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RESEARCH PAPER

Up-regulation of aldolase A and methylglyoxal production in adipocytes

Jianghai Liu[1], Kaushik Desai[1], Rui Wang[2] and Lingyun Wu[1,3]

[1]*Department of Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada,* [2]*Department of Biology, Lakehead University and Thunder Bay Regional Research Institute, Thunder Bay, ON, Canada, and* [3]*Department of Health Sciences, Lakehead University and Thunder Bay Regional Research Institute, Thunder Bay, ON, Canada*

Correspondence
Lingyun Wu, Department of Health Sciences, Lakehead University, Thunder Bay, ON, Canada P7B 5E1. E-mail: lwu@lakeheadu.ca

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BACKGROUND AND PURPOSE

We previously reported that up-regulation of aldolase B, a key enzyme in fructose metabolism, was mainly responsible for vascular methylglyoxal (MG) overproduction under different pathological conditions. Here we investigated whether aldolase A, an enzyme of the glycolytic pathway, also caused MG overproduction in insulin-sensitive adipocytes.

EXPERIMENTAL APPROACH

The relative contributions of different metabolic pathways or enzymes to MG generation were evaluated in cultured 3T3-L1 adipocytes.

KEY RESULTS

Glucose (25 mM) had no effect on aldolase A gene expression, but insulin (100 nM) up-regulated aldolase A mRNA and protein levels in the absence or presence of 25 mM glucose in adipocytes. Treatment with insulin increased levels of basal or glucose (25 mM)-induced MG and glucose 6-phosphate. However, insulin, glucose (25 mM) or their combination had no effect on cellular levels of sorbitol and fructose, but down-regulated gene expression of aldolase B to a similar extent, when compared with the control group. Incubation of 3T3-L1 adipocytes with fructose, acetone, acetol, threonine or glycine (25 mM), with or without insulin did not alter cellular MG levels. The elevated MG levels induced by insulin, glucose (25 mM) or their combination in adipocytes was completely reduced by siRNA knock down of aldolase A or application of 2-deoxy-D-glucose (a non-specific inhibitor of glucose uptake and glycolysis), but not by knock down of aldolase B.

CONCLUSION AND IMPLICATIONS

Insulin enhanced MG overproduction in insulin-sensitive adipocytes by up-regulating aldolase A, a mechanism that could be involved in the development of insulin resistance and obesity.

Abbreviations

2-DG, 2-deoxy-D-glucose; DHAP, dihydroxyacetone phosphate; ECs, endothelial cells; F-1-P, fructose 1-phosphate; F-1,6-BP, fructose 1,6-diphosphate; GA3P, glyceraldehyde 3-phosphate; MG, methylglyoxal; siRNA, small interference RNA; VSMCs, vascular smooth muscle cells

Introduction

Methylglyoxal (MG) is a highly reactive metabolite produced in mammalian cells. The glucose metabolites, glyceraldehyde 3-phosphate (GA3P) and dihydroxyacetone phosphate (DHAP), which spontaneously and non-enzymatically degrade to MG are considered the major sources for endogenous MG formation (Richard, 1993; Ramasamy *et al.*, 2006; Liu *et al.*, 2011). In the cytosol, glucose is physiologically metabolized through the glycolytic pathway into fructose 1,6-diphosphate (F-1,6-BP), which subsequently forms GA3P and DHAP catalysed by aldolase A (Liu *et al.*, 2011). Glucose can also metabolize through the aldose reductase pathway into sorbitol by aldose reductase and then to fructose by sorbitol dehydrogenase (Gabbay, 1973). In some cell types, such as vascular smooth muscle cells (VSMCs), the aldose reductase pathway becomes active when intracellular glucose is elevated (Gabbay, 1973; Yabe-Nishimura, 1998; Liu *et al.*,

2011). Fructose is phosphorylated to fructose 1-phosphate (F-1-P), which is cleaved by aldolase B to generate glyceraldehyde and DHAP. Glyceraldehyde is phosphorylated by triose kinase to form GA3P (Cox, 1994; Liu *et al.*, 2011). Secondary sources for MG formation include the degradation of aminoacetone generated from protein catabolism and ketone bodies (mainly acetone) from lipolysis by the action of semicarbazide-sensitive amine oxidase (SSAO) and cytochrome P450 2E1 (CYP 2E1) respectively (Koop and Casazza, 1985; Lyles and Chalmers, 1992).

The occurrence of high levels of glucose coupled with the accumulation of endogenous MG has received much attention in diabetes research due to the potentially pathogenic roles of MG and MG-induced advanced glycation end products in the development of diabetes and diabetic complications (Brownlee, 2001; Bierhaus *et al.*, 2012). Glucose enters insulin-insensitive VSMCs and endothelial cells (ECs) mainly through an insulin-independent glucose transporter 1 (GLUT1) (Shepherd and Kahn, 1999; Nishikawa *et al.*, 2000). Higher MG levels were observed in VSMCs and ECs treated with high glucose and in aorta of diabetic rats (Dhar *et al.*, 2010b; Liu *et al.*, 2011; 2012). We have identified aldolase B, but not aldolase A, as a key enzyme responsible for high fructose and glucose-induced MG overproduction in VSMCs and ECs (Liu *et al.*, 2011; 2012). Fructose up-regulates aldolase B expression while high glucose is converted into fructose, which results in MG overproduction in VSMCs (Liu *et al.*, 2011). Recently, MG formation in insulin-sensitive cells has received much attention as high levels of MG disturbed insulin signalling in 3T3-L1 adipocytes (Jia and Wu, 2007). Moreover, MG modified the insulin molecule and impaired its biological functions, such as insulin-stimulated glucose transport in adipocytes (Jia *et al.*, 2006). However, whether MG is over-produced in the insulin-sensitive cells under different pathological conditions is unknown. GLUT1 is expressed in adipocytes, but its intrinsic activity is >90% suppressed (Harrison *et al.*, 1991). An insulin-responsive GLUT4 is highly expressed in adipocytes but under conditions of low insulin, is sequestered in intracellular vesicles (Shepherd and Kahn, 1999). Hyperinsulinaemia is commonly observed in hypertension, obesity and the early stage of type 2 diabetes (Cusin *et al.*, 1992; Chen *et al.*, 1994; Henry, 1998; Wu *et al.*, 2009; Liu *et al.*, 2011). High levels of insulin promote membrane translocation of GLUT4 and stimulates glucose transport in adipocytes (Slot *et al.*, 1991). Treatment with insulin (100 nM) elevated basal glucose levels in adipose cells by 3.6-fold (Kovacic *et al.*, 2011). Insulin can also enhance glycolysis via up-regulating the transcription of glycolytic enzymes, such as hexokinase II, phosphofructokinase and glyceraldehyde 3-phosphate dehydrogenase (Alexander *et al.*, 1985; Ducluzeau *et al.*, 2001). We hypothesized that insulin would up-regulate aldolase A expression and enhance glucose-induced MG formation in adipocytes.

In this study, cultured 3T3-L1 adipocytes were treated with insulin (100 nM), glucose (25 mM) or their combination. The relative contributions of different enzymes and pathways to MG formation were evaluated by examining the gene expression of aldolase A and aldolase B and the glucose metabolism through glycolysis and the aldose reductase pathway, and by applying inhibitors for glycolysis, aldose reductase, SSAO and CYP 2E1 as well as siRNA targeting aldolase A or B.

Methods

Cell culture

Mouse 3T3-L1 fibroblasts (American Type Culture Collection, Manassas, VA, USA) were cultured in DMEM (25 mM glucose) containing 10% FBS and penicillin–streptomycin (1% v/v) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. 3T3-L1 fibroblasts were differentiated into adipocytes in 100-mm culture dishes, as described previously (Sakuma *et al.*, 2010). Two days after confluence, differentiation was initiated by incubation of cells for 3 days with DMEM (25 mM glucose, 10% FBS) supplemented with 0.25 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine and 1 $\mu g \cdot mL^{-1}$ insulin. The differentiation medium was changed every day. Thereafter, cells were grown in DMEM (25 mM glucose, 10% FBS) containing 1 $\mu g \cdot mL^{-1}$ insulin for 2 days and then in DMEM (25 mM glucose, 10% FBS) without insulin till >90% of cells differentiated into adipocytes. Adipocytes were cultured in FBS-free DMEM (containing 5 mM glucose) for 24 h before treatments. The untreated or control cells in this paper consisted of 3T3 cells cultured in FBS (10%) – DMEM (5 mM glucose), without adding agents such as 100 nM insulin or 25 mM glucose into medium.

Small interference RNA (siRNA) knock down

Knock down of aldolase A or B was established by transfection of adipocytes with a siRNA pool (a mixture of three or four different siRNA duplexes) targeting aldolase A or B (Santa Cruz Biotechnology Inc., Santa Cruz, CA) in siRNA Transfection Reagent (Santa Cruz Biotechnology). Briefly, transfection complexes were formed by incubating 30 μL siRNA pool (10 μM) with 30 μL of transfection reagent in 5 mL siRNA transfection medium (Santa Cruz Biotechnology) for 45 min at room temperature. Transfection complexes were added to cells grown in a 10 cm dish. After 6 h of incubation, 5 mL DMEM supplemented with 20% FBS was added for a final siRNA concentration at 30 nM and incubated for 18 h. After that, cells were cultured in FBS-free DMEM (containing 5 mM glucose) for 24 h and then treated with 10% FBS DMEM in the presence or absence of insulin or/and glucose for 12 h. >90% cells were viable at 60 h after transfection. Gene knock down was verified by semi-quantitative Nested RT-PCR with primers designed by Santa Cruz Biotechnology, according to the manufacturer's instructions.

Biochemical assays

Cell were sonicated and centrifuged at 15 000× *g* (10 min, 4°C). Total protein levels were determined with a bicinchoninic acid protein assay kit (Sigma, Oakville, ON, Canada). Intracellular fructose levels were measured by a fructose assay kit (BioVision, Mountain View, CA). Aliquots of supernatants were deproteinized with perchloric acid (PCA; 0.25 volumes, 1 N) and neutralized by 2.5 M K_2CO_3 to measure the levels of glucose 6-phosphate and sorbitol using enzymic fluorometric methods (Liu *et al.*, 2011).

MG measurement

MG levels in 3T3-L1 adipocytes were determined with an *o*-phenylenediamine (*o*-PD)-based assay (Wu and Juurlink, 2002; Wang *et al.*, 2004; Wang *et al.*, 2005). Briefly, cells were

sonicated (three times for 5 s each) and centrifuged at 15 000× *g* (10 min, 4°C); 240 µL of supernatant was mixed with 60 µL of PCA (1 N), kept on ice for 10 min, and deproteinized by centrifuging at 15 000× *g* (10 min, 4°C). Then 180 µL of supernatant was incubated with 90 µL of *o*-PD (100 mM) for 3 h at room temperature in the dark. The mixture was centrifuged at 15 000× *g* (5 min, 4°C). A portion of the supernatant (180 µL) was mixed with 20 µL of 5-methylquinoxaline (5-MQ, internal standard) and analysed by HPLC with a mobile phase buffer containing 17% acetonitrile, 8% 50 mM NaH_2PO_4 (pH 4.5) and 75% water.

MG production in digitonin-permeabilized adipocytes

Adipocytes were permeabilized with digitonin as previously described (Hardin and Finder, 1998). Cells were harvested by trypsin digestion, washed with PBS and then incubated in PBS containing 40 µg·mL^{-1} digitonin for 5 min, After centrifugation at 300× *g* for 5 min, cell pellets were washed twice with buffer A (in mM: 150 sucrose, 35 potassium acetate, 35 KCl, 5 $MgSO_4$, 5 NaH_2PO_4, and 40 HEPES, pH 7.55). Cell pellets were incubated at 37°C for 3 h in buffer A supplemented with 2 mM ATP, 1 mM NAD^+ and different glucose metabolites. After centrifugation at 300× *g* for 5 min, the supernatant was collected for MG measurement.

Analysis of gene expression

Total RNA was isolated using an RNeasy Mini Kit (Qiagen) and converted to cDNA with an iScript™ cDNA Synthesis Kit (Bio-Rad). Real-time PCR was performed using SYBR Green PCR Master Mix (Bio-Rad) with the following primers: mouse aldolase A forward 5′-CAACGGTCACAGCACTTC-3′, reverse 5′-CTTCCTCACTCTGCCCTC-3′; mouse aldolase B forward 5′-CCAGTTCCCTATGTTCCA-3′, reverse 5′-TTGCTGTGCC TCTTCTAT-3′. Primers of mouse 18S rRNA were purchased from Qiagen. Protein levels were analysed by Western blotting using antibodies as follows: aldolase A (1:5000, Sigma), aldolase B (1:500, Epitomics) and β-actin (1:5000, Santa Cruz Biotechnology).

Data analysis

Results are expressed as mean ± SEM from five independent experiments. Statistical analyses were performed using parametric Student's *t*-test (two-tailed) or one-way ANOVA.

Materials

Sorbinil was a generous gift from Pfizer Inc. (Groton, CT). (*E*)-2-(4-fluorophenethyl)-3-fluoroallylamine (MDL-72974) was a generous gift from Dr Peter Yu (Department of Pharmacology, University of Saskatchewan, Canada). 2-deoxy-D-glucose (2-DG) and diallyl disulfide (DADS) were purchased from Sigma-Aldrich (Ontario, Canada).

Results

Insulin enhanced MG formation in adipocytes

Application of insulin (100 nM) increased basal MG production in 3T3-L1 adipocytes (Figure 1A). Glucose (25 mM)

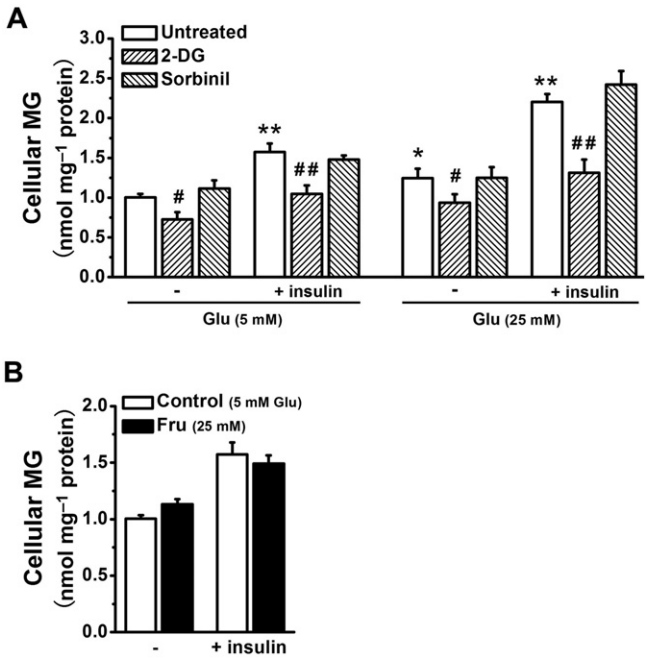

Figure 1

Insulin-enhanced MG formation in 3T3-L1 adipocytes. (A) Insulin (100 nM)-induced MG formation in adipocytes treated with or without 2-DG (5 mM) or sorbinil (10 µM) for 12 h. $^*P < 0.05$, $^{**}P < 0.01$ versus 5 mM glucose (Glu) and $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus untreated. (B) MG levels in adipocytes after 12-h incubation with fructose alone or plus insulin (100 nM). $n = 5$ in each group in panels A and B.

treatment elevated cellular MG levels, which was further augmented by co-treatment with insulin (Figure 1A). The glucose analogue, 2-DG, competitively inhibits glucose uptake and glycolysis (Pelicano *et al.*, 2006). Basal MG levels and the elevated MG levels induced by insulin (100 nM), glucose (25 mM) or their combination in adipocytes were reduced by application of 2-DG were but not affected by application of sorbinil, a specific aldose reductase inhibitor (Figure 1A). Incubation of 3T3-L1 adipocytes with fructose (25 mM) or fructose plus insulin (100 nM) did not increase MG levels (Figure 1B).

Insulin increased glucose 6-phosphate (G-6-P) levels in adipocytes

Glucose (25 mM) elevated levels of G-6-P (the first metabolite of glycolysis) in 3T3-L1 adipocytes. Insulin (100 nM) increased basal and glucose (25 mM)-induced G-6-P formation (Figure 2A). However, insulin (100 nM), glucose (25 mM), or their combination had no effects on cellular levels of sorbitol and fructose (Figure 2B, C).

We compared the generation of MG from metabolites in the glycolytic pathway upstream of aldolase A (G-6-P and F-1,6-BP) or in the aldose reductase pathway upstream of aldolase B (sorbitol, fructose and F-1-P) in digitonin-permeabilized adipocytes, as most of these metabolites are cell impermeable. The amount of MG produced by G-6-P or F-1,6-BP is 3.2- and 6.5-fold above the baseline levels of MG,

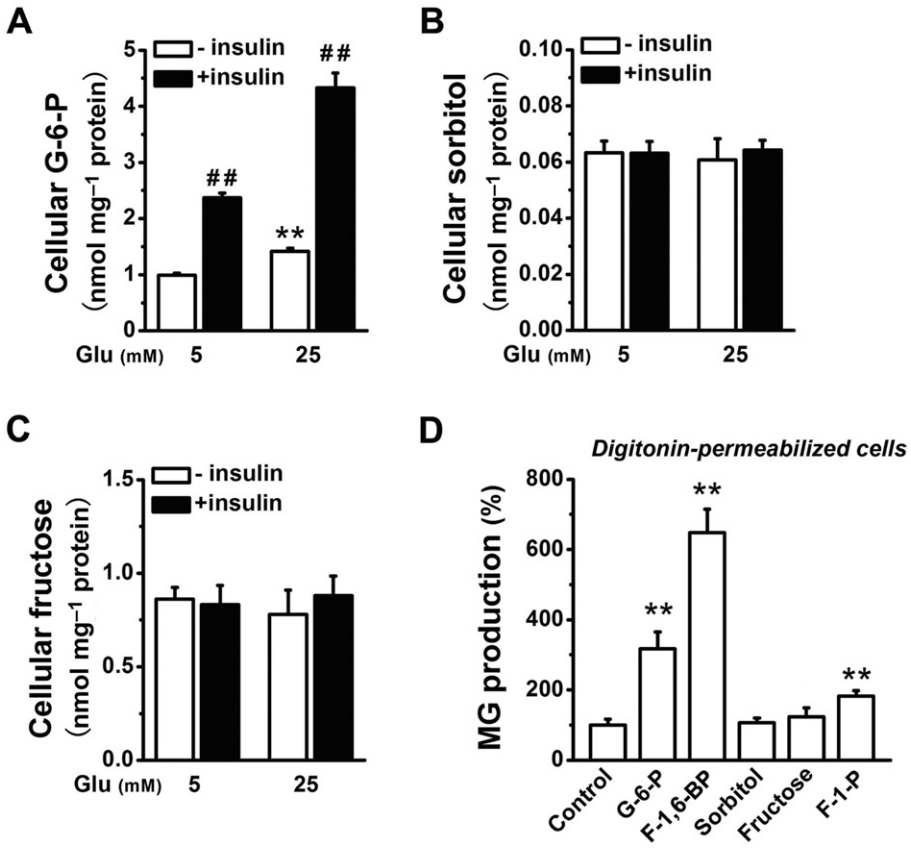

Figure 2

Glucose metabolism in 3T3-L1 adipocytes. (A) Levels of glucose 6-phosphate (G-6-P), (B) sorbitol and (C) fructose in adipocytes treated with or without insulin (100 nM) for 12 h. $^{**}P < 0.01$ versus 5 mM glucose (Glu) and $^{\#\#}P < 0.01$ versus glucose alone. (D) Relative MG production in digitonin-permeabilized adipocytes after treatment with G-6-P, fructose 1,6-diphosphate (F-1,6-BP), sorbitol, fructose and fructose 1-phosphate (F-1-P), at the same concentration of 1 mM for 3 h respectively. $^{**}P < 0.01$ versus control. $n = 5$ in each group in panels A–D.

respectively, and much higher than that produced by an equimolar concentration of F-1-P, which was 1.7-fold higher than control. Incubation with sorbitol or fructose did not elevate MG levels in digitonin-permeabilized adipocytes (Figure 2D).

Insulin up-regulated aldolase A gene expression in adipocytes

Insulin (100 nM) up-regulated mRNA and protein levels of aldolase A in 3T3-L1 adipocytes, but glucose (25 mM) had no effects on aldolase A gene expression, compared with that from control cells (Figure 3A, B). Co-application of glucose (25 mM) had no effect on insulin (100 nM)-enhanced aldolase A mRNA and protein levels (Figure 3A, B). However, gene expression of aldolase B (in mRNA and protein levels) was suppressed by insulin (100 nM), glucose (25 mM) or their combination in the same adipocytes (Figure 3A, B).

Knock down of aldolase A prevented MG formation in adipocytes

Transfection of 3T3-L1 adipocytes with single siRNA targeting aldolase A or aldolase B reduced mRNA levels of aldolase A by 70% and mRNA level of aldolase B by 78% respectively (Figure 4A). As single transfection with aldolase A siRNA elevated mRNA levels of aldolase B, we also used adipocytes cells double-transfected with aldolase A and aldolase B siRNAs in this study, which had 65 and 70% lower mRNA levels of aldolase A and aldolase B, respectively, in comparison with the transfection with control siRNA (Figure 4A). Basal MG levels were reduced in aldolase A siRNA-transfected cells and, although not significantly, in double siRNAs-transfected cells, but not in mock (transfected with only transfection agents), or control siRNA or aldolase B siRNA-transfected cells (Figure 4B). Excess MG production due to incubation with insulin (100 nM), glucose (25 mM) or their combination occurred in mock, control siRNA- or aldolase B siRNA-transfected cells, but was abolished in aldolase A siRNA- or double siRNA-transfected cells (Figure 4B).

CYP 2E1 and SSAO were not implicated in MG formation in 3T3-L1 adipocytes

Incubation of 3T3-L1 adipocytes with acetone, acetol, glycerol [converting to DHAP during triglyceride degradation (Pethe *et al.*, 2010)], glycine and threonine (glycine and threonine are precursors of aminoacetone) at concentrations of 25 mM, in the absence or presence of insulin (100 nM), did

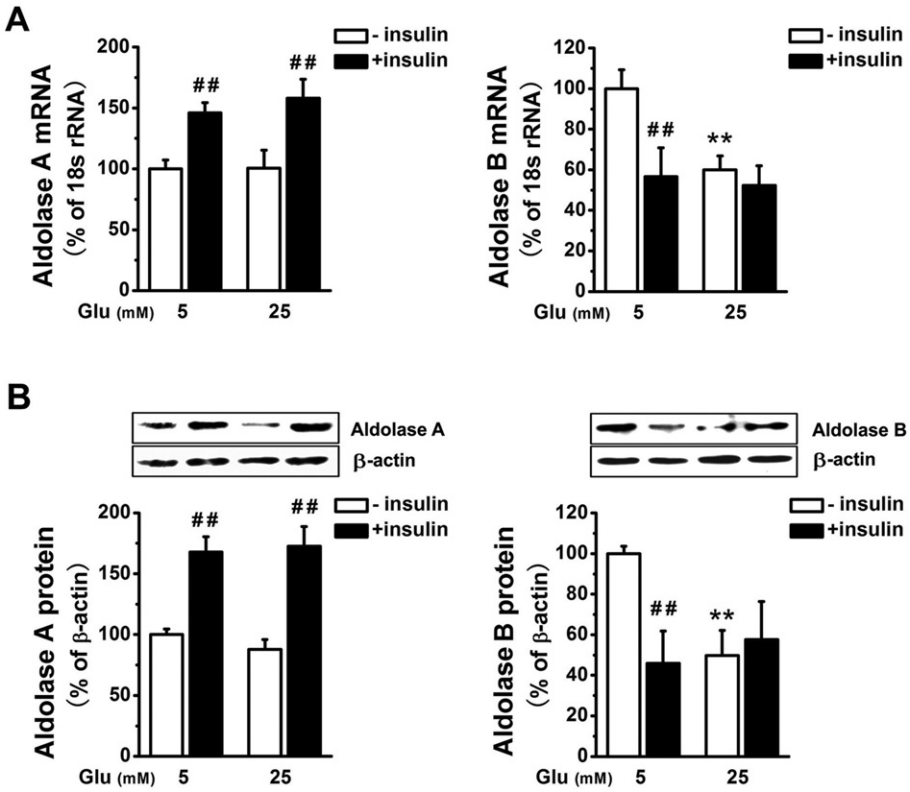

Figure 3

Gene expression of aldolase A and aldolase B in 3T3-L1 adipocytes. (A) Real-time PCR analysis of mRNA levels and (B) Western blot analysis of protein levels of aldolase A and aldolase B in adipocytes treated with or without insulin (100 nM) for 12 h. $^{**}P < 0.01$ versus 5 mM glucose (Glu) and $^{\#\#}P < 0.01$ versus glucose alone. $n = 5$ in each group in panels A and B.

not alter cellular MG levels (Figure 5A). Application of DADS or MDL-72974, the specific inhibitor of CYP 2E1 and SSAO, respectively, had no effect on basal MG formation and excess MG formation induced by insulin (100 nM), glucose (25 mM), or their combination in 3T3-L1 adipocytes (Figure 5B).

Discussion and conclusion

MG is an upstream pathogenic factor for the development of metabolic syndrome (Liu *et al.*, 2012). We previously found that in insulin-insensitive VSMCs and ECs, fructose (25 mM) or glucose (25 mM) up-regulated aldolase B and promoted MG overproduction (Liu *et al.*, 2011; 2012). In this study, we report a different mechanism for MG generation in insulin-sensitive adipocytes where fructose (25 mM) has no effect on MG production and glucose (25 mM) only slightly increases MG levels. However, insulin (100 nM) up-regulates aldolase A, an enzyme of the glycolytic pathway, and significantly elevates basal and glucose (25 mM)-induced MG formation in adipocytes.

Insulin-insensitive cells such as VSMCs and ECs, and insulin-sensitive cells, such as adipose cells, are traditionally distinguished by their sensitivity to insulin with respect to glucose transport. For example, glucose enters cells mainly through an insulin-independent GLUT1 in VSMCs while through an insulin-dependent GLUT4 in adipocytes (Shepherd and Kahn, 1999). MG formation in VSMCs is likely to be insulin independent, but here we found that insulin played a crucial role in MG production in adipocytes. In VSMCs, glucose (25 mM) raised cellular MG levels by threefold, but insulin (100 nM) had no effect on gene expression of aldolase A and aldolase B and cellular MG levels when compared with the control group (5 mM glucose alone) (Liu *et al.*, 2011). However, in 3T3-L1 adipocytes, we found that insulin (100 nM) treatment significantly increased basal MG formation and co-application of insulin and glucose (25 mM) significantly augmented glucose-increased MG levels (Figure 1).

Adipocytes rely less on fructose and aldolase B for MG formation. Fructose up-regulated gene expression of aldolase B and increased MG production in VSMCs (Liu *et al.*, 2011). However, fructose treatment did not alter MG generation in 3T3-L1 adipocytes (Figure 1). Fructose can be intracellularly produced from glucose through the aldose reductase pathway. Glucose (25 mM) treatment activated the aldolase reductase pathway and elevated sorbitol and fructose levels in VSMCs and ECs (Berrone *et al.*, 2006; Liu *et al.*, 2011), but not in 3T3-L1 adipocytes (Figure 2). In addition, insulin did not affect the conversion of fructose from glucose in 3T3-L1 adipocytes (Figure 2), which is consistent with the previous observations in erythrocytes, retina and kidney epithelial cells (Hutton *et al.*, 1974; Hotta *et al.*, 1991). More importantly, insulin, glucose (25 mM), or their combination

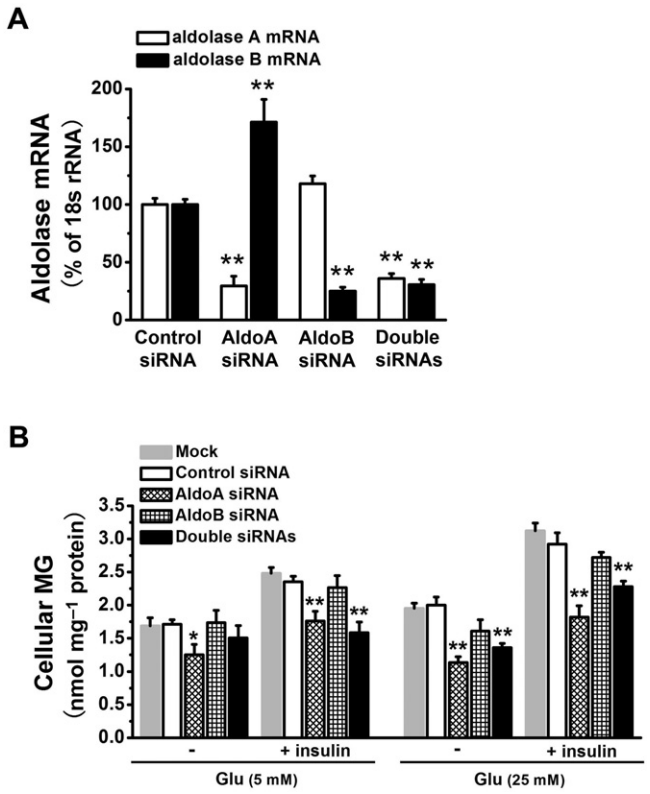

Figure 4

Knock down of aldolase A prevented MG formation in 3T3-L1 adipocytes. (A) mRNA levels of aldolase A or aldolase B, and (B) levels of MG in adipocytes transfected with transfection agents (mock), or with control, aldolase A or aldolase B siRNA, or double siRNAs. After transfection, cells were grown in medium with or without insulin (100 nM) for 12 h. *$P < 0.05$, **$P < 0.01$ versus respective control siRNA. $n = 4$ in each group in panel A and $n = 6$ in each group in panel B.

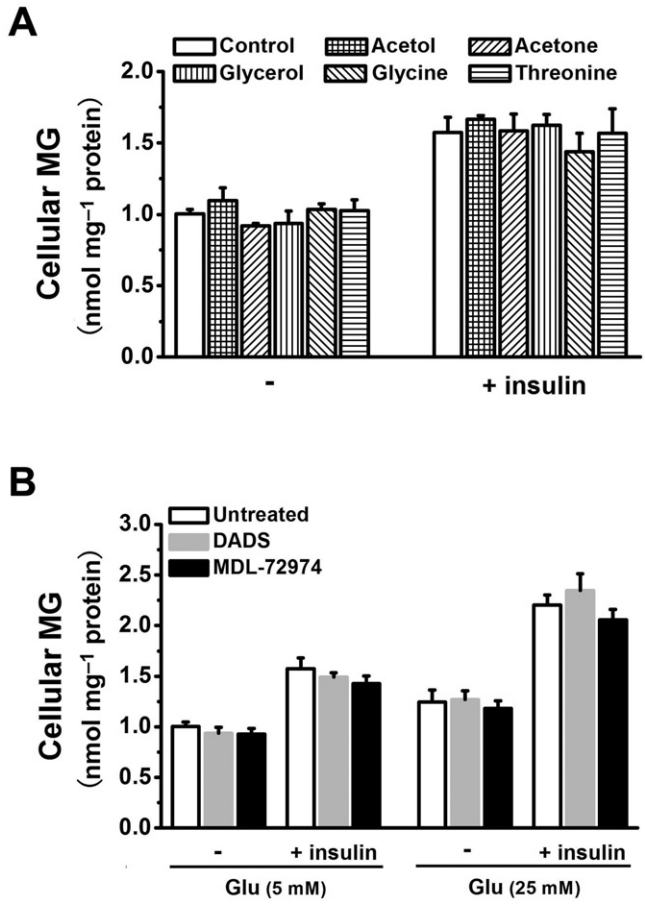

Figure 5

Effect of cytochrome P450 2E1 (CYP 2E1) and SSAO on MG formation in 3T3-L1 adipocytes. (A) MG levels in 3T3-L1 adipocytes treated with acetone, acetol, glycerol, glycine or threonine at the same concentration of 25 mM for 12 h. (B) Insulin (100 nM)-induced MG formation in the presence or absence of DADS (a CYP 2E1 inhibitor, 100 μM) or (E)-2-(4-fluorophenethyl)-3-fluoroallylamine (MDL-72974, a SSAO inhibitor, 5 μM) for 12 h. $n = 5$ for each group in panels A and B.

suppressed aldolase B expression in adipocytes (Figure 3). Inhibition of aldose reductase or knock down of aldolase B did not reduce basal or excess MG formation in adipocytes under our tested conditions (Figures 1, 4).

Instead, aldolase A becomes the dominant contributor to MG generation in adipocytes. Aldolase A cleaves F-1,6-BP in the glycolytic pathway to yield GA3P and DHAP, which are considered as the direct precursors of MG (Richard, 1993; Ramasamy *et al.*, 2006; Liu *et al.*, 2011). Knock down of aldolase A or application of 2-DG reduced basal MG formation in control 3T3-L1 adipocytes (Figures 1, 4), suggesting that aldolase A played a crucial role in maintaining basal MG formation in adipocytes. In cells, MG generation is elevated with the increase of its upstream metabolites, such as G-6-P and F-1,6-BP (Figure 2). Glucose (25 mM) elevated G-6-P and MG levels in adipocytes, although it had no effect on gene expression of aldolase A (Figures 1–3). However, insulin can up-regulate the gene expression of aldolase A independent of extracellular glucose concentrations (Figure 3). Co-application of insulin and glucose (25 mM) increased cellular G-6-P and MG levels when compared with the application of glucose alone (Figures 1, 2). When we blocked glycolysis with 2-DG or knocked down aldolase A expression in 3T3-L1 adipocytes, the enhancement of MG generation by insulin (100 nM), glucose (25 mM), or their combination, was completely prevented (Figures 1, 4). These data suggest that aldolase A is a key enzyme responsible for MG generation in 3T3-L1 adipocytes, and the basal or glucose (25 mM)-elevated MG formation was increased by insulin, which up-regulated aldolase A. We also noticed that aldolase B expression was up-regulated when aldolase A was knocked down (Figure 4). Reduced aldolase A activity would inhibit glycolysis, leading to an elevated glucose conversion to fructose via the polyol pathway. Increased fructose levels would then up-regulate the expression of aldolase B. The latter event has been shown in our previous study where fructose treatment directly increased mRNA and protein levels of aldolase B in cultured vascular smooth muscle cells (Liu *et al.*, 2011).

Blood insulin and MG levels are elevated in obesity, hypertension and diabetes (Cusin *et al.*, 1992; Henry, 1998;

Liu *et al.*, 2011; Lu *et al.*, 2011). Our work showing that treatment with insulin significantly elevated both basal and glucose (25 mM)-induced MG formation in adipocytes is of particular interest, as it indicates that MG in adipose tissues may be overproduced by hyperinsulinaemia in different metabolic syndromes, especially under conditions where significant insulin resistance does not blunt insulin signalling. Studies *in vivo* found that, at the early stage of obesity, blood insulin levels were increased while adipose tissues maintained their normal insulin sensitivity (Penicaud *et al.*, 1987; Litherland *et al.*, 2004). In addition, even in healthy people, the peak in plasma insulin following meals could be causing small transient elevations of MG production in adipocytes, which could become progressively more severe in cases of chronic high dietary carbohydrate intake and the subsequently increased secretion of insulin. The elevated MG, in turn, may play a role in the development of insulin resistance (Riboulet-Chavey *et al.*, 2006; Jia and Wu, 2007; Dhar *et al.*, 2011) and obesity (Jia *et al.*, 2012).

We have shown before that a single i.p. injection (240 mmol·kg^{-1}) of MG induced, after 2 h, insulin resistance with increased plasma insulin level and impaired glucose tolerance in Sprague-Dawley (SD) rats (Dhar *et al.*, 2010a). We also showed that, in chronically fructose-fed SD rats, serum MG level was elevated to around 4 μM (a value similar to that found in in diabetes) along with higher plasma insulin levels and insulin resistance-like syndrome (Jia and Wu, 2007). Glucose and fructose are the major precursors of MG formation (Dhar *et al.*, 2008; Liu *et al.*, 2011). In these whole animal studies, insulin resistance appears to be caused by MG, rather than a secondary event to hyperinsulinaemia (Riboulet-Chavey *et al.*, 2006; Jia and Wu, 2007; Dhar *et al.*, 2011). Our current study demonstrated that the insulin-induced MG overproduction in adipocytes, which suggests a reverse correlation between insulin and MG production. It is likely that MG causes insulin resistance, whereas insulin stimulates MG production in order to increase insulin resistance. Important limitations of our study were that it used cultures of rodent cells and that the insulin concentrations used were close to the upper limit of the physiological concentration range. Thus, the observations made in this cellular study can be extrapolated, only with caution, to insulin resistance in whole animals.

In conclusion, the metabolic pathway for MG formation in insulin-sensitive cells differed from that in insulin-insensitive cells. Insulin up-regulated aldolase A and enhanced MG formation in cultured 3T3-L1 adipocytes. Aldolase A would be a target to reduce excess MG generation in insulin-sensitive cells.

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Conflict of interest

The authors declare no conflicts of interest.

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