A role for PFKFB3/iPFK2 in metformin suppression of adipocyte inflammatory responses

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Abstract

Metformin improves obesity-associated metabolic dysregulation, but has controversial effects on adipose tissue inflammation. The objective of the study is to examine the direct effect of metformin on adipocyte inflammatory responses and elucidate the underlying mechanisms. Adipocytes were differentiated from 3T3-L1 cells and treated with metformin at various doses and for different time periods. The treated cells were examined for the proinflammatory responses, as well as the phosphorylation states of AMPK and the expression of PFKFB3/iPFK2. In addition, PFKFB3/iPFK2-knockdown adipocytes were treated with metformin and examined for changes in the proinflammatory responses. The following results were obtained from the study. Treatment of adipocytes with metformin decreased the effects of lipopolysaccharide on inducing the phosphorylation states of JNK p46 and on increasing the mRNA levels of IL-1β and TNFα. In addition, treatment with metformin increased the expression of PFKFB3/iPFK2, but failed to significantly alter the phosphorylation states of AMPK. In PFKFB3/iPFK2-knockdown adipocytes, treatment with metformin did not suppress the proinflammatory responses as did it in control adipocytes. In conclusion, metformin has a direct effect on suppressing adipocyte proinflammatory responses in an AMPK-independent manner. Also, metformin increases adipocyte expression of PFKFB3/iPFK2, which is involved in the anti-inflammatory effect of metformin.

Introduction

Chronic low-grade adipose tissue inflammation is a characteristic of obesity (Lumeng et al. 2007, Weisberg et al. 2003, Xu et al. 2003) and critically contributes to the pathogenesis of type 2 diabetes (Greenberg & Obin 2006, Shoelson et al. 2007). As shown in rodent models of obesity, adipose tissue–specific
metformin does not alter body weight and/or adipose tissue inflammation, in particular in rodents fed with an high-fat diet (HFD) (Anthony et al. 2013, Shin et al. 2013, Song et al. 2001, Woo et al. 2014). Given this discrepancy, there is a need to gain mechanistic insights of whether metformin has direct effects on adipocyte inflammatory and metabolic responses.

PFKFB3 is the gene that encodes for the inducible 6-phosphofructo-2-kinase (iPFK2). In adipose tissue, the expression of PFKFB3/iPFK2 is at high abundance (Huo et al. 2010). As a regulatory enzyme, iPFK2 generates fructose-2,6-bisphosphate, which in turn activates 6-phosphofructo-1-kinase to enhance glycolysis (Okar et al. 2004, Rider et al. 2004). PFKFB3/iPFK2 has been previously shown to critically regulate adipose tissue functions and systemic insulin sensitivity (Huo et al. 2010). For example, PFKFB3/iPFK2 disruption exacerbates diet-induced adipose tissue inflammation and systemic insulin resistance, whereas adipose tissue–specific PFKFB3/iPFK2 overexpression decreases adipose tissue inflammation and improves systemic insulin sensitivity (Huo et al. 2010, Huo et al. 2012). Limited research has suggested that the expression of PFKFB3/iPFK2 is increased in DB-1 melanoma cells upon metformin treatment, likely through a mechanism involving AMP-activated protein kinase (AMPK) (Mendoza et al. 2012). However, it is not clear whether metformin alters adipocyte expression of PFKFB3/iPFK2 in the context of adipose tissue inflammatory responses. The present study provides the evidence for the first time to support that metformin stimulates adipocyte expression of PFKFB3/iPFK2. The latter, in turn, is involved in the effect of metformin on suppressing adipocyte inflammatory responses.

Materials and methods

Adipocyte differentiation and treatment

3T3-L1 cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin as previously described (Huo et al. 2010). To differentiate 3T3-L1 cells, the 2d post-confluent cells were incubated in DMEM supplemented with 0.5 mM 3-isobutyl-1-methyl-xanthine, 1 µM dexamethasone and 10 µg/mL insulin for 48h. Thereafter, the cells were incubated in DMEM supplemented with 10 µg/mL insulin for an additional 8 d. At 10 days post differentiation, adipocytes were used for a dose-response study or a time-course study to examine the anti-inflammatory effect of metformin.
In the dose-response study, the differentiated adipocytes were treated with metformin at a dose of 5, 50 or 500µM (dissolved in phosphate-buffered saline, PBS) or PBS for 24h in the absence or presence of lipopolysaccharide (LPS, 100ng/ml) for the last 30min to harvest protein lysates or LPS (20ng/ml) for the last 6h to harvest total RNA samples. In the time-course study, the differentiated adipocytes were treated with metformin (50µM) for 0, 1, 6, 24 or 48h in the presence of LPS (100ng/ml) for the last 30min to harvest protein lysates or LPS (20ng/ml) and for the last 6h to harvest total RNA samples. To analyze the proinflammatory signaling, adipocyte lysates were examined for total amount and phosphorylation states of Jun N-terminal kinase (JNK) p46 and nuclear factor kappa B (NF-kB) p65. To analyze the expression of PFKFB3/iPFK2, adipocyte RNA was subjected to reverse transcription and real-time PCR to quantify PFKFB3 mRNA levels, whereas adipocyte lysates were subjected to Western blot analysis to examine iPFK2 amount. In addition, adipocyte RNA was used to examine the mRNA levels of proinflammatory cytokines including IL-1β, IL-6 and TNFα. Details were described in the pertinent assays.

**Adipocyte PFKFB3/iPFK2 knockdown and metformin treatment**

Stable PFKFB3/iPFK2 knockdown (iPFK2-KD) and control (iPFK2-Ctrl) 3T3-L1 cells were previously established (Huo et al. 2010). To examine the involvement of PFKFB3/iPFK2 in metformin actions, iPFK2-KD cells and iPFK2-Ctrl cells were differentiated in the same way as described above. After differentiation, the cells were treated with metformin (50µM) or PBS for 24h in the absence or presence of LPS (100ng/ml) for the last 30min to harvest protein lysates or LPS (20ng/ml) and for the last 6h to harvest total RNA samples. Some differentiated iPFK2-KD cells and iPFK2-Ctrl cells were treated with metformin (50µM) or PBS for 24h in the absence or presence of insulin (100nM) for the last 30min. Protein lysates were harvested and used to examine insulin signaling using Western blot analysis.

**Western blot analysis**

Adipocyte lysates were prepared in a lysis buffer containing 50mM HEPES (pH 7.4), 10mM EDTA, 50mM sodium pyrophosphate, 0.1M sodium fluoride, 10mM sodium orthovanadate, 2mM phenylmethylsulfonyl fluoride, 10µg/ml aprotinin, 10µg/ml leupeptin, 2mM benzamidine and 1% Triton X-100. After protein electrophoresis and transfer, immunoblots were performed using rabbit anti-serum as primary antibody at a 1:1,000 dilution. This dilution was used for each of the primary antibodies used for the present study. After washing, the blot was incubated with a 1:10,000 dilution of goat anti-rabbit horseradish peroxidase-conjugated secondary antibody followed by a chemiluminescent kit (Immobilon Western; EMD Millipore, Billerica, MA, USA) as previously described (Wu et al. 2005a). GAPDH was used as a loading control. The maximum intensity of each band was quantified using ImageJ software. Ratios of Pp46/p46, Pp65/p65, P-AMPK/AMPK or P-Akt/Akt were normalized to GAPDH and adjusted relative to the average of PBS-treated control, which was set as 1 (AU). Also, the amount of iPFK2 was normalized to GAPDH and adjusted similarly. Antibodies against Pp46, p46, Pp65, p65, P-AMPK and/or AMPK were products of Cell Signaling (Danvers, MA, USA). Antibodies against P-Akt and Akt and anti-rabbit IgG antisera were products of Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

**RNA isolation, reverse transcription and real-time PCR**

The total RNA was isolated from the adipocytes described above. Reverse transcription was performed using the GoScript Reverse Transcription System (Promega), and real-time PCR analysis was performed using SYBR Green (LightCycler 480 system; Roche) (Guo et al. 2013, Guo et al. 2012, Wu et al. 2006). The mRNA levels were analyzed for IL-1β, IL-6, TNFα and/or PFKFB3. Reverse products from a total of 0.1µg RNA were used for PCR determination of each of the interest genes. Results were normalized to 18s ribosomal RNA as plotted as relative expression to the average of PBS-treated control, which was set as 1.

**Statistical methods**

Numeric data are presented as means±s.e. (standard error). Two-tailed ANOVA and/or Student’s t-tests were used for statistical analyses. Differences were considered significant at the P<0.05.

**Results**

**Metformin suppresses adipocyte proinflammatory responses**

The effect of metformin on obesity-associated adipose tissue inflammation remains controversial. We sought to examine the direct effect of metformin on...
adipocyte inflammatory responses. In differentiated 3T3-L1 adipocytes, treatment with LPS, a powerful proinflammatory stimulus, caused significant increases in the phosphorylation states of JNK p46 and NF-KB p65 and in the mRNA levels of IL-1β and TNFα (Fig. 1A, B and C). Upon treatment with metformin, the effect of LPS on inducing adipocyte proinflammatory responses was significantly weakened. Specifically, treatment with metformin at a dose of 50 or 500 µM brought about a significant decrease in LPS-induced JNK p46 phosphorylation compared with metformin treatment at a dose of 5 µM (Fig. 1A). At a fixed dose of 50 µM, treatment with metformin for 24 or 48 h caused a marked decrease in the phosphorylation states of JNK p46 compared with control treatment or metformin treatment for 6 h (Fig. 1B). Unlike its effects on JNK p46, metformin treatment did not significantly alter adipocyte phosphorylation states of NF-KB p65 in either dose-response or time-course study. When cytokine expression was examined, treatment with metformin at a dose of 50 µM for 24 h significantly blunted the effect of LPS on increasing adipocyte mRNA levels of IL-1β and TNFα (Fig. 1C). Of note, treatment of adipocytes with metformin at a relatively low dose, e.g., 5 µM, or for a relatively short time, e.g., 6 h, appeared to not suppress or even increase LPS-induced JNK p46 phosphorylation states (Fig. 1A, and B). These effects, however, occurred prior to an increase in iPFK2 amount and/or in the presence of a decrease in iPFK2 amount (below, Fig. 3). Considering that decreased iPFK2 results in an increase in LPS-induced JNK p46 phosphorylation (Huo et al. 2010), it is likely that prior to full induction of iPFK2, metformin acts through certain mechanisms to increase LPS-induced JNK p46 phosphorylation. Once iPFK2 is increased, the anti-inflammatory effect of iPFK2 starts to dominate over the proinflammatory effect, thereby decreasing LPS-induced JNK p46 phosphorylation. Regardless, these
Metformin: involvement in insulin resistance.

Results suggest that metformin has a direct effect on suppressing adipocyte proinflammatory responses.

Metformin has limited effect on altering adipocyte AMPK phosphorylation

Metformin is shown to increase AMPK phosphorylation (activation) in various tissues/cells including the liver and muscle (Collier et al. 2006, Woo et al. 2014). However, the effect of metformin on adipose tissue AMPK phosphorylation remains controversial. Using adipocyte samples of the dose-response and time-course studies, we examined the direct effect of metformin on adipocyte AMPK phosphorylation. Consistent with our previous finding in adipose tissue of obese mice (Woo et al. 2014), treatment with metformin displayed limited effects on altering adipocyte AMPK phosphorylation (Fig. 2A, and B). This effect of metformin appeared to be different from that reported by Huypens and coworkers (Huypens et al. 2005). However, in the study by Huypens and coworkers, adipocytes were treated with metformin at a high dose (1 mM) for 48 h. Based on the results of the present study, it appears that metformin, within a relatively low dose range, is not capable of increasing adipocyte AMPK phosphorylation.

Metformin stimulates adipocyte PFKFB3/iPFK2 expression

In adipocytes, PFKFB3/iPFK2 has a role in protecting against overnutrition-induced proinflammatory responses (Huo et al. 2010, Huo et al. 2012). Therefore, we examined the effect of metformin on adipocyte PFKFB3/iPFK2 expression. In the dose-response study detailed above, PFKFB3 expression was increased by LPS stimulation, indicating a defensive response. Upon treatment with metformin at a dose of 5 µM for 24 h, the effect of LPS on increasing PFKFB3 expression was blunted. However, treatment with metformin at a higher dose, i.e., 50 or 500 µM, for 24 h caused a significant increase in the mRNA levels of PFKFB3 compared with control in the presence of LPS (Fig. 3A). Moreover, treatment with metformin at a dose of 50 µM appeared to cause the strongest induction of PFKFB3 mRNA levels. In the time-course study, treatment with metformin at a dose of 50 µM for 6 h, 24 h, or 48 h significantly increased the mRNA levels of PFKFB3. Within a period of time between 0 and 48 h, treatment with metformin for 24 h displayed the strongest effect on stimulating PFKFB3 mRNA levels (Fig. 3B). Consistently, treatment with metformin at a dose of 50 µM for 24 h or 48 h significantly increased protein amount of iPFK2.
compared with control treatment in the presence of LPS (Fig. 3C), although iPFK2 amount in adipocytes upon metformin treatment for 24 h is not statistically different from that upon metformin treatment for 6 or 48 h (Fig. 3C). Taken together, these results suggest that metformin has a direct effect on stimulating adipocyte expression of PFKFB3/iPFK2.

**PFKFB3/iPFK2 knockdown blunts the effect of metformin on suppressing adipocyte proinflammatory responses**

To elucidate a role for PFKFB3/iPFK2 in regulating metformin actions, we examined the effect of metformin on the proinflammatory responses in iPFK2-KD adipocytes. Consistent with the findings in differentiated 3T3-L1 adipocytes (Fig. 3), treatment with metformin increased iPFK2 amount in control cells (iPFK2-Ctrl) (Fig. 4A). However, this effect of metformin was nearly blunted in iPFK2-KD cells. Also, treatment with metformin did not significantly alter the phosphorylation states of AMPK in either iPFK2-KD or iPFK2-Ctrl cells. Although the phosphorylation states of AMPK in iPFK2-KD cells appeared to be increased in relative to those in iPFK2-Ctrl cells, the ratios of P-AMPK to total AMPK (normalized to GAPDH in iPFK2-KD cells were comparable with those in iPFK2-Ctrl cells in either the presence or absence of metformin treatment. Next, we examined the anti-inflammatory effect of metformin. In iPFK2-Ctrl cells, treatment with metformin caused a significant decrease in the phosphorylation states of JNK p46, although not significantly altering the phosphorylation states of NF-κB p65. However, in iPFK2-KD cells, the phosphorylation states of JNK p46 were significantly higher than those in iPFK2-Ctrl cells, and remained high upon treatment with metformin (Fig. 4B). When proinflammatory cytokine expression was analyzed, the mRNA levels of IL-1β, IL-6 and/or TNFα in iPFK2-Ctrl cells were significantly reduced by treatment with metformin under basal conditions (in the absence of LPS) compared with control (in the absence of metformin). In contrast, the mRNA levels of IL-1β, IL-6 and/or TNFα in iPFK2-KD cells were increased or remained high upon treatment with metformin under either basal or LPS-stimulated conditions (Fig. 4C). Indeed, the mRNA levels of IL-1β, IL-6, and/or TNFα were much more induced in iPFK2-KD cells upon LPS stimulation in the presence or absence of metformin compared with their respective levels in iPFK2-Ctrl cells (Fig. 4C). Taken together, these results suggest that intact PFKFB3/iPFK2 is required for
metformin to fully suppress adipocyte proinflammatory responses.

**PFKFB3/iPFK2 knockdown blunts the effect of metformin on improving adipocyte insulin signaling**

Adipocyte insulin signaling is reversely correlated with adipocyte proinflammatory status. We examined the involvement of **PFKFB3/iPFK2** in metformin actions on improving adipocyte insulin signaling at the level of Akt phosphorylation. In iPFK2-Ctrl cells, treatment with metformin enhanced the effect of insulin on increasing the phosphorylation states of Akt compared with control treatment (Fig. 5A and B). However, this effect of metformin was blunted in iPFK2-KD cells (Fig. 5A and B). Therefore, intact **PFKFB3/iPFK2** is needed for metformin to improve adipocyte insulin signaling.

**Discussion**

The anti-diabetic effect of metformin has been well established. However, it remains controversial whether metformin suppresses adipose tissue inflammation, thereby contributing to the anti-diabetic effect of metformin (Shin et al. 2013, Woo et al. 2014, Zhao et al. 2016, Zulian et al. 2011). Because the discrepancy of metformin actions on adipose tissue inflammation appears to be associated with various factors including the strains of rodents used and the doses and paths of metformin delivery, the present study sought to determine...
the direct effect of metformin on adipocytes. Using in vitro systems involving differentiated 3T3-L1 adipocytes, the present study validated a direct effect of metformin in improving adipocyte insulin signaling. PFKFB3/iPFK2 knockdown (iPFK2-KD) adipocytes and control (iPFK2-Ctrl) adipocytes were generated as described in Methods. (A, B). After differentiation, iPFK2-KD adipocytes and iPFK2-Ctrl adipocytes were treated with or without metformin (50 µM) for 24 h in the absence or presence of insulin (100 ng/mL) for the last 30 min. Adipocyte lysates were examined for the amount and phosphorylation states of Akt using Western blot analysis. Blots were quantified using densitometry. AU, arbitrary unit. A, representative bolts; B, quantification of blots. For B, data are means ± s.e. n = 4. **, P < 0.01 Met/Insulin vs Insulin (iPFK2-Ctrl cells); ††, P < 0.01 iPFK2-KD vs iPFK2-Ctrl under the same condition (Insulin or Met/Insulin); †††, P < 0.01 Insulin vs PBS or Met/Insulin vs Met/PBS for iPFK2-Ctrl cells.

In addition, metformin treatment at a fixed dose of 50 µM for 24 or 48 h caused significant decreases in LPS-stimulated adipocyte phosphorylation states of JNK p46. Consistently, treatment with metformin brought about a significant decrease in the mRNA levels of IL-1β and TNFα. Of note, metformin appeared to be more effective in adipocytes differentiated from un-transfected 3T3-L1 cells than in adipocytes differentiated from transfected and G418-selected 3T3-L1 cells while exhibiting anti-inflammatory effects. Specifically, metformin treatment caused a significant decrease in the mRNA levels of IL-1β and TNFα in un-transfected cells (Fig. 1C), but not in iPFK2-Ctrl cells (Fig. 4C) under LPS-stimulated conditions. This difference in metformin actions may be due to how adipocytes were prepared. Indeed, adipocytes differentiated from transfected cells exhibited a much stronger response to LPS than did adipocytes differentiated from un-transfected cells. Considering this, it is likely that there is a threshold of adipocyte proinflammatory status for metformin to exhibit anti-inflammatory effects.

In other words, metformin may not be effective when LPS-induced proinflammatory responses are too strong. This appeared to be the case. In the present study, under basal conditions where the proinflammatory status was low or mild, metformin treatment significantly decreased the mRNA levels of IL-1β, IL-6 and TNFα, as well as the phosphorylation states of JNK p46. In contrast, in iPFK2-KD cells where the basal proinflammatory status was strong, metformin failed to decrease proinflammatory signaling and cytokine expression. The threshold view, however, needs to be further investigated. Nonetheless, the results argue in favor of a role for iPFK2 in mediating the anti-inflammatory effect of metformin (see below).

Metformin suppression of adipocyte proinflammatory responses does not involve AMPK. Previous results from both human and rodent studies indicate that treatment with metformin stimulates the phosphorylation of states of adipose tissue AMPK (Boyle et al. 2011, Lu et al. 2016). In contrast, there are studies in which treatment with metformin does not significantly alter the phosphorylation states of adipose tissue AMPK (Woo et al. 2014). Similarly, the direct effects of metformin on adipocyte AMPK phosphorylation vary among studies. In the present study, treatment with metformin did not alter adipocyte AMPK phosphorylation as this was supported by the results from both does-response and time-course studies. These results were consistent with the findings of a previous study by Boyle and coworkers (Boyle et al. 2011), in which treatment with metformin at a dose of 1 mM for a time period of 0.5 to 24 h did not significantly alter adipocyte AMPK
phosphorylation, although treatment with metformin for 48h increased adipocyte AMPK phosphorylation states.

Of importance, in the present study, treatment with metformin significantly decreased the phosphorylation states of adipocyte JNK p46 and the mRNA levels of proinflammatory cytokines while exhibiting limited effect on increasing adipocyte AMPK phosphorylation states. Therefore, metformin appears to suppress adipocyte proinflammatory responses in an AMPK-independent manner. This is different from the effect of metformin on hepatocytes, in which treatment with metformin increases the phosphorylation states of AMPK and suppresses the proinflammatory responses (Woo et al. 2014). The mechanisms underlying the differential effects of metformin on AMPK phosphorylation states in hepatocytes versus in adipocytes are not clear, but may be due to the differences in the isoforms of the catalytic subunit of AMPK. In adipocytes, the α1 catalytic subunit is the predominant isoform and accounts for the major part of AMPK activity (Daval et al. 2005), whereas in hepatocytes, α2-subunit is the main functional isoform of AMPK (Andreelli et al. 2006, Jelenik et al. 2010). As shown in muscles, treatment with metformin increases the activities of AMPKα2, but not AMPKα1 (Collier et al. 2006). Considering this, lacking responses in AMPKα1 to metformin likely explains the observation of the present study. Alternatively, a relatively low dose, e.g., 50µM, is sufficient for metformin to exert anti-inflammatory effects, whereas a relatively high dose, e.g., 1mM, is needed for metformin to increase AMPK phosphorylation as reported by Huypens and coworkers (Huypens et al. 2005). In this case, metformin would still act to suppress adipocyte proinflammatory responses independent of AMPK phosphorylation.

It is a novel finding that treatment with metformin increased adipocyte expression of PFKFB3/iPFK2. Notably, treatment with metformin caused an increase in iPFK2 amount in both dose-response and time-course studies. In addition, treatment with metformin significantly increased the mRNA levels of PFKFB3 in the presence of LPS. Previously, AMPK activation upon lowering the pH values of the culture media is accompanied with increased PFKFB3/iPFK2 expression in glioblastoma cells (Mendoza et al. 2012). In the present study, metformin increased adipocyte PFKFB3/iPFK2 expression in the absence of stimulating AMPK phosphorylation. Considering this, metformin is likely capable of stimulating PFKFB3/iPFK2 expression in both AMPK-dependent and AMPK-independent manners. Regardless, increased amount of iPFK2 was correlated with the effect of metformin on decreasing adipocyte proinflammatory responses, suggesting the involvement of PFKFB3/iPFK2 in the anti-inflammatory effect of metformin. In adipocytes, PFKFB3/iPFK2 has been demonstrated as a metabolic regulator that links glucose and fatty acid metabolism and inflammatory responses. Specifically, PFKFB3/iPFK2, at an increase in its activity, is able to channel free fatty acids away from excessive oxidation to fat synthesis/storage, thereby reducing the generation of reactive oxygen species (Guo et al. 2010, Huo et al. 2012). Because of this, it is conceivable that metformin stimulation of PFKFB3/iPFK2 serves as a mechanism by which metformin suppresses adipocyte proinflammatory responses.

The involvement of PFKFB3/iPFK2 in the anti-inflammatory effect of metformin was further supported by the results from adipocytes upon PFKFB3/iPFK2 knockdown. In the present study, increased proinflammatory responses were confirmed in PFKFB3/iPFK2-knockdown adipocytes compared with those in control adipocytes. Of importance, metformin did not effectively suppress the proinflammatory responses in PFKFB3/iPFK2-knockdown adipocytes as did it in control adipocytes. As previously demonstrated by Guo and coworkers, PFKFB3/iPFK2 knockdown increased adipocyte proinflammatory responses, which was due to, in large part, increased adipocyte oxidative stress (Guo et al. 2010). In contrast, PFKFB3/iPFK2 overexpression promoted adipocyte fat deposition, leading to decreased adipocyte proinflammatory responses (Huo et al. 2012). Based on this, it is very likely that intact PFKFB3/iPFK2 is needed for a metabolic environment in which metformin is able to effectively suppress the proinflammatory responses. As additional evidence, metformin treatment increased insulin singling, indicated by insulin-induced Akt phosphorylation, in control adipocytes, but not in PFKFB3/iPFK2-knockdown adipocytes.

In summary, the present study provided the evidence to support a direct effect of metformin on suppressing adipocyte proinflammatory responses. This effect of metformin appeared to be AMPK independent. Moreover, the present study reported for the first time that metformin stimulated adipocyte expression of PFKFB3/iPFK2, which also was AMPK independent. In PFKFB3/iPFK2-knockdown adipocytes, the proinflammatory responses were much exacerbated and were not suppressed upon metformin treatment compared with those in control adipocytes. Taken together, metformin appears to act through simulating PFKFB3/iPFK2 expression to suppress adipocyte proinflammatory responses.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Authors’ contribution statement
C W, Y H and Y C designed the research; T Q, H L, Y R, S L W, X G and J Z conducted the research; T Q, H L and C W analyzed the data and wrote the manuscript. Y C, X Q and J A were involved in scientific discussion. C W and Y H had primary responsibility for the final content. All authors read and approved the final manuscript.

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