Curcumin promotes browning of white adipose tissue in a norepinephrine-dependent way

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ABSTRACT

Brown adipose tissue converts energy from food into heat via the mitochondrial uncoupling protein UCP1, defending against cold. In some conditions, inducible ‘brown-like’ adipocytes, also known as beige adipocytes, can develop within white adipose tissue (WAT). These beige adipocytes have characteristics similar to classical brown adipocytes and thus can burn lipids to produce heat. In the current study, we demonstrated that curcumin (50 or 100 mg/kg/day) decreased bodyweight and fat mass without affecting food intake in mice. We further demonstrated that curcumin improves cold tolerance in mice. This effect was possibly mediated by the emergence of beige adipocytes and the increase of thermogenic gene expression and mitochondrial biogenesis in inguinal WAT. In addition, curcumin promotes β3AR gene expression in inguinal WAT and elevates the levels of plasma norepinephrine, a hormone that can induce WAT browning. Taken together, our data suggest that curcumin can potentially prevent obesity by inducing browning of inguinal WAT via the norepinephrine-β3AR pathway.

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1. Introduction

The ongoing obesity epidemic in both Western and developing countries has propelled a major interest in the complex physiology of adipose cells and tissues. Adipose tissue is classified in mammals as brown adipose tissue (BAT) and white adipose tissue (WAT). WAT is the primary site of excess energy storage in the form of triglycerides, whereas BAT is a major site for non-shivering thermogenesis [1,2]. BAT was originally thought to be primarily found in infants and small mammals as a mechanism that facilitates adaptation to cold. More recently, studies have demonstrated that adult humans also contain functional BAT [3–5]. Brown adipocytes in BAT are packed with mitochondria that contain uncoupling protein-1 (UCP1), which is located in the mitochondrial inner membrane, and the unique thermogenic capacity of BAT results from mitochondrial energy uncoupling mediated by UCP1 [2]. When activated, UCP1 uncouples electron transport from ATP production, thus generating heat [6].

In addition to classical BAT, a type of inducible “brown-like” adipocyte can develop in WAT depots in response to specific stimuli, such as chronic cold exposure or β-adrenergic stimulation [7–10]. These UCP1-expressing adipocytes are referred to as beige or “brite” (brown in white) adipocytes. Similar to adipocytes in BAT, beige cells in white adipose tissues are characterized by a multilocular/small lipid droplet structure, high amounts of mitochondria and UCP1 expression. Thus, these cells also have the ability to “burn” fat. Moreover, the number of beige cells is inversely correlated with body mass index (BMI) in humans [11]. Therefore, identification of the factors that can induce the browning of white fat represents an attractive potential strategy for the management of obesity. 

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and treatment of metabolic disease, including obesity and type 2 diabetes.

Curcumin, also known as diferuloylmethane, is a natural flavonoid component in turmeric (Curcuma longa Linn.), a popular spice in Asian cuisine. Curcumin is safe and tolerable even at high doses (12 g/day) in humans [12]. Several studies have demonstrated that curcumin possesses anti-obesity and anti-diabetic properties [13]. When pharmacologically administered to insulin-resistant obese rodents, curcumin increased weight loss, improved insulin sensitivity and normalized carbohydrate and lipid parameters [14,15]. In addition, curcumin improved obesity-associated inflammation in mouse models [16]. Moreover, curcumin prevented type 2 diabetes mellitus (T2DM) development and improved overall function of β-cells in a prediabetic population [17]. Although curcumin inhibits adipogenesis [18,19] and induces adiponectin secretion [20], little is known about additional functions of curcumin in adipose tissue. In the present study, we report that curcumin treatment stimulates UCP1 gene expression and dramatically increases the appearance of brown-like adipocytes in subcutaneous WAT. Moreover, curcumin treatment elevates plasma norepinephrine levels that contribute to the browning of subcutaneous WAT.

2. Materials and methods

2.1. Reagents and antibodies

Curcumin was purchased from Sigma–Aldrich (St. Louis, MO, USA). Antibodies against β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies against UCP1 and peroxisome proliferator-activated receptor alpha (PGC1-α) were purchased from Millipore (Billerica, MA, USA). A mouse norepinephrine enzyme-linked immunosorbent assay (ELISA) kit was obtained from Cusabio Biotech (Wuhan, Hubei, China). All other reagents used were in the purest commercially available form.

2.2. Animals experiments

All experiments involving animals were performed according to the Guide for the Care and Use of Laboratory Animals and approved by the Fourth Military Medical University Committee on Animal Care. Male C57BL/6 mice (27 ± 2 g) obtained from the Animal Center of the Fourth Military Medical University were used in the experimental work. The mice were housed at 22 ± 2 °C, 55 ± 5% relative humidity and with a 12-h light/dark cycle. Food and water were provided ad libitum. The mice were randomly grouped into three groups, vehicle, 50 mg/kg curcumin or 100 mg/kg curcumin. Curcumin dissolved in corn oil (50 or 100 mg/kg body weight) was administered to the mice for 50 consecutive days by gavage. Corn oil was administered to vehicle group mice. Body weight was recorded every 5 days, and food intake was measured every other day throughout the study.

2.3. Cold exposure

After 50 days of curcumin administration, animals were placed for 6 h in a cold room (-4 °C) without access to food or water. Body temperature was recorded with a rectal probe connected to a digital thermometer.

2.4. Histology and immunohistochemistry

Inguinal and epididymal adipose tissues were harvested and fixed in 4% paraformaldehyde for 24 h. The next day tissues were washed with phosphate buffered saline (PBS), stored in 70% ethanol, and embedded in paraffin. Sections of the embedded tissues were subjected to hematoxylin and eosin (H&E) staining and immunostaining with a UCP1 antibody.

2.5. MitDNA content quantification

Total DNA was isolated from inguinal adipose tissues using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer’s instructions. Results were calculated as the difference in threshold cycle (ΔCt) values for mtDNA compared with nuclear DNA-specific amplification by quantitative real-time polymerase chain reaction (PCR) as previously described [21]. Data are expressed as 16S rRNA normalized to the hexokinase 2 gene. Primers used are listed in Supplementary Table 1.

2.6. Western blot analysis

Tissues were harvested and lysed in RIPA buffer containing a protease inhibitor cocktail (Roche). Total protein lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 5% non-fat milk, membranes were incubated with the appropriate primary antibodies against UCP1, β-actin or PGC1-α. Secondary antibodies conjugated to IRDye TM 800 were detected using an Odyssey infrared imaging system (LI-COR).

2.7. Quantitative real-time PCR analysis

Total RNA was extracted from tissues using the TRIzol method (Invitrogen) according to manufacturer’s instructions. cDNA was reverse transcribed from 2 μg of RNA by using the Quantitect Reverse Transcription Kit (Takara). Quantitative real-time PCR was performed on a BIO-RAD CFX96 with the Power SYBR Green PCR Master Mix (Takara). The relative amount of mRNA normalized to β-Actin was calculated by using the delta-delta method [22]. The sequences of the primers used in this study are described in Supplementary Table 1.

2.8. Plasma norepinephrine measurement

Blood sample from each mouse was centrifuged at 3000 rpm for 10 min immediately after collection. The extracted plasma was frozen at −80 °C until analysis. Plasma norepinephrine level was measured using a mouse norepinephrine ELISA Kit (Cusabio) following the manufacturer’s instructions.

2.9. Statistics analysis

All the data were expressed as the mean ± standard error of the mean (SEM). Comparisons between two groups were performed using Student’s t-test. P < 0.05 was considered significant.

3. Results

3.1. Curcumin attenuates weight gain and fat mass in mice

We examined the effect of curcumin on weight gain and fat mass in C57BL/6 mice. Mice treated with 50 or 100 mg/kg curcumin were noticeably protected from weight gain (Fig. 1B). Interestingly, we also noted that mice treated with curcumin (50 or 100 mg/kg) had less fat mass than control mice (Fig. 1C). The reduced weight gain could be a result of reduced calorie intake or increased energy expenditure. Therefore, we assessed food intake in mice treated with curcumin or vehicle. As shown in Fig. 1A, curcumin (50 or
100 mg/kg) did not alter food intake. Then, we performed a cold tolerance test to gauge adaptive thermogenesis as it is a major component of energy expenditure. As noted in Fig. 1D, curcumin-treated mice exhibited increased body temperature compared with control mice during 6 h of exposure to cold.

3.2. Curcumin induces browning of inguinal WAT

To determine whether increased adaptive thermogenesis was due to browning of white adipose tissue (WAT), we performed H&E staining and immunohistochemistry on sections of inguinal WAT (iWAT). As expected, curcumin (50 or 100 mg/kg)-treated mice exhibited unilocular white adipocytes with interspersed beige adipocytes that filled with multilocular small lipid droplets (Fig. 2A). Consistent with this finding, immunohistochemistry staining revealed that the Ucp1 immunoreactivity signal was considerably stronger in curcumin-treated iWAT compared with control iWAT (Fig. 2B). Western blot and quantitative PCR (qPCR) analysis further confirmed that curcumin (50 or 100 mg/kg) induced the expression of a number of brown fat-specific genes, including Ucp1, Pgc1a, Prdm16, Dio2, and Cidea (Fig. 2C and D). In addition, curcumin (50 or 100 mg/kg) increased mitochondrial biogenesis as determined by mtDNA copy number (Fig. 2E). Taken together, these data demonstrate that curcumin induces browning of iWAT.

3.3. Curcumin does not induce browning of epididymal WAT

We next tested whether curcumin also induced browning of epididymal WAT (eWAT). We observed that eWAT from mice treated with curcumin (50 or 100 mg/kg) had decreased white adipocyte size compared to controls (Fig. 3A). But, morphological beige adipocyte was not seen in the eWAT after curcumin (50 mg/kg or 100 mg/kg) treatment (Fig. 3A). Accordingly, western blot analysis showed that eWAT from curcumin-treated and control mice expressed similar levels of UCP1 (Fig. 3B). Moreover, qPCR analysis revealed that curcumin (50 or 100 mg/kg) did not induce the expression of a number of brown fat-specific genes, including Ucp1, Pgc1a, Prdm16, Dio2, and Cidea (Fig. 3C).

3.4. Curcumin increases plasma norepinephrine levels and β3AR gene expression in inguinal WAT

Norepinephrine, which acts on beta3 adrenoreceptors (β3AR) in WAT, plays a critical role in WAT browning. To address whether curcumin increased norepinephrine release, plasma norepinephrine levels were measured via ELISA. As noted in Fig. 4A, mice treated with curcumin (50 or 100 mg/kg) exhibited increased blood norepinephrine concentrations compared with control mice. Congruent with these results, treatment with curcumin (50 or 100 mg/kg) induced expression of β3AR in inguinal WAT.

4. Discussion

Obesity, an increasingly serious worldwide health concern, develops when energy intake exceeds energy expenditure [23]. Therefore, as a strategy to increase energy expenditure, stimulating the development of beige adipocytes in WAT (so called ‘browning’) represents an attractive concept for combating obesity and associated metabolic diseases [24]. Numerous factors known as “browning agents” have been described, including hormones, cytokines, food components and drugs [24,25]. The implied action of browning agents is that they increase UCP1 activity and consequently heat production, leading to slimming [24]. In this report we demonstrate that curcumin is also a “browning agent”. Mice treated with curcumin exhibited lower body weight gain and less fat mass. Moreover, UCP1-positive brown fat-like cells emerged in iWAT of mice after curcumin treatment.

It is widely recognized that curcumin possesses antiobesity properties. Dietary therapy with curcumin in ob/ob and DIO (diet-
induced obesity) mice resulted in significant weight loss and a decrease in fat mass [16]. Evidence from a study in high fat-fed mice demonstrated that curcumin supplementation (500 mg/kg diet) can also increase weight loss [18]. Consistent with these data, our results show that 50 days of intragastric administration of curcumin dissolved in corn oil (50 or 100 mg/kg body weight) decreased body weight gain and fat mass in C57BL/6 mice weighed 25–29 g at the start of the study. Body weight is determined by the balance of energy intake and energy expenditure. Any treatment for weight loss must reduce total energy uptake, increase energy expenditure, or affect both of these processes [26]. In agreement with a previous study in high fat-fed mice [18], we demonstrate that intragastric administration of curcumin (50 or 100 mg/kg/day) did not affect food intake in C57BL/6 mice, suggesting that curcumin alters energy metabolism. In contrast, another group detected a significant increase in food intake and a small but significant decrease in body

Fig. 2. Browning of inguinal adipose tissue by curcumin treatment for 50 days. (A) Representative pictures of hematoxylin and eosin–stained sections of inguinal WAT from mice treated with vehicle or curcumin. Scale bar, 20 μm. (B) UCP1 protein (brown stain) immunohistochemistry in inguinal WAT of vehicle- and curcumin-treated mice. Scale bar, 20 μm. (C) MtDNA copy number of inguinal WAT from vehicle- and curcumin-treated mice. (D) Representative Western blots demonstrating key protein changes in inguinal WAT after curcumin treatment. (E) mRNA levels of brown/brite-typical genes in inguinal WAT from mice treated with vehicle or curcumin. Values are mean ± SEM (n = 3–5 mice per group). *P < 0.05 for 50 mg/kg curcumin vs. vehicle. #P < 0.05 for 100 mg/kg curcumin vs. vehicle. Cur, curcumin (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).
weight in mice treated with curcumin at a high dose (30 g/kg diet) [16]. However, the dose of curcumin used in that study (approximately 1.5 g/kg/day) was 15- or 30-fold greater than that used in our study (50 or 100 mg/kg/day). Treatment with curcumin at a high dose reduced body weight with increasing food intake in mice, also suggesting alterations in energy expenditure. Evidence from in vitro and in vivo studies found that curcumin can increase the basal metabolic rate, thereby contributing to increased energy expenditure [27]. Here, we found that curcumin increased adaptive thermogenesis, another major component of energy expenditure. In addition, our data demonstrated that curcumin induces thermogenic gene expression, beige adipocyte emergence and mitochondrial biogenesis in iWAT. All these data suggest that curcumin drives browning of subcutaneous WAT. These findings may be an important mechanism by which curcumin increases energy expenditure.

Although curcumin treatment stimulates UCP1 gene expression and beige cells emerged in iWAT, we did not observe similar phenomena in eWAT. A previous study reported that the propensity to accumulate brite cells differs between WAT depots in rodents [28]. Several studies demonstrated that visceral/epididymal WAT is less susceptible to browning compared with inguinal/subcutaneous WAT [29,30]. For example, cold exposure, which stimulates chronic norepinephrine release, induced increased accumulation of beige cells in subcutaneous inguinal adipose tissue compared with epididymal adipose tissue [29].

The release of norepinephrine at sympathetic terminals in WAT and the adrenal medulla is mandatory for the immediate activation

Fig. 3. Curcumin administration for 50 days does not induce brown-like adipocyte in epididymal adipose tissue. (A) Representative hematoxylin and eosin staining of epididyma WAT histology after curcumin administration. Scale bar, 20 μm. (B) Representative Western blots revealing key protein changes in epididyma WAT. (C) Brown/brite-typical gene expression profile in epididyma WAT. Values are mean ± SEM (n = 3–5 mice per group). *P < 0.05 for 50 mg/kg curcumin vs. vehicle. #P < 0.05 for 100 mg/kg curcumin vs. vehicle. Cur, curcumin (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 50 days curcumin treatment on plasma norepinephrine levels (A) and β3AR gene expression in inguinal WAT (B). (A) Norepinephrine levels in plasma were measured by ELISA. (B) β3AR mRNA in inguinal WAT was measured by qRT-PCR. Values are mean ± SEM (n = 3–5 mice per group). *P < 0.05 for 50 mg/kg curcumin vs. vehicle. #P < 0.05 for 100 mg/kg curcumin vs. vehicle. Cur, curcumin.
of existing beige adipocytes and for the differentiation of beige adipocytes from their precursors [31]. Norepinephrine, acting through β-adrenergic (mostly β3) and cAMP-dependent pathways, is the major mediator of adaptive thermogenesis in brown and beige adipose tissues by increasing PGC-1α and UCP1 expression [24]. Many factors activate the β-adrenergic signaling pathway in WAT, thereby inducing the browning process. Previous studies demonstrated that cold exposure can induce WAT browning upon norepinephrine release from the sympathetic nerves innervating tissue in rodents [8,24]. Chronic treatment with a β-adrenergic agent also leads to browning of WAT in mice [32,33]. Moreover, a recent study revealed that elevated circulating catecholamine levels contribute to the browning of subcutaneous WAT in burn patients [34]. In this study, we found that curcumin increases plasma norepinephrine levels and β3AR gene expression in iWAT. These observations suggest that curcumin can induce WAT browning via the norepinephrine-β3AR pathway. A previous study reported that curcumin modulates the levels of norepinephrine in the various regions of the rat brain [35]. However, alternatively activated M2 macrophages in adipose tissue have been identified as another source of norepinephrine [36]. In addition, a recent study revealed that curcumin induces M2 macrophage polarization by secretion of interleukin (IL)-4 and/or IL-13 [37]. Thus, additional studies will be required to determine the source of the increased norepinephrine induced by curcumin.

The beneficial effects of curcumin on body weight, lipid metabolism and β-cell function have evoked substantial interest in curcumin as a potential treatment for diseases, such as obesity and diabetes [13]. In this study, we observe a clear function for curcumin in regulating adaptive thermogenesis by increasing the expression of thermogenic genes and beige cells emerging in inguinal WAT. These findings establish an important role for curcumin in browning white fat, and we propose that curcumin may be used for therapy in obese patients.

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Transparency document

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Appendix A. Supplementary data

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References


