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Chronic cold exposure results in subcutaneous adipose tissue browning and altered global metabolism in Qinghai-Tibetan plateau pika (*Ochotona curzoniae*)

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ABSTRACT

The plateau pika (*Ochotona curzoniae*), one of the indigenous animals of the Qinghai-Tibet Plateau, is adapted to life in a cold and hypoxic environment. We conducted a series of genomic, proteomic and morphological studies to investigate whether changes in energy metabolism contribute to adaptation of the plateau pika to cold stress by analyzing summer and winter cohorts. The winter group showed strong morphological and histological features of brown adipose tissue (BAT) in subcutaneous white adipose tissue (sWAT). To obtain molecular evidence of browning of sWAT, we performed reverse transcription and quantitative real-time PCR, which revealed that BAT-specific genes, including uncoupling protein 1 (UCP-1) and PPAR-γ coactivator 1α (PGC-1α), were highly expressed in sWAT from the winter group. Compared with the summer group, Western blot analysis also confirmed that UCP-1, PGC-1α and Cox4 protein levels were significantly increased in sWAT from the winter group. Increased BAT mass in the inter-scapular region of the winter group was also observed. These results suggest that the plateau pika adapts to cold by browning sWAT and increasing BAT in order to increase thermogenesis. These changes are distinct from the previously reported adaptation of highland deer mice. Understanding the regulatory mechanisms underlying this adaptation may lead to novel therapeutic strategies for treating obesity and metabolic disorders.

1. Introduction

Obesity is an epidemic condition that increases the risk for development of type 2 diabetes, metabolic syndrome and insulin resistance [1]. White adipose tissue (WAT) and brown adipose tissue (BAT) are the two major types of adipose tissue in mammals [2]. WAT primarily functions to store energy in the form of triglycerides [3] and excess WAT accumulation is associated with metabolic disorder, insulin resistance and cardiovascular disease. In contrast, BAT is specialized to dissipate energy by oxidative phosphorylation releasing heat via non-shivering thermogenesis (NST), which reduces fat deposition and promotes glucose disposal and triglyceride clearance [4]. Active brown adipocytes are found in adults [5] and increased BAT is thought to counteract obesity and reverse metabolic dysfunction [6].

WAT and BAT are derived from distinct progenitor cell lineages. WAT is derived from a Myf5 negative cell lineage [7], although some white adipocytes may be generated from Myf5 positive lineages [8]. WAT is characterized histologically by the presence of a

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single large lipid droplet per adipocyte and, functionally, by the ability to store and release fuel as needed [9]. Besides its metabolic function, WAT is considered a true endocrine organ, secreting a wide range of adipokines, which mediate many physiological functions [10]. BAT is derived from a Myf5 positive cell lineage, bears some similarity to skeletal muscle, and is characterized morphologically by abundant mitochondria and multiple small lipid droplets per adipocyte. BAT is specialized for NST and energy dissipation. The stored lipids and glucose in BAT are consumed for heat generation through the action of mitochondrial uncoupling protein 1 (UCP-1).

A third type of fat tissue is beige (also known as brite) adipose tissue, which represents the "browning" of WAT and is characterized by the presence of UCP-1 positive adipocytes in white fat depots [11]. In addition to thermogenesis, endocrine functions of brown/brite adipocytes have been demonstrated in recent years [12]. Cold exposure is the most physiologically relevant stimulus for browning of WAT [13]. Recently, several studies in both rats and humans have demonstrated that cold exposure can induce the browning of WAT and increase thermogenesis in BAT [14].

The plateau pika (*Ochotona curzoniae*) is a native mammal in the Qinghai-Tibet Plateau region [15] and is adapted to a harsh living environment characterized by extremely cold and hypoxic conditions [16]. Several studies have demonstrated that pikas have evolved multiple adaptive strategies to face these challenges, such as a high resting metabolic rate (RMR) and NST to keep warm, and lower rates of oxygen consumption as an adaptation to hypoxia [17]. Evidence indicates that with increasing altitude the pika increases fat accumulation [18] and that low temperatures at high altitude may be the primary environmental stimulus that triggers this adaptive phenotype [19]. Hence, the plateau pika is an interesting model organism for studying energy expenditure overall and the changes in lipid metabolism involved in adaptation to cold exposure [20].

In a previous study, plateau pikas were exposed to 5–6 °C for 4 h per day for 15 days in a hypobaric chamber simulating an altitude of 4100 m, which revealed that acute cold exposure induced visceral adipocyte browning that did not improve glucose and lipid metabolism [20]. In our present work, we studied two independent groups of animals, which were collected in summer and winter respectively, in order to investigate the mechanisms by which the plateau pika maintains thermal, metabolic, and energy balance in its natural environment. In the winter group, which consisted of pikas that were collected during a month with an average temperature under −5 °C, we found a browning phenotype of subcutaneous WAT (sWAT), which is believed to improve metabolic balance and insulin sensitivity [21]. Browning of sWAT in pikas serves to increase heat production as a defense against extremely cold temperatures. Understanding the regulatory mechanisms that mediate this adaptation might lead to novel therapies for obesity and metabolic-related diseases.

2. Materials and methods

2.1. Animals

Plateau pikas were collected from Menyuan county in Qinghai Province, at an altitude of approximately 3250 m in the summer (July) and winter (December) and were matched for gender. All animal studies complied with the Management Rules of the Chinese Ministry of Health and were reviewed and approved by the Research Centre for High Altitude Medicine, Qinghai University Medical College, Xining, Qinghai, China.

2.2. Tissue sample collection

For measuring blood metabolites and fasting blood glucose levels, pikas were fasted for 6–8 h. Blood samples were collected by puncture of the inferior vena cava or cardiac apex of pentobarbital-anesthetized animals. After euthanasia, adipose deposits were quickly isolated and weighed. Some tissues were quick-frozen in liquid nitrogen and stored at −80 °C until further extraction of RNA and protein. Other tissue was fixed in 4% paraformaldehyde and 2.5% glutaraldehyde for histology and electron microscopy.

2.3. Histology and immunohistochemistry

Hematoxylin and eosin (H&E) staining was performed according to standard methods. Fixed adipose tissue was embedded in paraffin and 5-μm-thick sections were used for immunohistochemical staining. After dewaxing and antigen retrieval, tissue slides were blocked with 5% goat nonimmune serum (SW3015, Solarbio) for 1 h at room temperature and incubated with primary antibodies against UCP-1 at 4 °C overnight. HRP-conjugated secondary antibodies were added the next day for 2 h at room temperature. A diaminobenzidine kit (ZLI-9017, ZSGB-Bio) was used for color development.

2.4. Reverse transcription (RT) and quantitative real-time PCR (qPCR)

Total RNA was extracted from adipose tissues using TRIzol Reagent (DP419, TIANGEN) according to the manufacturer's instructions. cDNA was synthesized using a PrimeScript RT reagent kit (KR106, TIANGEN). An ABI7500 system (Applied Biosystems) was used to perform qPCR. Relative RNA expression (E) was based on the cycle threshold (CT) using the formula $E = 2^{-\Delta\Delta CT}$. The nucleotide sequences of primers used for qPCR are shown in Table 1.

2.5. Western blot analysis

Total proteins from adipose tissues were fractionated by SDS-PAGE using 12% (for analysis of UCP-1, COX4) or 8% (for analysis of PGC-1α) polyacrylamide gels and transferred to a PVDF membrane, which was blocked with 5% nonfat milk for 1 h and incubated with primary antibodies at 4 °C overnight. Antibody binding was detected with a chemiluminescent reagent (Thermo Fisher

Table 1
RT-PCR primer sequences.

Name	Sequence (5′-3′)
18sRNA	AGGCCATGATTAAGAGGGAC
	TCTGATCGTCGTCGAACCTC
PGC1a	GTGACATCGAGTGTGCTGC
	TTGAGTCCACCCAGAAAGCTG
PRDM16	TCCTACACGCAGTTCTCCAAC
	GTAATGGTTCTTGCCCTCGC
UCP1	CCGGGACAATATGCGAGTGT
	TTCCATGATCCGAGTCGCAG
Dio2	GTAGCCTTTGAACGCGTGTG
	ATTCTTCTCCAGCCAACGCC
CideA	TGACTCTCGCTATTCCCGAC
	AGACCTTGGGAGACAACACG
Zfp516	GAACCACATGAAGGCACACG
	AAACAGGTTTCCGCACTTGG
Cox8	CCAAGTGAGCAGAGGACACTC
	ACCGAGATGCCTATGGCCTG
ATPase-5α	TACAGATCACCCAAGAACATAATGA
	CACTGGAGCATGCCAGAAAGA

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