role for epigenetic mediation of environmental influences on BAT thermogenesis.

Conclusion

Pgc-1α is a critical regulator of BAT thermogenesis that integrates environmental signals with physiological responses by regulating gene expression. Most research into Pgc-1α activity in BAT has focused on its expression in relation to downstream signaling via transcriptional coactivation and resulting physiological effects. The environmental signals responsible for Pgc-1α-dependent BAT thermogenesis act through discrete signaling pathways (Fig. 1). Most of these pathways, such as PKA, Akt, p38MAPK, are redundant in that they also regulate Pgc-1α protein levels.

In contrast, relatively little is known of the epigenetic mechanisms regulating Pgc-1α expression in BAT. Indeed, there is a paucity of data on how environment signals may induce epigenetic and other regulatory events to influence Pgc-1α expression and thermogenesis in BAT. The evidence indicates that modulation of Pgc-1α expression by the environment is regulated by integrated histone modifications and transcription factor activity. Most of this evidence arises from the cold environment. Whether the same interactions of histone modifications and transcription factor activity are also involved in diet-induced Pgc-1α expression in BAT remain to be seen. Human studies across pancreas, skeletal muscle, liver, placenta, and both cord- and adult-blood indicate Pgc-1α DNA methylation and RNA expression are inversely associated with each other in the context of thermogenic substrates and metabolic diseases. Future studies should evaluate these associations in thermogenic AT to determine if the surprising consistency across human tissues extends to BAT. There are a number of transcriptional regulatory features that have not been attributed to Pgc-1α activity in BAT yet, including noncoding RNAs and DNA methylation. The highly inducible nature of Pgc-1α suggests alternative mechanisms await discovery.

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