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Systemic inhibition of Janus kinase induces browning of white adipose tissue and ameliorates obesity-related metabolic disorders

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ABSTRACT

Browning of white adipose tissue is a promising strategy to tackle obesity. Recently, Janus kinase (JAK) inhibition was shown to induce white-to-brown metabolic conversion of adipocytes *in vitro*; however effects of JAK inhibition on browning and systemic metabolic health *in vivo* remain to be elucidated. Here, we report that systemic administration of JAK inhibitor (JAKi) ameliorated obesity-related metabolic disorders. Administration of JAKi in mice fed a high-fat diet increased UCP-1 and PRDM16 expression in white adipose tissue, indicating the browning of white adipocyte. Food intake was increased in JAKi-treated mice, while the body weight and adiposity was similar between the JAKi- and vehicle-treated mice. In consistent with the browning, thermogenic capacity was enhanced in mice treated with JAKi. Chronic inflammation in white adipose tissue was not ameliorated by JAKi-treatment. Nevertheless, insulin sensitivity was well preserved in JAKi-treated mice comparing with that in vehicle-treated mice. Serum levels of triglyceride and free fatty acid were significantly reduced by JAKi-treatment, which is accompanied by ameliorated hepatosteatosis. Our data demonstrate that systemic administration of JAKi has beneficial effects in preserving metabolic health, and thus inhibition of JAK signaling has therapeutic potential for the treatment of obesity and its-related metabolic disorders.

1. Introduction

The epidemic of obesity is increasing worldwide. Obesity often causes systemic metabolic disorders that accelerate atherosclerosis, and thus its deleterious effects on health have been a serious issue. An imbalance between energy intake and expenditure could simply accounts for obesity; therefore increasing energy expenditure is mostly effective to ameliorate obesity. Brown adipose tissue (BAT) is a thermogenic organ, and its high degree of specialization of lipolysis and fatty acid oxidation makes it a major contributor to the overall energy balance [1]. BAT dissipates extra energy to generate heat through uncoupled respiration mediated by uncoupling protein 1 (UCP1). Oxygen consumption in BAT is enormously high, hence its thermogenic activity can counteract the obese phenotype [2]. Also, metabolically active BAT has been identified in adult human, and its activity showed negative correlations with body mass index and fat depot [3–5]. Certain white adipose tissue (WAT) depots, called beige/brite adipocytes, are readily able to convert to a brown-like state upon prolonged cold exposure [6–8]. This white-to-brown conversion is referred to as browning, and browning has been linked to obesity in mouse models [9–11].

Recently, Janus kinase (JAK) inhibition has been revealed to induce browning in human adipocytes *in vitro* [12]; however, effects of JAK inhibition on browning and metabolic homeostasis *in vivo* remained to be elucidated. A growing body of evidence suggests that the JAK/signal transducer and activator of

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transcription (STAT) signaling pathway is dysregulated in obesity and metabolic disorders [13,14]. Furthermore, associations of genetic loci for JAK2 with obesity and metabolic syndrome have been reported [15,16]. Mouse studies targeting the JAK/STAT signaling pathway showed various metabolic phenotypes depending on target genes and tissues/cells. Inhibition of this signaling in immune cells appears to have beneficial effects on metabolism, while disruption of this pathway in adipose tissue, liver, muscle, and pancreas mostly showed deleterious effects on metabolic health [13]. Here we explored effects of systemic administration of JAK inhibitor on obesity and metabolic homeostasis in diet-induced obese mice.

2. Materials and methods

2.1. Materials

Tofacitinib was purchased from LC Laboratories. Ruxolitinib was purchased from Cayman Chemical. Osmotic pumps were purchased from Alzet.

3T3-L1 preadipocytes were obtained from JCRB cell bank in National Institutes of Biomedical Innovation, Health and Nutrition.

2.2. Cell culture

3T3-L1 pre-adipocytes were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum (Gibco). Adipogenesis was induced by treatment with insulin, dexamethasone, and isobutylmethylxanthine as described previously [17]. Mature adipocytes at Day 10–12 were used for experiments.

3T3-L1 adipocytes were incubated with either 2 μM tofacitinib, 1 μM ruxolitinib, or vehicle for 24 h, followed by RNA extraction. In some experiments, adipocytes were cultured in the absence of JAK inhibitors for 48 h after 24 h-treatment with tofacitinib.

2.3. Animal study

All animal experiments were performed in accordance with guidelines for the care and use of laboratory animals. Mice were kept under a 12 h light/dark cycle with *ad libitum* access to water and food. C57BL6J mice were fed with high-fat diet HFD (Oriental Bio #HFD-60) containing 35% fat, 25.3% carbohydrate and 23% protein, beginning at 6-week-old. After 4-week of HFD-feeding, tofacitinib was administered at the rate of 100 μg/day through osmotic pump implanted subcutaneously. For control mice, osmotic pumps loaded with vehicle were implanted in parallel. Because the osmotic pump could supply drug over a period to about 5 weeks, another osmotic pump was implanted at day 35, and phenotypic analysis was performed after ~10-week-treatment with tofacitinib. The Insulin tolerance tests (ITT) were performed as previously reported [18,19]. For the ITT, mice were given human insulin at 1 IU/kg by subcutaneous injection. Blood glucose was measured by the glucose oxidase method (Johnson & Johnson K.K.).

Core temperature was monitored with an electronic thermometer (Model BAT 12 R, Bio Research Center, Japan) equipped with a rectal probe (Model RET-3) [20]. For the acute cold exposure experiments, mice housed single mouse per cage were kept at ~4 °C, and their core temperature was measured every 30 min up to 120 min.

Subcutaneous inguinal WAT, visceral perigonadal WAT and interscaplar BAT were used for all of the analysis for WAT and BAT.

2.4. Histological analysis

Liver was extracted and embedded in paraffin, followed by sectioning. After H-E staining, sections were observed under microscopy.

2.5. Quantitative PCR

Nucleotide sequence for each primer was shown in Table-1. Total RNA was extracted using QIAzol (Invitrogen) followed by purification with RNeasy Lipid Tissue Mini Kit (QIAGEN) for adipose tissues and adipocytes, or with NucleoSpin RNA Clean-up kit (Macherey-Nagel) for other tissues. cDNA was synthesized from ~1 μg of total RNA using PrimeScript RT reagent Kit with gDNA Eraser (TAKARA). PCR reactions were prepared using FastStart SYBR Green Master (Roche Applied Science) followed by the real time PCR analysis using LightCycler96 (Roche Applied Science).

2.6. Statistical analysis

All data are presented as mean ± S.E. Differences between groups were analyzed using two-tailed Student's *t*-test. $P < 0.05$ was considered statistically significant.

Table-1
Nucleotide sequence for quantitative PCR primers.

PPAR-α	GAGGGTTGAGCTCAGTCAGG
	GGTCACCTACGAGTGGCATT
PPAR-γ	TTTTCAAGGGTGCCAGTTTC
	AATCCTTGGCCCTCTGAGAT
PPAR-δ	TGGAGCTCGATGACAGTGAC
	GTACTGGCTGTCAGGGTGGT
UCP-1	GGGCCCTTGTAAACAACAAA
	GTCGGTCCTTCCTTGGTGTA
UCP-2	GCCACTTCACTTCTGCCTTC
	GAAGGCATGAACCCCTTGTA
UCP-3	CCACACTTCCTCCTGCTCTC
	GTATAGGGCGCTCAAATGGA
AOX	GATCCCAAGAGATGAGCCTGGATCCT
	GCTCAGGTGTATCTGGAGTATCCTC
18 S	CCATCCAATCGGTAGTAGCCG
	GTAACCCGTTGAACCCCATT
CPT1a	GATTCCTACTCGTGTGCTAGCTGTAAC
	AGTGAAAGCCCACTCTTCTGCCTTGG
MCAD	TCAGCTAGCCACTGACGCCGTTCAGA
	CTGAAGCAGCAACAGTGCTTGGAGC
PRDM16	AACTCGGAGCACGCGTGTCATGCCGTG
	GTAGCCTGGACCACTGAGCATCAGC
FASN	GACCACTGTGAGAAGCATGTCCCTGG
	CATTGGCTGCTACTGTGGGTCCACT
SCD1	CCAACTCATGTGCCTCTGTCACAGTG
	GCTGTCTACTCCGACACACACCGAG
SREBP1	CTGGCCATCTGTGAGAAGGCCAGTG
	TGACTGCTGCCGCTGCCACAGACTG
SREBP2	TCCAGCAGTTCCGTGCTCAGGTCGG
	GGAGGCATGCATGGCTCTACAGGTA
PEPCK	CGAAATTGAGAGGGAGCTCCGAGCC
	GATCTACTCAGCATTGTGCCGCTATCTC
G6pc	TGCTGGACCCTGCTGTGTCTGGTAGGC
	ATGAGAGCTCTTGGATGGCTTGGGCT
GLUT1	CAATGGCGGCGGTCCTATAA
	GAGAGACCAAAGCGTGGTGA
GLUT4	ACTCTTGCCACACAGGCTCT
	CCTTGCCCTGTCAGGTATGT
IL-6	ACAACCACGGCCTTCCCTACTT
	CACGATTTCCCAGAGAACATGTG
TNF-α	TCCCAGGTTCTCTTCAAGGGA
	GGTGAGGAGCACGTAGTCGG
F4/80	GCTATGGGCTTCCAGTCCTGGTGGT
	GAAGGTCAGCAACCTCGTGTCCTTG
CCL-2	GGCTCAGCCAGATGCAGTTAA
	CCTACTCATTGGGATCATCTTGCT

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