Effect of Celecoxib in treatment on burn-induced hypermetabolism

Shubo Zhuang 1, Jiake Chai 2#, Lingying Liu 2, Huinan Yin 2, Yonghui Yu 2

1 Department of Dermatology & Plastic Surgery, the First Hospital of Tsinghua University, Beijing, China.

2 Burn Institute, Department of Burn & Plastic Surgery, the First Affiliated Hospital to PLA General Hospital, Beijing, China.

#Corresponding Author:
Jiake Chai, Burn Institute, Department of Burn & Plastic Surgery, the First Affiliated Hospital to PLA General Hospital, Beijing 100048, China. E-mail: sdkojhc@163.com.
Abstract

**Background:** Cyclooxygenase-2 (COX-2) catalyses the rate-limiting step of prostanoid biosynthesis. Under pathologic conditions, COX-2 activity can produce reactive oxygen species and toxic prostaglandin metabolites that exacerbate injury and metabolic disturbance. The present study was performed to investigate the effect of Celecoxib (the inhibitor of COX-2) treatment on lipolysis in burn mice.

**Methods:** One hundred male BALB/c mice were randomly divided into sham group, burn group, celecoxib group, and burn with celecoxib group (25 mice in each group). 30% TBSA full-thickness injury was made for mice to mimic burn injuries. Oxygen uptake (VO2), volume of carbon dioxide output (VCO2), respiratory exchange ratio (RER), energy expenditure (EE), COX-2 and uncoupled protein-1 (UCP-1) expression in BAT were measured for different groups.

**Results:** Adipose tissue (AT) activation was associated with the augmentation of mitochondria biogenesis, and UCP-1 expression in isolated iBAT mitochondria. In addition, VO2, VCO2, EE, COX-2 and UCP1 expression were significantly higher in burn group than in burn with celecoxib group ($P<0.05$).

**Conclusion:** BAT plays important roles in burn injury-induced hypermetabolism through its morphological changes and elevating the expression of UCP-1. Celecoxib could improve lipolysis after burn injury.

**Key words:** Celecoxib; UCP-1; Burn injury; Hypermetabolism; BAT
Introduction

Adipocytes can be broadly divided into white and brown fat cells. White fat cells are specialized to store chemical energy, when brown adipocytes produce heat, counteracting hypothermia, obesity, and diabetes [1]. Brown fat utilizes high mitochondrial content and high mitochondrial uncoupling protein 1 (UCP-1) to uncouple respiration and dissipate chemical energy as heat [2-4]. Meanwhile, small amounts of brown adipose tissue (BAT) may be found in the neck; in supra-clavicular and axillary regions; in paravertebral, perirenal/adrenal, and paraventral regions; and around major vessels (the aorta and its main branches: carotids, subclavian, intercostals, and renal arteries). BAT can also be found within white adipose tissue (WAT) and skeletal muscle tissues [5]. Notably, histological studies on humans suggest that brown and white adipocytes are mixed together [6-8]. Brown fat cells are characterized by multilocular lipid droplets and increased amount of mitochondria which express UCP-1 [9]. UCP-1 is located on the inner membrane of mitochondria and uncouples the rates of substrate oxidation and ATP production by favoring the loss of protons and subsequent energy release [10].

Cyclooxygenase (COX) catalyzes the rate-limiting step of prostanoid biosynthesis. Two COX isoforms have been identified, COX-1, the constitutive form, and COX-2, the inducible form [11]. COX-2 is implicated in body fat regulation, but underlying cellular mechanism remains to be elucidated [12].

In this study, burn injury model was constructed to explore the influences of Celecoxib, an inhibitor of COX-2, on fat catabolism and hypermetabolism after burn, as well as relevant molecular mechanisms.

Methods

Burn Injury Model and Grouping

This study was approved by Subcommittee on Research Animal Care of the First Affiliated Hospital to PLA General Hospital. A total of 100 Balbe/c mice (male, 20±3 g) were randomly divided into four groups: sham treated group (S), burned group (B), sham burn + Celecoxib group (C), and burn -
Celecoxib group (BC), 25 in each group. The animal experiments were performed in the animal experimental center of the First Affiliated Hospital to PLA General Hospital.

Thermal injury was produced in clean bench according to published protocols [13, 14], with minor modifications. Each mice was anesthetized with pentobarbital sodium (50 mg/kg body wt ip). After clipping back hair of the trunk, the animal was placed in a mold exposing 30% of total body surface area (TBSA), and the exposed area, which did not include the region expressing BAT, was immersed in 90°C water for 9 s, producing a full-thickness, third-degree thermal injury of 30% TBSA. Sham burn animals were similarly treated, with the exception that they were immersed in room temperature water. After burn or sham burn treatment, all animals immediately received fluid resuscitation with 40 ml/kg saline intraperitoneally. For mice in sham burn + Celecoxib group and burn + Celecoxib group, 1500ppm Celecoxib normal saline was given through gavage [15, 16]. All mice were caged individually throughout the study duration.

Measurement of rectal temperatures

Rectal temperature of mice in various groups. After the animal was placed on a hard surface, the probe was inserted, and rectal temperature was recorded after it was stabilized.

Histology

On post burn day 10, mice were sacrificed by pentobarbital sodium anesthesia. BAT lobules and contiguous or nearby normal WAT tissues were excised from posterior cervical-upper thoracic region and immersed in 10% formalin. After 24 h of fixation, excised fat was examined, comparative changes were noted and lobe sizes were measured. Tissues were then block sectioned, inserted into cassettes, processed to paraffin blocks, microtome sectioned into 6 μm and stained with H&E (hematoxylin and eosin) for microscopic examination. HE slides were microscopically evaluated for histological changes in BAT and adjacent WAT tissues. Lipid content was estimated as a percentage of “clear areas” relative to remaining areas in stained cellular components (nucleus and cytoplasm) and supporting connective tissue. A calibrated ocular grid was used for random fields, and percentages were calculated as statistical averages. This method was utilized in lieu of fat stained
frozen tissue sections cumbersome in evaluation and fraught with a host of staining artifacts. Additionally, this method guaranteed equal or higher accuracy.

**Transmission electron microscopy (TEM) protocol**

BAT was isolated from BALB/c mice of each group, and washed twice with PBS solution. Then the tissues were fixed with 2% glutaraldehyde for 4 weeks. Later, the tissues were sectioned into 6 μm using microtome, and the ultrastructure, endoplasmic reticulum and mitochondria of the cells were observed adopting TEM.

**Measurement of EE via Indirect Calorimetry**

Indirect calorimetry (TSE systems) was performed for 24 h at 7th day after burn treatment. The animals were fasted overnight. No food was offered in metabolic chamber during measurements. The metabolic chamber was controlled by a computer system. The rates of volume of oxygen uptake (VO2) and volume of carbon dioxide output (VCO2) were recorded, and respiratory exchange ratio (RER) and energy expenditure (EE) were computed automatically. Resting values for each parameter were defined as the 10th percentile of raw data. The animals were given free access to water during the measurements.

**Isolation of Mitochondria from iBAT**

Mitochondria were isolated from fresh iBAT using mitochondria isolation kit (Sigma, St. Louis, MO). Briefly, tissue was washed with extraction buffer containing 50 mM HEPES, pH 7.5, 1 M mannitol, 350 mM sucrose, and 5 mM EGTA and cut into small pieces. The tissue was then homogenized in extraction buffer containing 5 mg/dl fatty acid-free BSA and centrifuged at 600g for 5 min. Supernatant was centrifuged at 11,000g, and pellet was resuspended in extraction buffer and centrifuged at 60g for 5 min. The supernatant was centrifuged at 11,000 g, and the pellet was suspended in a small volume of storage buffer containing 50 mM HEPES, pH 7.5, 1.25 M sucrose, 5 mM ATP, 0.4 mM ADP, 25 mM sodium succinate, 10 mM K2HPO4, and 5 mM DTT. All of these
procedures were performed in a cold room. The concentration of mitochondrial protein was
determined through bicinchoninic acid method. The samples were kept in a 80°C freezer until
protein analysis.

**Measurement of BAT Uncoupling Protein-1 (UCP-1) and COX-2 mRNA with quantitative real-time polymerase chain reaction (qRT-PCR)**

RNA was extracted from iBAT tissues using QUAZO Lreagent (Gibco-BRL). Briefly, tissues were
put into a screw-cap vial ½ full of Zircona beads (Biospec). Tissue and beads were placed in
BeadBeater for 2 min. Lysate was transferred into a 2 ml tube where RNA was purified using Qiagen
RNeasy kit after chloroform treatment. Specific primers for mouse UCP-1, COX-2 and β-actin (as a
house-keeping gene) were designed by Invitrogen (California, USA). The primer sequences were as
follows: UCP-1 forward: 5’-AGGGTTTGTGGCCTTCTTTTCC-3’, reverse:
5’-TGGTTGGTTTTATTGCTGTTT-3’; COX-2 forward: 5’- GTGCCTGGTCTGATGATGTGATGATGATG-3’, reverse:
5’- TGAGTCTGCTTGTTGGAATAG-3’; β-actin forward: 5’-
AGAGGGAAATCGTGCGTGAC-3’, reverse: 5’-AGGAGCCAGGCAGTAATC
-3’. Real time RT-PCR quantified UCP-1 and COX-2 mRNA using Cycle IQ Mucicolor Real Time PCR Detection System (Bio-Rad, Hercules, CA) and SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). To normalize variations in mRNA extraction and cDNA synthesis, the expression of
β-actin, a house-keeping gene, was also measured. Thermal cycling conditions referred to an initial
94 °C for 10 min, and followed by 50 cycles of 94 °C for 30 s , 60 °C for 30s, and 72 °C for 30s.
Relative expression of UCP-1 and COX-2 was normalized to β-actin, and calculated using the
method of 2^{-∆∆Ct}.

**Western Blotting for UCP-1 and COX-2 proteins’ Expression**

iBAT mitochondrial protein (20g) was boiled in sample buffer (62.5 mM Tris·HCl, pH 6.8, 25%
glycerol, 2% SDS, 0.01% bromophenol blue, 710 mM-mercaptoethanol), separated by SDS-PAGE,
and transferred onto nitrocellulose membranes. The membranes were blocked with LI-COR blocking
buffer (diluted 1:1 in PBS; LI-COR Biosciences, Lincoln, NE) for 1 h, followed by overnight
incubation with primary antibody. Primary antibodies were used at the following dilutions: anti-UCP-1 rabbit monoclonal antibody (Sigma-Aldrich, St. Louis MO) 1:1,000, anti-COX-2 rabbit monoclonal antibody (Sigma-Aldrich, St. Louis MO) 1:1,000, anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH- a house-keeping protein) rabbit monoclonal antibody (Sigma-Aldrich) 1:1,000. After four washes with PBS-Tween 20 (PBS-T; 5 min each), the membranes were incubated with secondary antibody conjugated to horseradish peroxidase (HRP) in 1:1,000 blocking buffer for 1 h at room temperature. After four washes with PBS-T (5 min each) and two washes with PBS (5 min each) at room temperature, immunoreactivity was visualized and quantified. Densitometry values for anti-UCP-1 and anti-COX-2 blots were normalized to anti-GAPDH controls.

**Statistical Analysis**

All results were presented as mean±SEM. Differences in continuous variables between two groups were estimated via unpaired t-test. Nonlinear regression analysis was employed to identify correlations between continuous data. Two-way ANOVA was employed to compare data among three or more groups, and individual means were adjusted through Bonferroni test. All statistics were performed using SPSS 17.0. Differences with $P$ value less than 0.05 were considered to be significant.

**Results**

**Morphological Change in BAT Induced by Burn Injury**

We conducted tissue analyses to identify burn injury-associated iBAT activation. At macroscopic level, iBAT was much darker in burned animals than in sham-treated controls. However, in sham burn animals, it was difficult to distinguish iBAT from interscapular fat pad, which contains both iBAT and interscapular white adipose tissue (iWAT) based on gross observation. For further differentiation, we sectioned tissues, stained them with H&E, and performed histological analysis. At both low- and high-power light microscopic levels (Figure 1, A-D), two populations of adipocytes, white and brown, coexisted in iBAT in sham burn animals. Multilobular fat vacuoles were prominent
in brown adipocytes in sham burned animals (Figure 1, A and C) and occupied the majority of cell volume. There was little eosin-stained cytoplasm around fat droplets, and nucleus was located in peripheral area for each cell. In contrast, eosin-stained cytoplasm was prominent in brown adipocytes from burned rats, and nucleus was centrally located and surrounded by cytoplasm (Figure 1, B and D).

The size of fat droplets in burned animal (Figure 1, B and D) was much reduced compared with typical multilobular fat vacuoles seen in sham burn animals (Figure 1, A and C). Isolated BAT in interscapular area was weighted for mice. As shown in Figure 1E, BAT weight was significantly heavier in burned group (P<0.05), and Celecoxib treatment could obviously reduce BAT weight (P<0.05). Therefore, burn injury was associated with iBAT activation and increased the density of brown adipocytes in interscapular area. The ultrastructure of brown adipocytes was also evaluated using TEM. In sham burn animals (Figure 2A), fat droplets occupied the majority of cytoplasm, and round-shaped small mitochondria were scattered in cytoplasm. In burned animals (Figure 2B), fat droplets were relatively small, and cytoplasm was tightly packed by mitochondria. As illustrated in Figure 2C and D, morphometric analysis indicated that the ratio of fat droplet area to cytoplasmic area was significantly decreased after burn injury (68.41. vs. 14.41%, P<0.001). In addition, the number of mitochondria per brown adipocyte was increased after burn injury.

**Effect of Celecoxib on Treatment on Burn-Induced Hypermetabolism**

The effect of Celecoxib in reducing burn injury-induced hypermetabolism was further investigated in groups of sham burn and burned animals receiving continuous saline or Celecoxib infusion via implanted osmotic pumps. We explored metabolic rates of burned animals receiving 7 days of continuous Celecoxib infusion. The results were summarized in Figure 3. Two-way ANOVA demonstrated that in sham burn animals Celecoxib treatment did not cause significant difference in VO2, VCO2, or EE. The analysis also unveiled significantly increased metabolic rate after burn injury.

**Celecoxib Treatment Lowered the Expressions of UCP-1 and COX-2**
Possible mechanism of Celecoxib reducing EE in burned animals was investigated through its effects on UCP-1 and COX-2 expressions. QRT-PCR analysis suggested that compared to S (sham burn) group, the expression of COX-2 was significantly increased in B (burn) group (P<0.05). The treatment with Celecoxib could obviously suppress COX-2 expression in both sham and burn groups (P<0.05 for both). Moreover, compared to S group, the level of COX-2 did not show obvious changes in BC (burn + Celecoxib) group (P>0.05), suggesting that Celecoxib treatment could inhibit COX-2 expression during burn (Figure 4A). QRT-PCR analysis on UCP-1 suggested that burn treatment could induce the expression of UCP-1, and that Celecoxib treatment was able to suppress UCP-1 expression (P<0.05 for both). Moreover, UCP-1 level did not show significant differences between S and BC groups (P>0.05), revealing that Celecoxib treatment might completely suppress UCP-1 activation induced by burn (Figure 4B).

In addition, the protein levels of UCP-1 and COX-2 in isolated mitochondria from iBAT were estimated using western blot. Represented blotting images were shown in Figure 4C. Quantitative analysis demonstrated that compared to S group, burn treatment (B group) significantly induced the expression of COX-2 (P<0.05). Celecoxib could obviously inhibit COX-2 expression in both sham and burn groups (P<0.05 for both). Moreover, the protein levels of COX-2 in C and BC groups did not show significant difference (P>0.05), revealing that Celecoxib treatment inhibited COX-2 expression during burn (Figure 4D). As for UCP-1 protein in isolated mitochondria from iBAT, B group showed a significantly increasing tendency compared to S group (P<0.05). Celecoxib could obviously inhibit UCP-1 expression in both sham and burn groups (P<0.05 for both). Moreover, UCP-1 expression did not show obvious differences between C and BC groups (P>0.05), suggesting an inhibiting effect of Celecoxib treatment on UCP-1 expression during burn (Figure 4E).

Discussion

Two major observations were completed in two studies in this investigation. First, BAT was activated by burn injury and was associated with increased expression of UCP-1 and augmented amount of mitochondria. Second, mitochondria-targeted peptide Celecoxib attenuated burn injury-induced hypermetabolism, which was correlated with decreased expression of COX-2 and UCP-1. These observations clearly demonstrated that burn injury significantly increased resting EE at 7th day after
burn. It was worth mentioning that in study 2, animals underwent surgical procedures for the implantation of catheters and Celecoxib delivery pumps; however, both burned groups showed similar increments in EE compared to sham burn animals, indicating that the above-mentioned surgical procedures did not exacerbate hypermetabolism at 7th day following burn injury. Thus, the observed alterations in metabolic rate reflected hypermetabolism induced merely by burn injury.

Our previous study [17] revealed morphological changes in BAT after burn injury. The present study further explored those changes through immunohistochemistry and TEM, confirming that burn injury increased BAT mitochondria. All the observed changes were correlated with increased mitochondrion biogenesis and lipolysis. Increased mitochondrion biogenesis and lipolysis in BAT might be a possible mechanism for the development of burn injury-induced hypermetabolism.

In the present study, mechanisms for increased BAT energetics were further studied through measuring UCP-1 and COX-2 expressions in isolated mitochondria from BAT. In the present study, we observed a significant alleviation in hypermetabolism following Celecoxib treatment in burned animals. The present study also revealed that reduction in resting EE was correlated with the reduction of brown adipocytes and reduced UCP-1 expression. These findings provided further evidence supporting that BAT and UCP-1 were associated with burn injury-induced hypermetabolic state. Mechanism underlying the effect of Celecoxib on energy metabolism can be explained by its role in modulating mitochondrial function after thermal injury. Significant increase in superoxide level following burn injury and oxidative damage to tissues are implicated in inflammation, systemic inflammatory response syndrome, severe injury, infection, sepsis, and multiple organ failure. Recent studies have demonstrated that superoxide induces uncoupling process in mitochondria, and that uncoupling is correlated with UCP-1 expression in different tissues, but not in those not expressing UCPs, such as liver [18-20]. The expression of UCP-1 in BAT occurs in mitochondria and is a nucleotide-sensitive process [21]. Mitochondria-targeted antioxidants could abolish superoxide-induced uncoupling through lowering UCP-1 expression [22]. Celecoxib is a scavenger of ROS that ameliorates lipid peroxidation, reduces mitochondrial ROS levels, inhibits mitochondrial permeability transition, and prevents the swelling of isolated mitochondria [23, 24]. In addition, COX-2 is an essential factor for UCP-1 synthesis, which is a necessary product to induce the transformation of white adipocytes into brown adipocytes. Celecoxib is an inhibitor of COX-2, and the treatment with Celecoxib could suppress the expressions of COX-2 and UCP-1, thus inhibiting
BAT activation and hypermetabolism. Taken together, mechanism for Celecoxib functioning was associated with reducing burn injury-induced hypermetabolism which was mediated by the inhibition of superoxide-induced UCP-1 expression in BAT.

In recent years, BAT has become a target tissue in developing strategies for treating diseases associated with hypometabolic states such as diabetes and obesity [25]. The present study demonstrated that BAT might also play a role in burn injury-induced hypermetabolism. Therefore, BAT might be a potential target in treating burn injury-induced hypermetabolism. Ultrastructural analysis on BAT could serve as an indicator for treatment response in both hypo- and hyper-metabolic diseases and injuries. In conclusion, our studies have demonstrated that burn injury-induced hypermetabolism is associated with the activation of BAT with significant upregulation of UCP-1 expression and mitochondria biogenesis. The inhibition of this hyper-metabolic state by Celecoxib may be related to reduced mitochondrial UCP-1 expression. Therefore, altered mitochondrial function and increased uncoupling process are possible important contributors to burn injury-induced hypermetabolism. In the future, alteration in BAT could be a therapeutic target in reducing hypermetabolism and associated protein wasting in metabolic care of severely burned patients.

Limitations in this study should be noted. Firstly, the sample size was not large enough. Secondly, the influences of Celecoxib treatment on the ultrastructure of brown adipocytes were not estimated by TEM in our study, which might reduce the veracity of final result. Thirdly, mechanism for Celecoxib treatment functioning on burn-induced hypermetabolism was not explored. In addition, the influences of Celecoxib on COX-1, the constitutive form of COX, were not investigated in our study. Besides, Celecoxib could inhibit SUP-1 expression via multiple ways, but whether other inhibitors of COX-2 could suppress the expression of UCP-1 was not estimated. Therefore, further studies would be necessary in the future to verify our findings and investigate relevant mechanisms.

Acknowledgments
None.

Conflict of Interest
None.
Author Contribution
Conceived and designed the experiments: S.Z. and J.C.
Performed the experiments: all authors.
Analyzed the data: S.Z., J.C. and L.L., H.Y., Y.Y.
Wrote the paper: all authors.

Declaration
This study was supported by the Ethics Committee of Research Animal Care of the First Affiliated Hospital to PLA General Hospital.

Funds
None.
References

[1] Schoettl T, Fischer IP and Ussar S. Heterogeneity of adipose tissue in development and metabolic function. J Exp Biol 2018; 221:

[2] Lim S, Honek J, Xue Y, Seki T, Cao Z, Andersson P, Yang X, Hosaka K and Cao Y. Cold-induced activation of brown adipose tissue and adipose angiogenesis in mice. Nat Protoc 2012; 7: 606-615.

[3] Reverte-Salisa L, Sanyal A and Pfeifer A. Role of cAMP and cGMP Signaling in Brown Fat. Handb Exp Pharmacol 2018;

[4] Biswas HM. Effects of alpha-(prazosin and yohimbine) and beta-receptors activity on cAMP generation and UCP1 gene expression in brown adipocytes. J Basic Clin Physiol Pharmacol 2018;

[5] Clarke JR, Brglevska S, Lau EW, Ramdave S and Hicks RJ. Atypical brown fat distribution in young males demonstrated on PET/CT. Clin Nucl Med 2007; 32: 679-682.

[6] Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J and Cinti S. The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. FASEB J 2009; 23: 3113-3120.

[7] Heaton JM. The distribution of brown adipose tissue in the human. J Anat 1972; 112: 35-39.

[8] Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Tahtinen M, Laine J, Savisto NJ, Enerback S and Nuutila P. Functional brown adipose tissue in healthy adults. N Engl J Med 2009; 360: 1518-1525.

[9] Ikeda K, Mareteh P and Kajimura S. The Common and Distinct Features of Brown and Beige Adipocytes. Trends Endocrinol Metab 2018; 29: 191-200.

[10] Bouillaud F, Raimbault S and Ricquier D. The gene for rat uncoupling protein: complete sequence, structure of primary transcript and evolutionary relationship between exons. Biochem Biophys Res Commun 1988; 157: 783-792.

[11] Li X, Mazaleuskaya LL, Ballantyne LL, Meng H, FitzGerald GA and Funk CD. Genomic and lipidomic analyses differentiate the compensatory roles of two COX isoforms during systemic inflammation in mice. J Lipid Res 2018; 59: 102-112.

[12] Oh S, Kim DY, Baek MK, Byun K and Woo JH. The Effect of Human Adipose Tissue-Derived Mesenchymal Stem Cells in Rat's Subglottic Stenosis Model. Ann Otol Rhinol Laryngol 2018; 127: 5-12.

[13] Beffa DC, Carter EA, Lu XM, Yu YM, Prelack K, Sheridan RL, Young VR, Fischman AJ and Tompkins RG. Negative chemical ionization gas chromatography/mass spectrometry to quantify urinary 3-methylhistidine: application to burn injury. Anal Biochem 2006; 355: 95-101.

[14] Carter EA, Tompkins RG, Babich JW, Correia JA and Fischman AJ. Decreased cerebral glucose utilization in rats during the ebb phase of thermal burn. J Trauma 1996; 40: 930-935.

[15] Pawlik TM, Carter EA, Bode BP, Fischman AJ and Tompkins RG. Central role of interleukin-6 in burn induced stimulation of hepatic amino acid transport. Int J Mol Med 2003; 12: 541-548.

[16] Zhang Q, Carter EA, Ma BY, White M, Fischman AJ and Tompkins RG. Molecular mechanism(s) of burn-induced insulin resistance in murine skeletal muscle: role of IRS phosphorylation. Life Sci 2005; 77: 3068-3077.

[17] Zhuang S, Chai J, Yu Y, Yin H, Liu L, Fan J, Wang Y and Hou Y. [Experimental study on effect of burn on brown adipose tissue in mice]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2014; 28: 988-992.

[18] Echtay KS. Mitochondrial uncoupling proteins--what is their physiological role? Free Radic Biol Med 2007; 43: 1351-1371.

[19] Dikov D, Aulbach A, Muster B, Drose S, Jendrach M and Bereiter-Hahn J. Do UCP2 and mild uncoupling improve longevity? Exp Gerontol 2010; 45: 586-595.
[20] Ruiz-Ramirez A, Lopez-Acosta O, Barrios-Mayra M and El-Hafidi M. Uncoupling Protein Overexpression in Metabolic Disease and the Risk of Uncontrolled Cell Proliferation and Tumorigenesis. Curr Mol Med 2017; 17: 598-607.

[21] Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, Harper JA, Roebuck SJ, Morrison A, Pickering S, Clapham JC and Brand MD. Superoxide activates mitochondrial uncoupling proteins. Nature 2002; 415: 96-99.

[22] Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RA and Murphy MP. Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. J Biol Chem 2001; 276: 4588-4596.

[23] Villa V, Thellung S, Bajetto A, Gatta E, Robello M, Novelli F, Tasso B, Tonelli M and Florio T. Novel celecoxib analogues inhibit glial production of prostaglandin E2, nitric oxide, and oxygen radicals reverting the neuroinflammatory responses induced by misfolded prion protein fragment 90-231 or lipopolysaccharide. Pharmacol Res 2016; 113: 500-514.

[24] Senbel AM, AbdelMoneim L and Omar AG. Celecoxib modulates nitric oxide and reactive oxygen species in kidney ischemia/reperfusion injury and rat aorta model of hypoxia/reoxygenation. Vascul Pharmacol 2014; 62: 24-31.

[25] Fruhbeck G, Becerril S, Sainz N, Garrastach P and Garcia-Vellos MO. BAT: a new target for human obesity? Trends Pharmacol Sci 2009; 30: 387-396.
Figure legends:

**Figure 1.** Burn injury-induced morphological changes in iBAT. Gross views on interscapular fat pad from sham burn (A) and sham Celecoxib (B) and burn saline (C) and burn Celecoxib (D) animals. E showed the weight of isolated BAT in interscapular area in mice. *: P<0.05

**Figure 2.** Ultrastructure of brown adipocytes in iBAT. A (×1000) and C (×3000) from sham burn animals; B (×1000) and D (×3000) from burned animals.

**Figure 3.** The effect of Celecoxib in reducing burn injury-induced hypermetabolism. A, VO2 was, increased in B group compared with S, C and BC groups; B, VCO2 was, increased in B group compared with S, C and BC groups; C, EE was, increased in B group compared with S, C and BC groups; D, RER had no significant difference between four groups. B group: burned animals; S group: sham treated animals; C group: sham burn + Celecoxib animals; BC group: burn + Celecoxib animals; VO2: volume of oxygen uptake; VCO2: volume of carbon dioxide output; RER: respiratory exchange ratio; EE: energy expenditure; *P<0.01.

**Figure 4.** Expressions of UCP-1 and COX-2 mRNA and protein in iBAT. Relative expressions of COX-2 (A) and UCP-1 (B) mRNA in BAT samples from the four groups. Represented western blotting images for UCP-1 and COX-2 proteins in iBAT (C). Densitometry values were calculated based on GAPDH expression, and shown in D and E. Values were shown as mean±SEM in densitometry units. *P<0.05.
